

**U.S. Wheat and Barley Scab Initiative
 FY00 Preliminary Final Performance Report
 (approx. May 00 – April 01)
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Cover Page

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Project

Program Area	Project Title	Requested Amount
Biotechnology	Develop rapid testing of anti-fungal proteins against Fusarium graminearum.	\$56,355.00
	Requested Total	\$56,355.00¹

 Principal Investigator

 Date

¹ Note: The Requested Total and the Award Amount 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?

Pre-testing of anti-fungal proteins (AFPs) in suspension plant cell cultures was the desired goal. This was because whole-plant transformation and head blight testing of adult transformants is expensive and time-consuming [Chen *et al.*, 1999; Smith *et al.*, 2000]. It takes a minimum of eighteen months or more to test the efficacy of anti-fungal genes using whole plant transformation and adult plant screening procedures [Van de Mortel *et al.*, 1999]. Whole plant transformation is a very expensive method of screening potentially useful AFPs.

In addition, individual AFPs are often not broad-spectrum; therefore many isoforms need to be tested. Plant suspension cell expression systems that accept a wide range of constructs are more desirable than other expression systems that require additional cloning steps. Bacterial expression systems may not properly process biologically active eukaryotic anti-fungal proteins. Therefore, protocols using a plant suspension cell assays for pre-testing eukaryotic anti-fungal protein, biolistic constructs (AFPs) useful in genetic engineering of fungal disease resistant cereals [Bushnell *et al.*, 1998] were developed. In these protocols, plant suspension cell cultures were transformed using biolistic constructs of AFPs, and the efficacy of the AFPs was tested directly against growth of the *Fusarium* Head blight fungus.

PROTOCOL # 1 (Visible Fungal Growth Approach)

The first system was based on creating plant cell suspension culture lines containing individual AFP's (Black Mexican Sweet Corn cell suspensions- BMS). BMS cells were easily transformed by microprojectile bombardment and grew well on both MS solid and liquid medium [Murashige and Skoog, 1962]. BMS cells responded equally well to promoters like cauliflower mosaic virus 35s, maize ubiquitin and the sugar cane badanvirus promoter [Hilburn *et al.*, 2000]. We tested "lawns" of these BMS-AFP cell lines for their ability to stop or slow visible colony growth of the *Fusarium* Head blight fungus, *Fusarium graminearum*.

PROTOCOL # 2 (Microassay Approach)

The second system based on use of a hand-held biolistic gene gun. In this protocol BMS cells were placed directly on cellulose filters and were co-transformed with using a biolistic hand-held gene gun. BMS lawns were transformed with visual transgene marker that upregulated maize anthocyanin [Bowen B. 1992], and one or more AFPs. AFP-anthocyanin co-transformed BMS cells were inoculated with a *F. graminearum* isolate previously transformed with an *Aequorea victoria* green fluorescent protein (GFP) reporter gene. Thirty hours later, BMS cell filters were examined microscopically. The interactions between co-transformed cells (seen as brown or red cells or cell clumps) and for GFP fungal hyphae were scored for fungal hyphal contact or fungal hyphal avoidance.

2. What were the most significant accomplishments?

The microassay approach (Protocol # 2) proved to be the most rapid and effective method of pre-testing AFPs.

Results from the microassay protocol revealed that specific anti-fungal proteins repelled *F. graminearum* contact. These AFPs were Arabidopsis PR5, Fusarium Tri 101 and wheat WIR 2. However, barley chitinase, barley glucanase, rice chitinase and oat Tlp1 AFPs were ineffective in repelling *F. graminearum* contact. Also, combinations of chitinase/glucanase or chitinase/oatTlp 1 were ineffective (See Listing of AFPs Used)

The microassay of AFP efficacy has great potential for evaluating AFPs for later use in whole plant transformation. It can also be used with other non-biotrophic fungal pathogens of wheat and barley to test the efficacy of various AFP candidates. All tested AFP constructs were given to G.J. Muehlbauer at the University of Minnesota and were placed into the whole-plant transformation project involving barley and wheat. Selected constructs were also used to transform oat.

AFP's Used & Construct Information

Anti-fungal protein genes tested were in biolistic constructs driven by the Sugar cane Badna Virus (ScBV [Tzafir et al. 1998]), or maize ubiquitin promoters followed by a maize alcohol dehydrogenase intron or a maize ubiquitin 1 intron, the particular AFP coding sequence and the *Agrobacterium* NOS terminator. pBScBV Rchit- rice chitinase transgene [Zhu and Lamb, 1991]. Singly, they were:

- pBScBV TLP1- oat thaumatin like protein cDNA [Lin *et al.*, 1996]
- pBScBV Barchit- barley chitinase cDNA [Leah *et al.*, 1991]
- pBScBV Barglu- barley glucanase cDNA [Leah *et al.*, 1991]
- pBScBv ArabPR5- Arabidopsis PR5 cDNA [Uknes *et al.*, 1992]
- pUBK Tri101-Trichothecene 3-O-acetyltransferase cDNA [Kimura *et al.*, 1998]
- pAHC WIR 2- wheat thaumatin-like protein cDNA [Rebman *et al.*, 1991]

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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.

Bushnell WR, Somers DA, Giroux RW, Szabo LJ, & **Zeyen R.J.** 1998. Genetic engineering of disease resistance in cereals. *Canadian Journal of Plant Pathology* **20**:137-149.

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Hilburn KLB. 2001. A plant suspension cell assay for anti-fungal transgene efficacy. MS Thesis. University of Minnesota 210 pp.

Smith L, Wyckoff M, Baldrige G, **R. Zeyen R.**, & Muehlbauer GJ. 2000 Antifungal protein gene expression in transgenic wheat (*Triticum aestivum*). *Agronomy Abstracts*

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