U.S. Wheat and Barley Scab Initiative
FY01 Final Performance Report (approx. May 01 – April 02)
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Cover Page

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Grant Number:	**N/A**
Grant Title:	**Fusarium Head Blight Research**
FY01 ARS Award Amount:	**$ 48,673**

Project

Program Area	Project Title	Requested Amount
Biotech	Marker and Plasmid-free Transgenic Barley Encoding Antifungal Proteins	$ 58,100
	Total Amount Requested	**$ 58,100**

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Principal Investigator Date

(Form – FPR01)

Project 1: Marker and Plasmid-free Transgenic Barley Encoding Antifungal Proteins

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

Fusarium head blight (FHB) of barley in the upper Midwest has reached epidemic proportions. Yield and quality losses have caused economic hardships for producers and users. Various sources of resistance in barley germplasm have been identified and are being introgressed into elite germplasm, but resistance is only partial and additional sources of high resistance are desirable. We seek to produce FHB resistance by introducing novel genes, or novel expression patterns of genes, encoding antifungal proteins via recombinant DNA technologies. These genes include thaumatin-like genes cloned from oat, *Avena sativa,* and genes from *Fusarium* itself, *e.g.*, *TRI 101* and *TRI 12*. Our work will result in the production of transgenic barley plants containing *Ds*-bordered, putative antifungal protein genes, which by cross-hybridization with *Ac*-transposase stocks, will relocate the introduced gene to new, plasmid- and marker-free locations that will support stable expression of these genes. We are most interested in introducing these genes into the elite 6-rowed barley cultivar, Drummond, to facilitate resistance testeing and the practical application of this technology, but we will also work with the easily-transformed cultivar, Golden Promise, to ensure the production of transformed plants.

2. What were the most significant accomplishments?

Pathogenesis-related genes from oat, *tlp1* and *tlp4*, and two trichothecene pathway genes, *TRI 101* and *TRI12*, were put into *Ds*-bordered, maize *ubiquitin*-promoter-driven or rice *actin*-promoter-driven expression cassettes, and introduced via particle bombardment into immature embryos of Golden Promise, and into green, regenerative tissues of Drummond. Also introduced were plasmids encoding either bialophos or hygromycin resistance for selection of transformed tissues.

Fifteen, independent, bialaphos-resistant, *tlp1*-transformed Golden Promise callus lines and 22 independent, bialaphos resistant, *tlp4*-transformed Golden Promise callus lines were obtained. Regenerated plants were recovered from four *tlp1* lines and seven *tlp4* lines and plants from one *tlp1* and two *tlp4* lines were shown by PCR to contain the appropriate *tlp* gene. Selection and regeneration attempts continue on additional lines. Similar transformation efforts with all four gene constructs are in process for Drummond, but selections are in the early phase at present and definitive proof of transformation success has not been obtained at this time. The gene encoding maize *Ac*-transposase, under control of the maize *ubiquitin* or its own putative promoter, has been introduced into the Drummond background via backcrossing, and introduction via transformation is being attempted.

To develop tools for measuring the expression of the introduced proteins encoded by *tlp 1, tlp 4*, *Tri 101* and *Tri 12*, these genes were inserted in the protein expression vector, pGEX4T3, and transformed into *E. coli*. The proteins, TLP1, TLP4 and TRI 101 were expressed at high levels after IPTG induction and the proteins purified. Rabbits were injected with the purified proteins and serum is currently being prepared to test antibodies for efficacy in immunoblots. TRI 12, a known membrane protein, was produced in low levels in *E. coli* and is currently being scaled up to obtain sufficient protein for antibody production.

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.

Publications and Presentations

Yu, X-H., P. Bregitzer, M-J. Cho, and P.G. Lemaux. 2001. Using the maize *Ac-Ds* system to gernerate marker-free transgenic barley plants that stably express putative antifungal proteins. Poster presentation at the 2001 National Fusarium Head Blight Forum, December, 2001, Cincinnati.