U.S. Wheat and Barley Scab Initiative
FY02 Final Performance Report (approx. May 02 – April 03)
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Cover Page

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Grant Title:	**Fusarium Head Blight Research**
FY02 ARS Award Amount:	**$ 49,756**

Project

Program Area	Project Title	USWBSI Recommended Amount
BIO	Marker- and Plasmid-free Transgenic Barley Encoding Antifungal Proteins.	$51,000
	Total Amount Recommended	**$51,000**

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Principal Investigator Date

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 testing and the practical application of this technology, but we early efforts utilized the easily-transformed cultivar, Golden Promise, to ensure the production of transformed plants.

2. What were the most significant accomplishments?

1) Novel contructs were produced containing four putative antifungal proteins: *tlp1* and *tlp4*, encoding thaumatin-like proteins derived from oat; and *TRI 101* and *TRI 12* derived from *Fusarium sporotrichioides* and which, respecitively, detoxify or transport out of cells the mycotoxin deoxynivalenol (DON). These plasmids were introduced singly, in combination with a selectable marker (either pAHC20 for glufosinate-ammonium resistance or pHpt4 for hygromycin resistance) via bombardment into immature embryos of Golden Promise or into green, regenerative tissues of the 6-row malting cultivar Drummond.

2) Barley transformants were produced in Golden Promise*: tlp1* (3 independent lines, 9 plants); *tlp4* (3 independent lines, 14 plants); *TRI 12* (one line, one plant). We are initiating crosses of T_1 plants to a Drummond-derived line containing *Ac* transposase.

3) Drummond transformants have been produced: *TRI 101* (19 independent, hygromycin-resistant, *TRI 101* lines); *tlp1* (3 independent hygromycin-resistant lines; *tlp4* (6 independent, hygromycin-resistant lines. Plants were recovered from 3 *TRI 101*, 2 *tlp1* and 2 *tlp4* lines. PCR analysis in progress has confirmed at least one *TRI 101* and one *tlp1* line as transgenic. Direct introduction of *Ac*-transposase into Drummond via particle bombardment has also been successful, and several regenerated plants have been confirmed as transgenic.

4) To enable detection and quantification of antifungal proteins, polyclonal antibodies have been produced to TLP1, and TRI 101. Antibodies to TLP1 also recognize TLP4. These antibodies have been available to other USWBSI-funded researchers (Blechl, Dahleen), and we have confirmed antifungal protein production via Western analysis in some of their transgenic lines Antibody production to TRI 12, a membrane protein, has been hampered by poor expression in several *E. coli* expression vectors.

5) In related, but unfunded research, we are organizing an effort to screen transgenic plants with constitutive expression of antifungal proteins against a spectrum of other diseases. There is sufficient interest among pathologists at several institutions to enable screening against smuts, bunts, mildew, septoria, spot and net blotch, and rusts. Wheat lines produced by two USWBSI-funded researchers are currently being screened for resistance to smuts and powdery mildew. These efforts will greatly enhance the impact of USWBSI-funded research at minimal cost.

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.

X-H. Yu, P. Bregitzer, M-J. Cho, M.L. Chung, and P.G. Lemaux. 2002. Transposon-mediated generation of marker-free barley plants expressing putative antifungal proteins. pp. 52-53, Proceedings, National Fusarium Head Blight Forum, Dec. 7-9, Elanger, Kentucky.