USDA-ARS / USWBSI
FY03 Final Performance Report (approx. May 03 – April 04)
July 15, 2004

Cover Page

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Year:	**FY2003 (approx. May 03 – April 04)**
FY03 ARS Agreement ID:	**NA**
FY03 ARS Agreement Title:	**FHB-resistant transgenic barley: marker-free plants and chloroplas transformation.**
FY03 ARS Award Amount:	**$ 62,439**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	FHB-resistant transgenic barley: marker-free plants and chloroplast transformation.	$ 62,439
	Total Amount Recommended	**$ 62,439**

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Principal Investigator Date

* BIO – Biotechnology
CBC – Chemical & Biological Control
EDM – Epidemiology & Disease Management
FSTU – Food Safety, Toxicology, & Utilization
GIE – Germplasm Introduction & Enhancement
VDUN – Variety Development & Uniform Nurseries

Project 1: ***FHB-resistant transgenic barley: marker-free plants and chloroplast transformation.***

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 bordered by maize *Ds* sequence: *Ds-tlp1* and *Ds-tlp4*, encoding thaumatin-like proteins derived from oat; and Ds-*TRI 101* and *Ds TRI 12* derived from *Fusarium sporotrichioides* and which, respectively, detoxify or transport out of cells the mycotoxin deoxynivalenol (DON). These plasmids were introduced singly, in combination with a selectable marker (either *bar* or *hpt*), 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); and *tlp4* (3 independent lines, 14 plants). Crosses to a Drummond-derived line containing *Ac* transposase are being used to induce transposition.

3) Drummond transformants have been produced, a significant accomplishment in that this system allows the direct transformation of an economically important, 6-rowed malting cultivar. Transformed plants have been recovered for: *TRI 101* (12 independent lines); *tlp1* (3 independent hygromycin-resistant lines; *tlp4* (2 independent lines); and *tlp4* (2 independent lines). Direct introduction of *Ac*-transposase into Drummond via particle bombardment has also been successful, and several regenerated plants have been confirmed as transgenic.

4) Polyclonal antibodies have been produced to TLP1, and TRI 101. Antibodies to TLP1 also recognize TLP4. The TRI101 antibodies have been made available to other USWBSI-funded researchers (Blechl, Dahleen), and we have confirmed antifungal protein production via Western analysis in some of their transgenic lines. An indirect ELISA protocol has been developed for TRI101. Antibody production to TRI 12, a membrane protein, has been hampered by poor expression in several *E. coli* expression vectors.

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 you grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Yu, X-H., 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.

Bregitrzer, P., P.G. Lemaux, and X-H. Yu. 2003. Transposons and meristematic cultures: tools to improve transgene stability, agronomic performance, and consumer acceptance. pp 5-6, Proceedings, National Fusarium Head Blight Forum, Dec. 13-15, Bloomington, Minnesota.

Yu, X-H., P. Bregitzer, M-J. Cho, L-C. Hsueh, H-S. Yu, and P.G. Lemaux. 2003. Introduction of putative antifungal genes into two-row and six-row barley through genetic engineering. p. 54, Proceedings, National Fusarium Head Blight Forum, Dec. 13-15, Bloomington, Minnesota.