U.S. Wheat and Barley Scab Initiative
FY01 Final Performance Report (approx. May 01 – April 02)
July 15, 2002

Cover Page

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Year:	**FY2001 (approx. May 01 – April 02)**
Grant Number:	**N/A**
Grant Title:	**Fusarium Head Blight Research**
FY01 ARS Award Amount:	**$ 26,284**

Project

Program Area	Project Title	Requested Amount
Biotech	Spike-specific promoter isolation from Bowman and near-isogenic marker lines	$ 27,062
	Total Amount Requested	**$ 27,062**

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Principal Investigator Date

(Form – FPR01)

Project 1: Spike-specific promoter isolation from Bowman and near-isogenic marker lines

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

Barley transformation has the potential to help combat Fusarium head blight by introducing anti-fungal and anti-toxin genes. Promoters currently in use for barley transformation give transgene expression in all plant tissue throughout development, which is not an efficient use of plant resources. This project will isolate spike-specific promoters to target gene expression to the spike tissues that are attacked by Fusarium head blight. RNA differential display technology is being used to identify genes that are expressed in spike tissues of Bowman and ten near-isogenic lines with morphological mutations in spike tissues. Regulatory regions, i.e. promoters, of these genes will be identified by DNA sequencing and 5'-rapid amplification of cDNA ends (5'RACE). Candidate spike-specific promoters will be inserted into marker gene constructs and tested for transgene expression patterns. Differential display comparisons include Bowman spike tissue vs. non-spike tissue, expressing vs. non-expressing tissue of the morphological marker lines, and morphological line tissue expressing the trait vs. the comparable normal tissue in Bowman.

2. What were the most significant accomplishments?

Differentially expressed sequences have been identified by comparison of eight of the near-isogenic lines with Bowman. These sequences were expressed in tissues showing the morphological trait but not in normal tissues, or were found in spike tissues but not in leaf tissue. Differential display has been completed on six populations, resulting in dot blot comparisons of 84 sequences from orange lemma (rob1.a), 95 from glossy sheath and spike (gsh2.f), 58 from glossy spike (cer-i.16), 88 from globosum (glo-a.1003), 78 from glossy sheath and spike (gsh6.s), and 88 from glossy lemma and pericarp (Gle1.a). At the end of this grant cycle, fifteen clones have been confirmed as differentially expressed using the dot blots. Ten of these have been sequenced; comparison to sequences in GenBank showed no known gene matches, but several matches to sequences in the barley and wheat EST database. Three of these sequences showed a single copy on Southern blots (Southerns on other clones underway) and will be sent to Dr. Kleinhofs to identify the homologous BAC clones, after northern analysis to confirm spike-specific expression. Primers for 5'RACE have been designed for all ten sequences to obtain the full gene sequence and the promoter. Sequences from albino lemma (alm1.a) and yellow head (yhd1.a) have been isolated from differential display gels and are being prepared for re-amplification and cloning. RNA extractions have been completed for the last two problematic lines (red lemma and pericarp, Pre2.b; black lemma and pericarp, Blp1.b) and differential display analyses will begin shortly.

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.

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