U.S. Wheat and Barley Scab Initiative
Annual Progress Report
September 18, 2000

Cover Page

PI:	**Lynn Dahleen**
Institution:	**USDA-ARS**
Address:	**Northern Crop Science Lab** **P.O. Box 5677 SU Station** **Fargo, ND 58105**
Email:	**dahleen@badlands.nodak.edu**
Phone:	**701-239-1384**
Fax:	**701-239-1369**
Year:	**FY2000**
Grant Number:	
Grant Title:	**Fusarium Head Blight Research**
Amount Granted:	**$50,000.00**

Project

Program Area	Objective	Requested Amount
Biotechnology	Isolate spike-specific promoters from Bowman barley and near-isogenic morphological marker lines.	$50,348.00
	Requested Total	$50,348.00[1]

Lynn Dahleen Sept. 18, 2000
Principal Investigator Date

[1] Note: The Requested Total and the Amount Granted are not equal.

Project 1: Isolate spike-specific promoters from Bowman barley and near-isogenic morphological 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 changes 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. Please provide a comparison of the actual accomplishments with the objectives established.

RNA has been extracted from expressing and non-expressing tissue of ten plants each of six mutant lines and from corresponding normal tissue from Bowman. Two replicate differential display comparisons have been conducted for each mutant and multiple candidate sequences identified. These are being re-amplified and purified for reverse Northern blot hybridization to confirm differential expression. We are on target for our objectives for these six mutants. For the other four mutants, RNA has been extracted from expressing and non-expressing tissue of fewer than ten plants, delaying differential display experiments.

3. What were the reasons established objectives were not met? If applicable.

Problems with greenhouse conditions have prevented some albino lemma (alm1.a) and yellow head (yhd1.a) plants from developing spikes for tissue collection and RNA extraction. Differential display experiments will begin as soon as tissue for RNA extraction is available. The black (Blp1.b) and red (Pre2.b) lemma and pericarp mutants contain high levels of phenolic compounds. Removing these compounds from RNA samples has been challenging and different methods are being tried to obtain RNA clean enough for differential display experiments.

4. What were the most significant accomplishments this past year?

Multiple differentially expressed sequences have been isolated from six mutant lines. These sequences will be further tested to confirm differential expression and will be used to isolate spike-specific promoter sequences.

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.

Dahleen, L.S., P.A. Okubara and A.E. Blechl. 1999. Transgenic approaches to combat Fusarium head blight in wheat and barley. Agron. Abstr. p. 83.