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

Cover Page

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Year:	**FY2000**
Grant Number:	
Grant Title:	**Fusarium Head Blight Research**
Amount Granted:	**$50,000.00**

Project

Program Area	Objective	Requested Amount
Biotechnology	Develop Fusarium-responsive promoters for use in transformation of barley and wheat.	$50,000.00
	Requested Total	$50,000.00

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Principal Investigator Date

(Form – PR1)

Project 1: Develop Fusarium-responsive promoters for use in transformation of barley and wheat.

1. What major problem or issue is being resolved and how are you resolving it?
Currently, there are no barley lines with biochemical resistance to *Fusarium graminearum*. To produce resistant lines, it may be necessary to introduce *F.g.* resistance through genetic transformation. Antifungal protein genes must be expressed in the most appropriate tissue and subcellular compartment to avoid placing a metabolic burden on the plant and to limit selection for resistant strains. To do this most effectively, it is also necessary to understand the process of *F.g.* infection. The proposed research incorporates subcellular targeting research and research on *F. g.* infection. Results from these studies will be incorporated with ongoing research, which has produced a lemma-specific promoter and several other floret-specific candidate genes. The planned product is a precise antifungal gene targeting vector that can be used in barley and wheat. The most useful antifungal protein would be hordothionin (HTH), which is highly toxic to *F.g.*

2. Please provide a comparison of the actual accomplishments with the objectives established.

Obj. A: Determine how to reroute HTH to the intracellular space by conducting transient expression assays of HTH subcellular targeting sequences: Targeting constructs were produced and tested by transient expression assays following particle bombardment of etiolated coleoptiles. Although weak, all secreted GUS activity (MUG assay) into the apoplasm, whereas no MDH activity was secreted (cell leakage control). The unaltered GUS control did not secrete, and had only localized GUS staining spots. We are now trying to determine whether artifacts could have caused these results.
Obj. B: Determine *F.g.* penetration route into lemma and pericarp using confocal microscopy: Not done yet.
Obj. C: Develope HTH antibodies: The HTH signal/GUS reporter vector did not produce high levels of GUS. Also, the HTH clone that was placed into the pET expression vector did not produce HTH protein. A new HTH clone was produced in which the first Met and all codons leading to a second Met were eliminated. This produced high levels of full-length HTH protein, which was used to produce HTH antibodies. These did not react with the purified (45 amino acid) HTH mature protein, possibly due to its high positive charge and tightly condensed folding pattern. However, they did react with only two seed homogenate proteins. These were of the proper size to represent the full-length pre-processed HTH and its processed C-terminal protein.

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

Progress is sufficient to meet grant objectives. The presence of the first Met codon in the HTH clone inhibited expression, and recloning of the HTH gene caused delays in the program.

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

    a. Construction and testing of subcellular targeting sequences.
    b. Production of HTH antibodies.

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.

Sathish, P, ML Federico, J Fu, R Skadsen, HF Kaeppler. 2000. Targeted expression of antifungal protein genes in barley. 6th Internatl. Congress of Plant Mol. Biol., Quebec, Canada. June 2000. Plant Mol. Biol. Rep. 18:2 (suppl), Abs. S03-104.