U.S. Wheat and Barley Scab Initiative

Annual Progress Report
September 15, 1999

Cover Page

PI:	**Ron Skadsen**
Institution:	**USDA/ARS, Cereal Crop Research Unit**
Address:	**501 N. Walnut St.** **Madison, WI 53705**
Email:	**rskadsen@facstaff.wisc.edu**
Phone:	**608-262-3672**
Fax:	**608-264-5528**
Year:	**FY1999**

Project

Program Area	Objective	Requested Amount
Biotechnology	Develop Fusarium-responsive promoters for use in transformation of barley and wheat.	$35,000
	Requested Total	$35,000[1]

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Principle Investigator Date

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

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?

Problem - Production of barley that is resistant to Fusarium graminearum. Since barley varieties do not have adequate resistance to Fusarium, it is necessary to introduce resistance genes through genetic transformation. We are modifying barley in order to redirect the expression of its native antifungal genes so that they will provide a barrier to Fusarium. This expression must ultimately be confined to seed spike tissues in order to avoid selection pressures which would result in a population of Fusarium that is resistant to the antifungal proteins. We are a) developing gene promoters to confine transgene expression to the lemma, palea and pericarp tissues that surround the developing seed and serve as primary colonization tissues for Fusarium, b) determining the cellular and subcellular route of Fusarium infection, c) (new) developing vectors for the subcellular targeting of antifungal protein gene expression, and d) identifying genes that are activated upon Fusarium infection.

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

a) A gene (D5) that is expressed only in lemma/palea tissue has been identified, cloned, and sequenced. The putative promoter region of the gene has been subcloned and used in the construction of a promoter/reporter recombinant gene which causes the production of a green fluorescent protein whenever the promoter is activated in a given tissue. We have transiently expressed this recombinant in lemma tissue and found that it is active. b) Conditions for growing and visualizing gfp/Fusarium (Fusarium that has been transformed with the green fluorescent protein gene) have been established at the tissue level. Histological examination of gfp/Fusarium has been started, and preparations have been made in which it is possible to see attachment of germinating Fusarium to individual lemma cells. Studies to follow this initial penetration event at the subcellular level will soon begin. c) Constructs have been developed for the subcellular targeting of antifungal genes to the vacuole and intracellular space. d) Fusarium gene response studies have not yet begun.

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

Not applicable. The funded research has not been under way for one year.

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

Production of a lemma-specific gene promoter
Determination of the growth characteristics of Fusarium in invading the lemma and pericarp
Construction of subcellular targeting 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 the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

P Sathish, ML Federico, HF Kaeppler, R Skadsen. Isolation of the promoter for a gene preferentially expressed in barley lemma/palea tissue. Abs SP-1000. Proceedings of the 1999 Congress on InVitro Biology, June, 1999, New Orleans.