USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY06 Final Performance Report (approx. May 06 – April 07)
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Cover Page

PI:	Ronald W. Skadsen
Institution:	USDA-ARS
Address:	Cereal Crop Research Unit 501 N. Walnut St. Madison, WI 55726
E-mail:	rskadsen@wisc.edu
Phone:	608-262-3672
Fax:	608-264-5528
Fiscal Year:	2006
USDA-ARS Agreement ID:	NA
USDA-ARS Agreement Title:	Fusarium Head Blight Research.
FY06 ARS Award Amount:	$ 32,803

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Award Amount
GET	Overexpression of Antifungal Genes with Tissue-Specific and Fusarium-Inducible Promoters.	$ 32,803
	Total Award Amount	**$ 32,803**

Ronald W Skadsen

June 7, 2007

Principal Investigator Date

* CBCC – Chemical, Biological & Cultural Control
EEDF – Etiology, Epidemiology & Disease Forecasting
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GET – Genetic Engineering & Transformation
HGR – Host Genetics Resources
HGG – Host Genetics & Genomics
PGG – Pathogen Genetics & Genomics
VDUN – Variety Development & Uniform Nurseries

Project 1: *Overexpression of Antifungal Genes with Tissue-Specific and Fusarium-Inducible Promoters.*

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

Fusarium graminearum infects developing spikes of barley mainly through the lemma and epicarp. Currently, there are no adequate host defenses, and no antifungal protein genes have been shown to provide adequate protection. In addition, the introduction of foreign genes into the barley genome is a sensitive product issue. This research attempts to lessen GMO issues by utilizing native barley genes, both as promoters and as antifungal protein genes. We have cloned, synthesized and tested a thionin antifungal gene from barley (LemThio1) and found it to be toxic to F. graminearum. We have also produced a barley gene promoter (Lem2) that is specific for the lemma/palea and epicarp. Combining these two should limit expression of the transgene to the lemma/palea and epicarp. Our research attempts to genetically modify barley with a Lem2::LemThio1 construct through transformation.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment: Barley was transformed using both the Agrobacterium method and the biolistic method. Both appeared to work equally well. In addition, the Lem2 promoter was compared with the constitutive Ubi promoter (maize ubiquitin plus first intron). LemThio1 antibodies were developed and used to analyze lemma protein on western blots. The target protein was detected in almost all transformants but not in untransformed controls. The Ubi promoter produced roughly eight times as much LEMTHIO1 protein as the Lem2 promoter, although both appeared equally strong with respect to GFP production. The Lem2 promoter also caused expression in the endosperm in some transformants but not in others. Initial antifungal testing will occur in Aug. ’07 using T1 plants. Testing will be done on homozygous positives, relative to homozygous negatives, as determined from Southern blots.

Impact: This study shows that the strategy of using barley sequences exclusively for promoter::antifungal gene constructs can lead to successful expression in transformants. The strategy can easily be broadened to other promoter::antifungal gene combinations. This will be especially beneficial to barley growers who presently do not have available stocks with sufficient genetic resistance to withstand ongoing F. graminearum infections.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn’t have before?

Additional knowledge has been generated on the use of tissue-specific gene promoters for genetic transformation. An additional antifungal gene clone has been added to the collection of genes available to barley and wheat researchers.

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.

None - project is in final phase.