USDA-ARS / USWBSI
FY04 Final Performance Report
July 15, 2005

Cover Page

PI:	**Ronald W. Skadsen**
Institution:	**USDA-ARS**
Address:	**Cereal Crops Research Unit** **501 N. Walnut St.** **Madison, WI 53726**
E-mail:	**rskadsen@wisc.edu**
Phone:	**608-262-3672**
Fax:	**608-890-0302**
Year:	**FY2004**
FY04 ARS Agreement ID:	**NA**
FY04 ARS Agreement Title:	**Transformation of Barley with an Antifungal Gene Under Control of an Epicarp Promoter.**
FY04 ARS Award Amount:	**$ 61,463**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Transformation of Barley with an Antifungal Gene Under Control of an Epicarp Promoter.	$ 61,643
	Total ARS Award Amount	**$ 61,463**

_____

Principal Investigator Date

* BIO – Biotechnology
CBC – Chemical & Biological Control
EDM – Epidemiology & Disease Management
FSTU – Food Safety, Toxicology, & Utilization
GIE – Germplasm Introduction & Enhancement
VDUN – Variety Development & Uniform Nurseries

(Form – FPR04)

Project 1: ***Transformation of Barley with an Antifungal Gene Under Control of an Epicarp Promoter.***

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

There are no known barley lines with biochemical resistance to *Fusarium*. Thus, it may be necessary to introduce resistance through genetic transformation. Many technical obstacles must be overcome before stable pathogen-resistant transgenic cereals can be introduced into the field. It is necessary to learn the requirements for strong re-directed expression of endogenous antifungal genes in specific tissues. Antifungal proteins must be expressed in the most appropriate tissue and subcellular compartment to avoid placing a metabolic burden on the plant and to minimize pressures which select for resistant pathogen strains. The long-range goal is to produce an antifungal gene/targeting vector that can be used in both barley and wheat.

2. What were the most significant accomplishments?

Previously, we cloned a gene, *Ltp6*, a novel lipid transfer protein gene that is preferentially expressed in the pericarp epithelium (epicarp). Vectors were synthesized that contained a shortened *Ltp6* promoter, followed by the *Ltp6* signal sequence that is responsible for secretion of the LTP6 peptide. A modified hordothionin gene (*Hth5*) was fused in-frame behind this, followed by an in-frame *gfp* gene. Transformants were obtained in which GFP could be detected in the epicarp of T1 developing seed. However, GFP was also detected in the endosperm, beginning in the dough phase. The transgene mRNA was detected in epicarps using a PCR approach. We were unable to detect HTH protein in epicarps by homogenizing the tissue in buffer containing SDS (Laemmli sample buffer.)

We have recently learned that HTH can be extracted using H2SO4. Since the positive transformants also produce GFP in the germinating coeoptiles, we extracted these with H2SO4 and got positive bands on western blots using an antibody produced from the C-terminal half of the HTH mature peptide. We are in the process of raising T2 seeds of these and previous transformants to re-test for HTH production.

In related studies, analysis of the T2 generation of Lem2 (lemma/palea-specific promoter) promoter transformants showed that most transformed lines developed gfp expression in the endosperm as well as the lemma/palea. As with the Ltp transformants, this began in the doughy stage. Only one line, e-1, maintained the original expression patter (lemma, palea and epicarp) seen in the T1 generation. This may have been caused by position effects or cryptic regulatory sequences in the rest of the vector. Ths shows the need for generating many events. Thionin genes expressed in the lemma were cloned by a PCR approach. Over 20 clones were been sequenced. These fell into 3 main groups, but small sequence differences occurred between group members. Only one of the clones was highly homologous with the leaf cell wall form (GenBank X05576), while the others were more homologous to leaf vacuolar forms. Synthetic peptides were ordered for the mature peptides of vacuolar form (ThClone18) and seed-specific HTH. Both will be tested for anti-Fusarium activity. HTH peptide will serve as a control for the synthesis process.

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 you grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Abebe T, R Skadsen, M Patel, H Kaeppler. 2005. The *Lem2* gene promoter of barley directs cell- and developmental-specific expression of *Gfp* in transgenic plants. Plant Biotechnology J. (In press).

Federico ML, Kaeppler HF, Skadsen RW. 2005. The complex developmental expression of a novel stress-responsive barley *Ltp* gene is determined by a shortened promoter sequence. Plant Mol Biol 57: 35-51.

Federico ML, T Abebe, HF Kaeppler, RW Skadsen. 2004. Development of Tissue-Specific Gene Promoters for Targeting Anti-Fusarium Gene Expression in Barley. 9th International Barley Genetics Symposium, Brno, Czech Republic, June 2004. In Czech J. of Plant Breeding 40: 57.

Skadsen RW, ML Federico, T Abebe, M Patel. 2004. Development of tissue-specific promoters for targeting anti-Fusarium gene expression. 2nd Internatl. Symp. on Fusarium Head Blight and 8th European Fusarium Seminar, Orlando, FL Dec., 2004. p. 260.

Skadsen RW, ML Federico, T Abebe, M Patel. 2004. Targeting of anti-Fusarium gene expression in barley. Same as above. p. 261.