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

Cover Page

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Year:	**FY2004** (approx. May 04 – April 05)
FY04 ARS Agreement ID:	**58-5442-2-314**
FY04 ARS Agreement Title:	**Control of Scab with Wheat Puroindoline Proteins.**
FY04 ARS Award Amount:	**$ 40,000**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Control of Scab with Wheat Puroindoline Proteins.	$ 40,000
	Total ARS Award Amount	**$ 40,000**

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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

Project 1: ***Control of Scab with Wheat Puroindoline Proteins.***

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

Head scab of wheat and barley, caused by *Fusarium graminearum* and *F. culmorum*, has been a major disease problem in parts of the country for more than a decade. We have been studying the anti-fungal properties of the puroindoline proteins, which are found in wheat endosperm and contribute to grain softness. Infection of transgenic wheat that over-express the puroindoline gene *pinB* with *F. culmorum* and *F. graminearum* was greatly reduced in severity and the percentage of tombstone kernels was reduced by 90%. The fact that these proteins are natural products and already found in wheat flour might help allay fears of unintended consequences resulting from the addition of these proteins to the food supply.

The goal of this research is to confirm and strengthen the evidence that the puroindolines can provide control of wheat and barley scab caused by *F. graminearum* and *F. culmorum*. The specific aims include:
1. Evaluate disease tolerance of transgenic wheat in the filed and greenhouse.
2. Molecular evaluation of transgenic barley plants and begin analysis of disease resistance.
3. Evaluate the mode of action of puroindoline proteins using a heterologous yeast expression system. This will be done to determine if the anti-fungal and seed texture functions of the proteins can be separated.

What were the most significant accomplishments?

a. Evaluate disease tolerance of transgenic wheat in the filed and greenhouse. Three seasons of field and greenhouse data have confirmed the increased tolerance of transgenic wheat the constitutively expresses *pinB* to both *F. graminearum* and *F. culmorum*.

b. Molecular evaluation of transgenic barley plants. Approximately 4,500 barley calli were bombarded with two plasmids, *pinA* with the constitutively-expressed maize ubiquitin promoter (*Ubi1*), and the hygromycin phosphotransferase (*hph*) promoter driven by the CaMV 35S promoter. Thirty-five putative transgenic plants derived from 25 calli grew in soil. Some plants tested by PCR were positive for *pinA* and *hph*. Southern blots of T1 barley genomic probed with *hph* were negative. Northern blots probed with *pinA* and *hph* using mRNA from T1 leaf tissue were also negative. The conclusion is that no stably transformed plants were obtained.

c. Evaluation of the mode of action of puroindoline proteins using a heterologous yeast expression system. This is being done to determine if the anti-fungal and seed texture functions of the proteins can be separated. During this first year, *pinA* was cloned by PCR with primers modified to allow direct ligation into the plasmid vector. This vector uses an inducible promoter, necessitated by asking a yeast to produce and anti-fungal protein and a secretion sequence. The proper insertion of *pinA* into the vector was confirmed by sequencing. The plasmid was then transformed into *Pichia pastoris*. Induction of the transformed yeast produces a detectable protein of the appropriate size; western blots are being performed to confirm that the proper protein is being made.

Accomplishment: This research has focused on three major objectives: evaluation of scab resistance of wheat transformed with a puroindoline protein expressed throughout the plant or over expressed in the seed endosperm; transformation of barley with a puroindoline gene to see if this can be expanded to that crop; and examination of the mode of action of the anti-fungal activity by expression of the puroindoline genes in yeast so that mutations in the gene can be easily expressed. We have demonstrated conclusively in the field and glass house that wheat that constitutively expresses *pinB* has reduced levels of scab, both in percentage of infected seed and spikelets and reduced number of tombstones. This reduction was seen with both *F. graminearum* and *F. culmorem.* More than thirty hygromycin –resistant barley plants were regenerated following transformation with *pinB.* None of the transformation events were stable through the next generation, a common problem with barley transformation. Heterologous expression of the *pin* genes in yeast will allow the determination of the moieties critical for anti-fungal activity. It appears that successful expression of *pinA* in yeast has been accomplished.

Impact: Confirmation that transgenic wheat expressing the puroindoline genes have increased tolerance to Fusarium head scab opens the possibility that this approach could be used to fight this disease. Determination of the active portion of the puroindoline protein would allow us to potentially separate anti-fungal and grain texture-altering properties of the puroindoline proteins.

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?:

We have demonstrated and confirmed that the puroindoline proteins, normal constituents of the wheat endosperm, possess anti-fungal activity that is active against *Fusarium graminearum* and *F. culmorum*, two of the major scab-causing species. A transgenic wheat that constitutively expresses one of the puroindoline proteins is substantially more resistant to head scab than the wild type and transgenic controls. These proteins are available for use as a potential tool in the control of head scab.

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.

1. Balconi, C. and J.E. Sherwood. 2001. *In vitro* anti-fungal activity of the wheat puroindoline proteins. Phytopathology 91:S5.
2. Gerhardt, S.A., C. Balconi and J.E. Sherwood. 2002. Control of *Fusarium* Scab with Puroindoline-Containing Transgenic Wheat. Phytopathology 92:S28.
3. Giroux, M.J., T. Sripo, S. Gerhardt, and J. Sherwood. 2003. Puroindolines: their role in grain hardness and plant defense. Biotech. Genet. Eng. Rev. 20: 265-278.
4. Giroux, M., J. Sherwood and B. Beecher. 2002. Puroindolines confer grain texture changes and anti-fungal properties in transgenic cereals. American Association of Cereal Chemists. Montreal, Oct 13-17 (Abstract).
5. Sherwood and Giroux. 2003. Controlling scab with puroindoline-expressing wheat and barley Scab meeting. Minneapolis, MN. Dec 12-17.
6. Trujillo, K., T. AL-Niemi, S. Gerhardt, T. Koepke and J. Sherwood. 2005. Wheat puroindolines: a mutational study of anti-fungal activity. Phytopathology 95:S104