USDA-ARS / USWBSI
FY04 Final Performance Report
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Cover Page

PI:	**Lynn Dahleen**
Institution:	**USDA-ARS**
Address:	**Northern Plains Cereal Crop Research - Fargo, ND** **P.O. Box 5677 SU Station** **State University Station** **Fargo, ND 58105**
E-mail:	**dahleenl@fargo.ars.usda.gov**
Phone:	**701-239-1384**
Fax:	**701-239-1369**
Year:	**FY2004**
FY04 ARS Agreement ID:	**NA**
FY04 ARS Agreement Title:	**Developing and Testing of Transgenic Barley and Wheat for FHB Resistance.**
FY04 ARS Award Amount:	**$ 92,878**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Development and Testing of FHB Resistant Transgenic Plants in Barley.	$ 55,317
EDM	Monitoring Infection of GFP-marked *Fusarium graminearum* in Transgenic Barley.	$ 37,651
	Total ARS Award Amount	**$ 92,878**

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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

(Form – FPR04)

Project 1: ***Development and Testing of FHB Resistant Transgenic Plants in Barley.***

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

Fusarium head blight (FHB) in barley is a major disease of devastating economic impact. The fungus also produces the mycotoxin deoxynivalenol (DON) in infected grains which poses safety concerns for human and livestock. Currently, there are no reports of barley genotypes that are highly resistant to FHB. Resistance sources to FHB in barley is limited with only a few sources providing partial resistance. Our goal is to provide a combination of additional genes (through particle bombardment) for FHB resistance and low DON for breeding resistant barley cultivars, using the following seven genes: 3-OH acetyltransferase (*Tri101*) and ATP-binding cassette transporter (*Tri12*) from *F. sporotrichoides*: thaumatin-like protein (*tlp*) and chitinase (*chi*) from *Oryza sativa*: glucanase (*glu*), endochitinase (*endo*), and exochitinase (*exo*) from *F. venenatum*. T_2 or T_3 homozygous lines that shows stable expression of *chi* and *tlp*, *tlp* and *Tri101*, and other gene combinations genes have been developed. Homozygotes from newly produced transgenic lines expressing both antitoxin and antifungal genes are under development. All lines will be tested in multi-year field trials.

2. What were the most significant accomplishments?

Plant transformation. For 2004-2005, we have successfully transformed Conlon with all the gene combinations as planned. T_2 and T_3 plants are now being grown in the greenhouse for PCR, Southern, northern and western analyses. The number of regenerated plants expressing different transgenes and the number of minimum transformation events for each combination are:

Antitoxin or Antifungal Gene/s	No. of Plants	No. of Events	Progenies Analyzed
chi	2	2	T_0 to T_2
Tri101	23	3	T_0 to T_5
Tri12	3	3	T_0
glu	33	4	T_0
endo	1	1	T_0
chi + *tlp*	91	13	T_0 to T_3
chi + *Tri101*	8	8	T_0 to T_2
chi + *Tri12*	3	3	T_0 to T_2
chi + *glu*	3	3	T_0 to T_2
chi + *endo*	1	1	T_0 to T_2
chi + *exo*	2	2	T_0 to T_2
tlp + *Tri10*1	45	8	T_0 to T_3
tlp + *Tri12*	11	7	T_0 to T_2
tlp + *glu*	11	10	T_0 to T_2
tlp + *endo*	3	2	T_0 to T_2

Disease analysis. 2004 field trials included replicated T_5 and BC_1F_3 lines expressing *Tri101* or *PDR5*. Most of the Conlon BC lines did not differ from wild-type Conlon. Several of the Lacey BC lines showed changes in FHB and DON. These trials, with additional lines, will continue in 2005.

Chimeric constructs. Chimeric constructs harboring a spike-specific promoter and antitoxin genes (*mTri101* or *Tri12*) were derived but clones derived from two chimeric constructs needed to be analyzed further for restriction sites and sequence comparison. Five clones with Lem2-*Tri101* and four with the Lem2-*Tri12* combination were selected for restriction site and sequence analyses.

Project 2: ***Monitoring Infection of GFP-marked Fusarium graminearum in Transgenic Barley.***

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

Fusarium head blight (FHB), predominantly caused by *Fusarium graminearum*, is an important disease of all classes of wheat and barley throughout much of the small grains-producing regions of the world. The more recent scab outbreak in the United States caused huge economic losses, estimated in billions of dollars. Various plant responses to pathogen attack include the deposition of mechanical barriers to infection (lignin, papillae, suberin), synthesis of phytoalexins and PR proteins, and the activation of various stress/wound related pathways, or even a combination of these responses. Temporal expression patterns of PR genes during the early stage infection of *F. graminearum* in barley need further investigation.

A combination of antitoxin and antifungal genes may offer greater protection and it is important to make *in vivo* observations on the response of the fungus and the mechanism of plant defense. Likewise, the pathogen response to antitoxin and antifungal genes that are expressed in transgenic host plants during early infection has not been determined. Our objective is to use a GFP-expressing *F. graminearum* strain as inoculum in resistant and susceptible barley lines as well as transgenic lines that express antifungal and/or antitoxin genes. We have been developing a sensitive and rapid fungal detection/quantification method as a component in the study of host-pathogen interactions, which will facilitate real-time observations during early- and post-infection events *in vivo*, having no need for additional substrates and time consuming staining.

The first technical staff (Ph.D.) hired, although experienced, was able to work on the project for only six months as she had to return to her home country. The protocol was established testing non-transgenic plants. Another experienced person (MS grad) was hired to continue working on the first set of transgenic Conlon. However, after two months the person left for an internship. New observations of infection process in target tissues for susceptible and resistant barley varieties should be continued. We are currently looking for a student who is interested in pursuing a Master's degree to continue with this study and who could do the work full-time in the lab.

2. What were the most significant accomplishments?

Identification of the infection routes of Fusarium graminearum in barley spikes using a strain expressing a green fluorescent protein. Infection pathways in cv Conlon (susceptible) was further characterized by using the green fluorescent protein (*gfp*)-expressing GZT501 *F. graminearum* strain and visualizing its green fluorescence with a stereoscope, an epifluoresence and a confocal microscope. Preliminary results show that adaxial surfaces of palea and lemma are the candidate primary sites of invasion in these tissues. In accordance with initial results, we are monitoring the early invasion at the upper pericarp tissue and at the adaxial faces of the lemmas and paleas.

Working protocols. Working protocols were established using Conlon and preliminary studies were done using CI4196, Steptoe and a barley transgenic line expressing both *chi* and *tlp* genes. The infection process in lemma/palea tissues from 0-24 hrs to 2 wks after inoculation was monitored using aseptic techniques and microscopy. Description and comparison of the infection process and route in lemma/palea tissues of non-transgenic Conlon was presented at the 2nd International Symposium on FHB. A rapid and sensitive method for studying host-pathogen interaction is available and can be applied in barley wild-type and transgenic plants.

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.

Tobias, D.J., C. Pritsch, A.K. Jha and L.S. Dahleen (2004) Expression patterns of chitinase and thaumatin-like proteins in three transformation events of barley (*Hordeum vulgare* cv. Conlon), Proc 2nd Intl Symposium on Fusarium Head Blight 1:269-272.

Tobias, D.J., A.K. Jha and L.S. Dahleen (2004) Transformation of barley with antifungal and antitoxin genes. J Soc In Vitro Biology 40:53A.

Tobias, D.J., C. Pritsch, L.S. Dahleen and R. Skadsen (2004) Monitoring the infection process of *gfp*-marked *Fusarium graminearum* in barley (*Hordeum vulgare* cv. Conlon) lemma/palea, Proc 2nd Intl Symposium on Fusarium Head Blight 2:493

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