USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY07 Final Performance Report (approx. May 07 – April 08)
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Cover Page

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USDA-ARS Agreement Title:	Genes Regulated by the Gpmk1 Pathway and Pathogenesis in *Fusarium graminearum*.
FY07 ARS Award Amount:	$ 46,829

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
PGG	Isolate and Characterize Mutants Defective in DON Accumulation in Wheat Tissues.	$46,829
	Total Award Amount	**$ 46,829**

7-14-2008

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
IIR – Integrated/Interdisciplinary Research
PGG – Pathogen Genetics & Genomics
VDUN – Variety Development & Uniform Nurseries

Pro`ject 1: *Isolate and Characterize Mutants Defective in DON Accumulation in Wheat Tissues.*

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

Trichothecene DON produced by *Fusarium graminearum* poses serious health concerns and is an important virulence factor for plant infection. Although most of the genes encoding enzymes for metabolic steps in DON biosynthesis have been characterized, it is not clear about the transcriptional regulatory systems and plant factors controlling DON accumulation in infested wheat tissues. In this study, one approach we used was to screen the REMI (restriction enzyme mediated integration) transformants generated in our lab for mutants defective in mycotoxin accumulation. We have screened 650 REMI transformants and identified eight mutants that produced significant less DON in infected wheat kernels. Further characterization of these mutants and corresponding genes is in progress and may lead to the identification of important regulatory factors for DON production. For the second approach, we have generated and characterized gene replacement mutants of the *FCC1* and *AMY1* homologues in *F. graminearum*. These two genes have been implicated in regulating fumonisin production under certain conditions in *F. verticilloides*. The *CID1* (Cyclin C-like gene required for infection and DON production 1) gene is homologous to *F. verticilloides FCC1* and yeast *SSN8*. Deletion of *CID1* resulted in a reduction in conidiation but an increase in pigmentation. The *cid1* deletion mutant was defective in colonizing flowering wheat heads and significantly reduced in DON production. Expression of the *CID1* gene in the *F. verticilloides fcc1* mutant complemented its defect in fumonisin production, indicating that the Cid1 cyclin C-like protein may be one component of a well-conserved transcription regulatory complex involved in the biosynthesis and accumulation of Fusarium mycotoxins.

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: We have screened 650 REMI transformants and identified eight insertional mutants that are defective in DON accumulation in infected wheat kernels. In addition, we have generated and characterized gene replacement mutants of the *FCC1* and *AMY1* homologues in *F. graminearum*. These two genes may be well conserved in regulating the production and accumulation of mycotoxins in Fusarium species.

Impact: Our effort represents the first attempt to screen for random insertional mutants that are defective in DON production in infected wheat kernels. Further characterization of the REMI mutants identified in this study may lead to the identification of regulatory factors for DON production.

Our results indicated that the *CID1* cyclin C-like gene plays a critical role in regulating DON synthesis and plant infection in *F. graminearum*. This is the first functional characterization of the *FCC1* homologue in filamentous fungi (other than *F. verticilloides*). The Fcc1/Cid1 cyclin C-like protein may be part of a well-conserved transcription regulatory complex involved in the biosynthesis and accumulation of mycotoxins in Fusarium species.

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?:

Although most of the genes encoding enzymes for metabolic steps in DON biosynthesis have been characterized, it is not clear about the transcriptional regulatory systems and plant factors controlling DON accumulation in infested wheat tissues. In this study, we used both the forward and reverse genetic approaches to identify and characterize genes that play critical roles in regulating DON production in *F. graminearum*. A total of 650 random insertional mutants were screened. Eight REMI mutants defective in DON production were identified. Further characterization of these REMI mutants and genes disrupted by transforming DNA is in progress and may lead to the identification of important regulatory factors for DON production. The approach used in this study can be applied to additional insertional mutants that are available in our and other labs.

We also have generated and characterized gene replacement mutants of the *FCC1* and *AMY1* homologues in *F. graminearum*. Our results indicated that the *CID1* cyclin C-like gene plays a critical role in regulating DON production and plant infection in *F. graminearum*. Because *CID1* could functionally complement the defect of *F. verticilloides fcc1* mutant in fumonisin production, it is likely that the Fcc1/Cid1 protein may be part of a well-conserved transcription regulatory complex involved in the biosynthesis and accumulation of mycotoxins in different Fusarium species. Therefore, it will be important to identify and characterization other components of this Fcc1/Cid1 complex. Understanding molecular mechanisms of *CID1* in regulating pathogenesis and DON production in *F. graminearum* may eventually lead to the development of novel scab disease control or toxin reduction strategies.

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.

1. Ding, S., Mehrabi, R., Koten, C., Kang, Z., Wei, Y., Seong, K., Kistler, H. C., and Xu, J. -R. 2008. The transducin beta like gene *FTL1* is essential for pathogenesis in *Fusarium graminearum.* Submitted to *Cellular Microbiology*.
2. Barhoom, S., Kupiec, M., Zhao, X., Xu, J. -R., and Sharon, A. 2008. Functional characterization of *cgCTR2*, a vacuole copper transporter that is involved in germination and pathogenicity of *Colletotrichum gloeosporioides*. *Eukaryotic Cell.* 7: 1098–1108.
3. Mehrabi, R., Zhao, X., and Xu, J. –R. 2008. The cAMP signaling and MAP kinase pathways in plant pathogenic fungi. Invited book chapter for Mycota V. Plant Relationship. Springer. Germany. In press (invited review).
4. Seong, K, Zhao, X., Xu, J. –R., Güldener, U., and Kistler, H. C. 2008. Conidial germination in the filamentous fungus *Fusarium graminearum*. *Fungal Genetics and Biology.* 45: 389-399.
5. Zhao, X., Mehrabi, R., and Xu, J. –R. 2007. MAP kinase pathways and fungal pathogenesis. *Eukaryotic Cell*. *Eukaryotic Cell*. 10: 1701-1714.
6. Cuomo, C., Güldener, U., Xu, J. –R. , Trail, F., ...…and Kistler, H. C. 2007. The genome sequence of *Fusarium graminearum* reveals localized diversity and pathogen specialization. *Science.* 317: 1402-1405.
7. Anderson, J. M., Cambron, S. E., Crane, C., Goodwin, S. B., Scofield, S., Schemerhorn, B., Shukle, R. H., Williams, C. E., Ohm, H. W., Deb, M., Kong, L., Shen, X., Buechley, G., Shaner, G., Xu, J. -R., and Stuart, J. 2007. Annual Wheat Newsletter. Volume 53: 106-110.