USDA-ARS/ U.S. Wheat and Barley Scab Initiative FY06 Final Performance Report (approx. May 06 – April 07) July 16, 2007

Cover Page

PI:	Jin-Rong Xu
Institution:	Purdue University
Address:	Department of Botany and Plant Pathology
	Lilly Hall
	West Lafayette, IN 47907
E-mail:	jinrong@purdue.edu
Phone:	765-494-6918
Fax:	765-494-0363
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USDA-ARS Agreement ID:	59-0790-6-071
USDA-ARS Agreement	Genes Regulated by the Gpmk1 Pathway and Pathogenesis in
Title:	Fusarium graminearum.
FY06 ARS Award Amount:	\$ 41,353

USWBSI Individual Project(s)

USWBSI Research		ARS Award
Area [*]	Project Title	Amount
PGG	Genes Regulated by the Gpmk1 Pathway and Pathogenesis in	\$ 41,353
	Fusarium graminearum.	+
	Total Award Amount	\$ 41,353

FSX

7/14/2007

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: Genes Regulated by the Gpmk1 Pathway and Pathogenesis in Fusarium graminearum.

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

The *GPMK1* MAP kinase and its homologs in phytopathogenic fungi are well conserved for regulating different plant infection and developmental processes. In Fusarium graminearum, the gpmk1 mutants are defective in colonization of flowering wheat heads and in spreading from inoculated florets to neighboring spikelets. Because there is only limited knowledge of transcription factors and other downstream targets regulated by this important MAP kinase pathway, in this study we functionally characterized several genes found in the microarray analysis with the *gpmk1* mutant. Real-time RT PCR was used to confirm the expression patterns of FG01298, FG01636, FG07310, FG02516, FG09286, FG03783, FG04683, FG06593, FG07674, FG09884, FG10740, and FG11165 with RNA samples isolated from the gpmk1 mutant and wild-type PH-1 cultures starved for nitrogen source. All but two of these genes were confirmed to have significantly altered expression levels in the *gpmk1* mutant. Gene replacement mutants were generated for FG01298, FG01636, FG10740, and FG11165. While deletion of the other three genes had no significant effect on pathogenesis, the FG10740 deletion mutant was drastically reduced in virulence. Macroconidia produced by this mutant had reduced viability and were more sensitive to several stress conditions tested, such as desiccation. Putative gene replacement mutants for other three genes have been identified and will be further characterized for their role in pathogenesis.

In addition, we have conducted microarray analysis with the fst12 deletion mutant. A total of 131 and 126 genes were commonly up- and down-regulated in the fmk1 and fst12 mutants, respectively. We also compared genes identified in this study with microarray data of the pmk1 and mst12 mutants of the rice blast fungus Magnaporthe grisea.

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 confirmed the altered expression pattern of 10 genes identified in the microarray analysis with the fmkl mutant. Targeted deletion mutants were generated for seven of these genes. At least one of them has been shown to be important for plant infection.

We also have conducted microarray analysis with the fst12 mutant and identified genes that are commonly up- and down-regulated in the *fmk1* and *fst12* mutants. A total of 29 putative functionally conserved genes were identified by comparative analysis with the microarray data of the *pmk1* and *mst12* mutants of *M. grisea*.

Impact:

Our results represent the first microarray analysis with this important MAP kinase pathway in plant pathogenic fungi. Genes characterized in this study will be helpful to improve our

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understanding of the Gpmk1 pathway in *F. graminearum* and phytopathogenic fungi in general.

Our results indicate that the autophagy process is regulated by this MAP kinase pathway and it plays a critical role in pathogenesis. Disrupting the autophagy process with the chemical or transgenic plant approach may provide an alternative strategy for disease control.

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 the importance of Gpmk1 and its homologs in plant infection have been confirmed in two different Fusarium species and over a dozen other plant pathogenic fungi, currently there is only limited knowledge of genes regulated by this MAP kinase pathway. Genes identified and characterized in this study will be helpful to improve our understanding of molecular mechanisms regulating pathogenesis in *F. graminearum*, and may eventually lead to the development of novel scab disease control strategies. Further characterization of genes commonly regulated by this MAP kinase pathway in *F. graminearum* and *M. grisea* also will be important to understand the conserved and unique features of fungal pathogenesis in these two important pathogens and other fungal pathogens with similar infection mechanisms. FY06 (approx. May 06 – April 07) PI: Xu, Jin-Rong USDA-ARS Agreement #: 59-0790-6-071

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., and Xu, J. –R. 2007. Genes regulated by the *FMK1* MAP kinase in the wheat scab fungus *Fusarium graminearum*. In preparation

2. Seong, K, Zhao, X., Xu, J. – R., Güldener, U., and Kistler, H. C. 2007. Conidial germination in the filamentous fungus Fusarium graminearum. *Fungal Genetics and Biology*. Submitted

3. Zhao, X., Mehrabi, R., and Xu, J. -R. 2007. MAP kinase pathways and fungal pathogenesis. *Eukaryotic Cell*. Submitted.

4. Cuomo, C., Güldener, U., Xu, J. –R., ... and Kistler, H. C. 2007. The genome sequence of *Fusarium graminearum* reveals localized diversity and pathogen specialization. Submitted to *Science*. (The genome paper) Under revision.

5. Bluhm, B. H., Zhao, Z., Flaherty, J., Xu, J. –R., and Dunkle, L. D. 2007. *RAS1* regulates growth and pathogenesis in *Fusarium graminearum*. *Molecular Plant-Microbe Interactions*. 20: 627-636. (18031)

6. Ramamoorthy, V., Zhao, X., Snyder, A. K., Xu, J. –R., and Shah, D. M. 2007. Two Mitogenactivated protein kinase signaling cascades regulate sensitivity to antifungal plant defensins in *Fusarium graminearum*. Cellular Microbiology. 9: 1491–1506.

7. Xu, J. -R., Peng, Y., Dickman, M. B., and Sharon, A. 2006. The dawn of fungal pathogen genomics. *Annual Reviews of Phytopathology* 44: 337-366.

8. Anderson, J. M., Cambron, S. E., Crane, C., Goodwin, S. B., Johnson, A., Nemacheck, J. A., Scofield, S., Schemerhorn, B., Shukle, R. H., Williams, C. E., Ohm, H. W., Deb, M., Kong, L., Sharma, H. C., Shen, X., Buechley, G., Huber, D., Shaner, G., Xu, J. R., and Stuart, J. 2006. Annual Wheat Newsletter. Volume 52: 144-149.