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

# Cover Page

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| **Fiscal Year:** | 2006 |
| **USDA-ARS Agreement ID:** | 59-0790-6-071 |
| **USDA-ARS Agreement Title:** | Genes Regulated by the Gpmk1 Pathway and Pathogenesis in *Fusarium graminearum.* |
| **FY06 ARS Award Amount:** | $ 41,353 |

**USWBSI Individual Project(s)**

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

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 *fmk1* 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

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.

**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 Mitogen-activated 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.