USDA-ARS / USWBSI
FY04 Final Performance Report
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Cover Page

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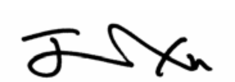
Year:	**FY2004** (approx. May 04 – April 05)
FY04 ARS Agreement ID:	**58-3640-2-139**
FY04 ARS Agreement Title:	**Genomics, Population Genetics and Development of Gibberella zeae.**
FY04 ARS Award Amount:	**$ 78,853**

USWBSI Individual Project(s)

USWBSI Research Area*	**Project Title**	**ARS Adjusted Award Amount**
EDM	Further Characterization of Two Pathogenicity Factors Identified in Fusarium graminearum.	$ 37,390
EDM	Genomics of Gibberella zeae, the Head Scab Fungus.	$ 41,463
	Total ARS Award Amount	**$ 78,853**



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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: ***Further Characterization of Two Pathogenicity Factors Identified in Fusarium graminearum.***

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

Fusarium head blight or scab caused by *Fusarium graminearum* is a destructive disease on wheat and barley. Infested cereals are reduced in yield and contaminated with harmful mycotoxins. The *GPMK1* MAP kinase pathway is known to regulate essential plant infection processes in *F. graminearum*. In this study, we functionally characterized two putative G-protein coupled receptors (GPCR), FgSte3 and FgGpr1. The FgSte3 knockout mutant is reduced in fertility but still pathogenic on flowering wheat heads, indicating that FgSte3 is not the receptor for the *GPMK1* pathway during plant infection. Mutants deleted of the FgGpr1 gene had no obvious phenotypes. However, we have identified another two GPCR genes that are homologous to Gpr1 in the *F. graminearum* genome. These three Gpr1 homologs may be functionally redundant and play important roles in relaying various upstream signals to the downstream *GPMK1* pathway. We are currently investigating the function of other two FgGpr1 homologs. For the second objective, the expression patterns of predicted genes within 15 kb upstream or downstream from the *PKS1* gene (FG01790) were examined by RT-PCR. We found that seven genes located nearby the *PKS1* gene had similar expression levels at different culture conditions. Mutants deleted of a 10 kb fragment containing four upstream genes and the N-terminal part of *PKS1* have been identified and will be verified for their defect in plant infection.

2. What were the most significant accomplishments?

The *GPMK1* MAP kinase pathway is known in many fungal pathogens to be essential for regulating various plant infection processes. However, no fungal receptor gene involved in recognizing plant signals has been identified in any plant pathogen. The results with the FgSte3 and FgGpr1 indicate that *Fusarium graminearum* has more complex upstream signal inputs than yeast to activate the *GPMK1* pathway during plant infection. To our knowledge, this study is the first time GPCR genes were functionally characterized in plant pathogens. The expansion of the Gpr1 homologs in *F. graminearum* indicates that these GPCR genes may play important roles in relaying various upstream signals to the downstream *GPMK1* pathway. Considering the importance of GPCRs in signal transduction, this study will be very helpful for scientists to understand the function of GPCRs in fungal-wheat interactions during scab disease development.

Project 2: ***Genomics of Gibberella zeae, the Head Scab Fungus.***

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

Fusarium graminearum lineage 7 is the major fungal species causing Fusarium head blight of small grains in North America. Infested cereals are reduced in yield and contaminated with harmful mycotoxins. In the past decade, it has resulted in severe economic loss to U.S. agriculture. However, there are only limited studies on molecular mechanisms of fungal pathogenesis in *F. graminearum*. As a pilot study to conduct large-scale functional genomic analyses in this pathogen, we have generated gene replacement mutants of six non-ribosomal peptide synthase (NRPS) genes with the split-marker approach. Over 50% of the hygromycin-resistant transformants were true gene knockout mutants. Preliminary data indicate that none of these NRPS gene was essential for plant infection and vegetative growth under the laboratory conditions. Detailed phenotype characterization of these mutants is in progress. In addition, we have generated targeted deletion mutants of the *PTH12* and *Ku70* homologs in *F. graminearum*. Deletion of Ku70 has been shown in *Neurospora crass* to significantly increase homologous recombination frequency. In *Magnaporthe grisea*, *PTH12* is essential for appressorium formation and plant infection. Mutants deleted of the *PTH12* homolog were reduced in virulence but still pathogenic on flowering wheat heads.

2. What were the most significant accomplishments?

Like many other *Fusarium* species, *F. graminearum* produces various mycotoxic compounds. In fungal pathogens, non-ribosomal peptide synthases (NRPS) are well known to be responsible for producing various toxic compounds. Six NRPS genes characterized in this study is the first step to systematically illustrate the functions of all NRPS genes in *F. graminearum.* Detailed analysis of these mutants will be useful to understand the roles of NRPS genes in the wheat scab disease. The mutant deleted of Ku70 homolog will improve the efficiency of gene knockout for scientists working with *F. graminearum*.

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.

Seong, K., Hou, Z., Kistler, H. C., and **Xu, J. –R**. 2005. Random Insertional Mutagenesis Identifies Genes Associated with Virulence in the Wheat Scab Fungus *Fusarium graminearum. Phytopathology*. 95 (7): 744-750.

Seong, K., Li, L., Kistler, H. C., and **Xu, J. –R**. 2005. Cryptic promoter activity of the *HMR1* coding region in the wheat scab fungus *Fusarium graminearum.* Submitted to *Fungal Genetics and Biology*. (Under revision)

Seong, K., Li, L., Kistler, H. C., and **Xu, J. –R**. 2004. Cryptic promoter activity of the *HMR1* coding region in *Fusarium graminearum.* Presented at the Wheat and Barley Scab Forum, Orlando, FL., Dec. 11-15, 2004.

Genomics of the wheat scab fungus *Gibberella zeae*. Invited presentation at the APS annual meeting, Anaheim, CA. July 30-August 3, 2004.

Genomic studies in *Fusarium graminearum*. Invited presentation at the Society of Industrial Microbiology annual meeting. Anaheim, CA. July 25-29, 2004.