USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY13 Final Performance Report
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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
Fiscal Year:	FY13
USDA-ARS Agreement ID:	59-0200-3-009
USDA-ARS Agreement Title:	Exploring Novel Approaches to Reduce the Impact of Fusarium Head Blight and DON.
FY13 USDA-ARS Award Amount:	$ 90,237

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Exploring Molecular Mechanisms Conferring DON Tolerance in F. graminearum.	$ 44,710
PBG	Testing the Feasibility of Using the HIGS Approach to Reduce Heat Blight.	$ 45,527
	FY13 Total ARS Award Amount	**$ 90,237**

7/15/2014

Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Soft Winter Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: *Exploring Molecular Mechanisms Conferring DON Tolerance in F. graminearum.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Fusarium head blight not only causes severe yield losses but also contaminates infected grains with harmful mycotoxins such as trichothecene deoxynivalenol (DON). Two genes in the trichothecene biosynthesis clusters, *TRI12* and *TRI101*, have been implicated in providing some degree of DON-tolerance but the *tri12* and *tri101* mutants still produce DON. Other genes must exist to be responsible for conferring self-protection against DON in *F. graminearum*. In this study, we firstly generated the T5-12-101 triple mutant by deletion of the *TRI5*, *TRI12*, and *TRI101* genes simultaneously. The T5-12-101 mutant was defective in DON production and plant infection. We then used the RNA-seq approach to identify genes induced by DON treatment. Because DON is inhibitory to protein synthesis, cycloheximide (CHX) treatment was used as the control. We screened 264 genes specifically induced by DON but not CHX treatment. Through gene annotation and GO enrichment analysis, we selected 21 genes that were likely involved in DON resistance for further functional characterization. Their expression profiles were verified by qRT-PCR. For the five genes with the highest DON-specific induced expression levels, FG02248 (transporter), FG12438 (monooxygenase), FG13426 (transporter), FG00572 (transporter), and FG03414 (oxidase), we have constructed vectors to test their function to increase DON tolerance in the yeast *pdr5* strain. Knockout mutants have been generated for four of them. Their functions in conferring DON tolerance in *F. graminearum* are being tested. We expected that all the planned experiments will be finished by September, 2014.

2. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

Accomplishment:
The most important accomplishments include the generation of the *tri5 tri12 tri101* triple deletion mutant and the identification of 21 candidate genes that are likely involved in conferring DON tolerance to *F. graminearum*. Five of them are been further characterized for their functions in conferring tolerance to DON in the budding yeast and *F. graminearum*.

Impact:
Some of these genes that were found to be specifically induced by DON treatment but not by a general protein synthesis inhibitor are likely involved in conferring self-protection against DON in *F. graminearum*. After confirming their functions in response to DON treatment, they have the potentials for being used to developing transgenic plants with improved FHB resistance and reduced DON contamination. In addition, the *tri5 tri12 tri101* triple deletion mutant generated in this study can be used by the FHB community to further characterize the response of *F. graminearum* to exogenous DON (without *tri12* and *tri101* in a non-DON producer), which is similar to wheat plants.

Project 2: *Testing the Feasibility of Using the HIGS Approach to Reduce Heat Blight.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

To test the feasibility of applying the host-induced gene silencing (HIGS) of fungal genes approach to control Fusarium head blight (FHB) in wheat, we first assayed the uptake and silencing effects of synthetic siRNA oligos targeting the FGSG_10037, 10066, 04947, 10228, and 07251 genes. The wild-type strain PH-1 could inefficiently take up the fluorescence labelled oligos because high concentrations of fluorescence oligos were required before fluorescence was observed in germ tubes. However, no obvious silencing effects were observed after repeated tests with any of these four genes. In similar tests with the *PMK1* and *SUM1* genes, the uptake of silencing oligos was much efficient in *Magnaporthe oryzae*. We also tested with the *GzBUD32* (FGSG_10037) gene by the virus-induced gene silencing (VIGS) approach but did not observe any effect on FHB. We then decided to directly rest the HIGS effects with 15 *F. graminearum* genes (FGSG_06878, 10228, 07329, 02795, 010037, 08691, 07295, 06385, 13711, 05171, 13464, 11564, 05775, 08731, and 09897) that are essential for growth or plant infection. HIGS constructs have been generated for all of them and will be first tested with Brachypodium for protection against FHB. The ones tested positive will be further characterized on wheat.

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2. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

Accomplishment:

We found that *F. graminearum* could take up siRNA oligos but it is an inefficient process. With the FGSG_10037, 10066, 04947, 10228, and 07251 genes tested, no silencing effects were observed in germ tube growth when treated with siRNA oligos. There results indicate that it will be technically challenging to generate sufficient amount of silencing oligos in transgenic plants to silence the target fungal genes. To directly test the feasibility or efficiency of HIGS in reducing Fusarium infection, we have generated HIGS constructs for 15 *F. graminearum* genes that are known to be essential for growth or plant infection. These HIGS constructs will be tested for protection again Fusarium infection in Brachypodium and wheat plants.

Impact:

Our results indicated that could take up siRNA oligos but it is an inefficient process. Also, we failed to observe silencing effects with siRNA oligos targeting five genes important for growth. Therefore, it will be difficult to generate sufficient amount of siRNA oligos in transgenic plants to silence most of the target genes in *F. graminearum*. Nevertheless, we are in progress to directly test HIGS effects and efficiency in protection again FHB with 15 genes essential for growth or plant infection.

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 FY13 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

1) Jiang, C., Zhang, S. J., Zhang, Q, Yin, T., and **Xu, J. –R**. 2014. *FSK7* and *FTF1* have overlapping functions in ascosporogenesis, pathogenesis, and stress responses in *Fusarium graminearum*. Environmental Microbiology. In press.
2) Luo, Y. P., Qi, L. L., Zhang, H. C., Zhang, S., Zhou, X. Y., Zhang, Y. M., and **Xu, J. –R.** 2014. The FgKin1 kinase localizes to the septal pore and differentially regulates the localization of two beta-tubulins in *Fusarium graminearum*. New Phytologist. In Press
3) Hu, S., Zhou, X. Y., Gu, X. Y., Cao, S., Wang, C. F., **Xu, J.-R**. 2014. The cAMP-PKA pathway regulates growth, sexual and asexual differentiation, and pathogenesis in *Fusarium graminearum.* Molecular Plant-Pathogen Interactions. 27: 557–566.
4) Zheng, Q., Hou, R., Zhang, J., Ma, J., Wu, Z., Wang, G., Wang, C., and **Xu, J. –R.** 2013. The *MAT* locus genes play different roles in sexual reproduction and pathogenesis in *Fusarium graminearum.* PLoS One. 8(6): e66980.