USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY14 Final Performance Report
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Cover Page

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Fiscal Year:	FY14
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.
FY14 USDA-ARS Award Amount:	$ 47,287

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Different Roles of Two Beta-Tubulins in Fungicide Resistance and DON Production.	$ 47,287
	FY14 Total ARS Award Amount	**$ 47,287**

7-15-2015

Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
WES-CP – Western 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: *Different Roles of Two Beta-Tubulins in Fungicide Resistance and DON Production.*

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

The goal of this study is to characterize the functions of two beta-tubulin Tubl and Tub2 in DON production and benzimidazole fungicide resistance. We have generated the *TUB1*-GFP and *TUB2*-mCherry transformants and showed that both Tub1- and Tub2-microtubules were sensitive to fungicide treatment but the latter was more sensitive. Unlike *tub2* mutants, *tub1* mutants were blocked in ascospore formation. Because fungicide resistance mutations in field isolates have only been identified in *TUB2*, we introduced the resistance mutation E198L into *TUB1* in PH-1 and $TUB2^{E198L}$ mutant. The resulting $TUB1^{E198L}$ transformants had no significant changes but $TUB1^{E198L}$ $TUB2^{E198L}$ mutants were hyper-sensitive to benzimidazole fungicides. Nevertheless, these transformants with the E198L mutation in *TUB1* were blocked in ascospore formation, and still respond to low dose of benzimidazole fungicides for stimulating DON production.

In addition, we have generated transformants of PH-1, *cpk1*, *fac1*, *tub1*, and *tub2* mutants expressing the *TRI12*-mCherry and *TRI4*-GFP constructs. The *cpk1* and *fac1* mutants were blocked in the toxisome formation but not intercalary hyphal swelling. Toxisome formation and mobilization in the other transformants have been examined under fluorescence microscope and will be carefully examined to determine their functions by confocal microscopy.

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: Our data showed that *TUB1* and *TUB2* differed in sensitivity to benzimidazole fungicides. They also play different roles in hyphal growth, sexual reproduction, and sexual reproduction. More importantly, we found that fungicide resistance mutations in TUB1 had a significant cost effect by blocking ascospore formation. We also showed that the cAMP-PKA pathway is essential for toxisome formation.

Impact: Because the cAMP-PKA pathway is essential for toxisome formation, it should be a great molecular targets for disrupting DON production in *F. graminearum*. Mutants with fungicide resistance mutations in both *TUB1* and *TUB2* are blocked in sexual reproduction. Therefore, it is impossible for *F. graminearum* to become completely resistant to benzimidazole fungicides in nature due to the fitness cost associated *TUB1* mutation on ascospore formation. (That may explain why MBC fungicides are still effective in controling FHB after being used in some countries over 30 years)

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY14 award period. The term "support" below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student's stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY14 award period?** No

   If yes, how many?

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY14 award period?** No

   If yes, how many?

3. **Have any post docs who worked for you during the FY14 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?** Yes

   If yes, how many? Dr. Cong Jiang whom participated in this project has taken a faculty position in Northwest A&F University in China.

4. **Have any post docs who worked for you during the FY14 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?** None

   If yes, how many?

Include below a list of all germplasm or cultivars released with full or partial support of the USWBSI during the FY14 award period. List the release notice or publication. Briefly describe the level of FHB resistance. *If not applicable because your grant did NOT include any VDHR-related projects, enter N/A below.*

N/A

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 FY14 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

1) Guo, L., Breakspear, A., Zhao, G., Gao, L., Kistler, H. C., Xu, J. -R, and Ma, L. J. 2015. Comparative transcriptomics uncovers conservation and divergence of cAMP-PKA pathway in two filamentous fungi. MPMI. doi: 10.1111/mpp.12272.
2) Liu, H. Q., Zhang, S. J., Ma, J., Dai, Y., Li, C. H., Lyu, X., Wang, C. F., and Xu, J. R. 2015. Two Cdc2 kinase genes with distinct functions in vegetative and infectious hyphae in *Fusarium graminearum.* PLoS Pathogens. DOI:10.1371/journal.ppat.1004913.
3) Hou, R., Jiang, C., Zheng, Q., Wang, C., and Xu, J. R. 2015. AreA mediate the regulation of DON synthesis by ammonium and cAMP signaling in *Fusarium graminearum*. Molecular Plant Pathology. doi: 10.1111/mpp.12254.
4) Yang, C., Liu, H. Q., Li, G. T., Liu, M., Yun, Y., Ma, Z. H., Wang, C., and **Xu, J. –R**. 2015. The MADS-box transcription FgMcm1 regulates cell identity and fungal development in *Fusarium graminearum.* Environmental Microbiology. DOi:10.1111/1462-2920.12747.
5) Qin, J., Wang, G. H., Jiang, C., **Xu, J. –R**., and Wang, C. F. 2015. Fgk3 glycogen synthase kinase is important for development, pathogenesis, and stress responses in *Fusarium graminearum.* Nature Scientific Reports. 5: 8504. doi:10.1038/srep08504.
6) Jiang, C., Zhang, S. J., Zhang, Q, Yin, T., and **Xu, J. –R**. 2015. *FgSKN7* and *FgATF1* have overlapping functions in ascosporogenesis, pathogenesis, and stress responses in *Fusarium graminearum*. Environmental Microbiology. 17: 1245-1260.
7) Zhao, Z. T., Liu, H. Q., Luo, Y. P., Zhou, S. Y., Zhou, M. G., Wang, C. F., and **Xu, J. –R**. 2014. Evolutionary analysis of tubulin family reveals molecular mechanism driving functional divergence of α- or β-tubulin paralogs in fungi. Nature Scientific Reports. 4: 6746 | DOI: 10.1038/srep06746.
8) Luo, Y. P., H. C., Qi, L. L., 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. 204:943-954.
9) 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.