USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY15 Final Performance Report
Due date: July 15, 2016

Cover Page

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Fiscal Year:	2015
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.
FY15 USDA-ARS Award Amount:	$ 47,241
Recipient Organization:	Purdue University AG Spnsored Program Services 615 W. State Street West Lafauette, IN 47907
DUNS Number:	07-205-1394
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Project/Grant Reporting Period:	07/01/15-06/30/16
Reporting Period End Date:	06/30/16

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.	$ 47,241
	FY15 Total ARS Award Amount	**$ 47,241**

7/15/2016

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
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
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 are the major goals and objectives of the project?**
   Previous studies have showed that MBC fungicides targeting at beta-tubulins are stimulatory to DON production and microtubules are likely involved in toxisome movement. The wheat scab fungus *Fusarium graminearum* has two beta-tubulin genes, *TUB1* and *TUB2*, that are differentially regulated by the Kin1 kinase in microtubule organizations and they play different roles in resistance to MBC fungicides and sexual reproduction. These two beta-tubulin genes also likely differ in functions related to the formation of DON-producing swollen hyphal structures and production or movement of toxisomes (vesicles related to DON production and translocation in the cytoplasm). The goal of this study is to determine different functions of *TUB1* and *TUB2* in DON production and MBC resistance and determine the underlying mechanisms. One objective is to further characterize the functions of *TUB1* and *TUB2* in DON production, MBC fungicide resistance, and ascospore development. The second objective is to determine different roles played by *TUB1* and *TUB2* in the formation of intercalary DON-producing hyphal structures and the production or movement of toxisomes.

2. **What was accomplished under these goals?**
   1) major activities: The *TUB1*-GFP and *TUB2*-mCherry constructs were transformed into the wild-type and mutant strains to observe microtubules and response to fungicide treatment. The expression levels of *TUB1* and *TUB2* in different genetic backgrounds and culture conditions were assayed. The E198L mutation and mutations at the editing sites were introduced into *TUB1* and assayed for their effects in *F. graminearum*. Suppressor strains of the *tub2* mutant were isolated and characterized. Mutants deleted of all the four beta-tubulin and alpha-tubulin genes were generated and characterized for DON production and related cellular differentiation. The *TRI12*-mCherry and *TRI4*-GFP constructs were introduced into the *tub1* and *tub2* mutants and examined for toxisome formation under different conditions. The effects of E198L mutation in the *TUB1* and *TUB2* genes on toxisome formation and DON production were also assayed. The *Fgkin1* deletion mutant was assayed for cellular differentiation, toxisome formation, and responses to MBC fungicides.

   2) specific objectives: We aim to further characterize overlapping and distinct functions of the two beta-tubulin genes, *TUB1* and *TUB2,* in DON production, MBC fungicide resistance, and ascospore development by characterizing the effects of MBC treatments on Tub1/2 localization and point mutations or deletion of *TUB1/2*. Another objective is to determine the difference between *TUB1* and *TUB2* in the formation of intercalary DON-producing hyphal structures and the production or movement of toxisomes by detailed characterization with the *tub1*, *tub2*, *kin1*, *TUB1*^{E198L}, and *TUB2*^{E198L} mutants.

   3) significant results: Both Tub1-GFP and Tub2-mCherry fusion proteins formed microtubules although Tub2-mCherry was more sensitive to fungicide treatment. In *F. graminearum*, deletion of *TUB1* increased the expression of *TUB2*. The E198L mutation in *TUB1* had no effects on hyphal growth but increased resistance to MBC fungicides and blocked ascospore development. The *tub2* deletion mutant was unstable when cultured on

V8 agar plates and over a dozen spontaneous fast-growing suppressors have been identified. Genome sequencing analyses showed that non-sense or deletion mutations in the *KAR9* and Alpha2 genes suppressed the growth defects of *TUB2*. None of the suppressors sequenced had mutations in the *TUB1* gene.

Deletion of either *TUB1* or *TUB2* did not block the formation of hyphal swollen structures related to DON production. The *tub1* and *tub2* mutants expressing the *TRI12*-mCherry and *TRI4*-GFP constructs still formed toxisomes. Whereas the *tub1* mutant was normal, DON production and the formation of hyphal swollen structures and toxisomes were reduced in the *tub2* mutant. The E198L mutation in either *TUB1* or *TUB2* had no effect on toxisome formation and DON production. The regulatory effect of FgKin1 kinase on Tub1 was found to be specific to sexual reproduction but not cellular differentiation related to DON biosynthesis. Interestingly, the terminator sequence of *TUB1* was found to be essential for its functional specificity in ascus and ascospore development. *TUB1* but not *TUB2* had three missense A-to-I editing events occurring specifically during sexual reproduction.

4) key outcomes or other achievements: Our results showed that although *TUB1* and *TUB2* are highly similar in sequences and overall protein structures, they differ significantly in functions, localizations, and regulations in *F. graminearum*. We also showed that *TUB1* and *TUB2* have overlapping functions but *TUB2* likely plays a more significant role in cellular differentiations associated with DON production and *TUB1* has sexual stage-specific transcriptional regulation and amino acid sequence variations.

One other achievement is that our group recently found that genome-wide A-to-I editing occurs specifically during sexual reproduction in *Fusarium graminearum*. A-to-I RNA editing has not been reported in fungi and plants and is assumed to be unique to animals. Our discovery is important to studies with the wheat scab fungus because ascospores are the primary inoculum for head blight and RNA editing affects ascospore formation and release.

3. What opportunities for training and professional development has the project provided?

Two graduate students and one postdoc research associates have been involved in this project. Participation in this project provide them solid training in various molecular biology techniques, fungal genetics, and cell biology. They were also trained to present their results in professional meetings. In addition, one undergraduate student was hired to help preparing fungal cultures and conidia. This experience is helpful to the student to be familiar with a research laboratory lab environment.

4. How have the results been disseminated to communities of interest?

Targeted audience of this study mainly are other researchers working with Fusarium and fungal pathogens. The PI presented two last-minute posters on our most recent results related to tubulin genes and RNA editing at 2015 scab forum. In addition to publications, the PI was invited to give presentations on our *Fusarium graminearum* research at scientific conferences and universities.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY15 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 FY15 award period?**

   If yes, how many? NO

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY15 award period?**

   If yes, how many? Yes, One

3. **Have any post docs who worked for you during the FY15 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**

   If yes, how many? Yes. One. Dr. Jianhua Wang participated in this project has taken a faculty position in Shanghai Academy of Agricultural Sciences in China.

4. **Have any post docs who worked for you during the FY15 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**

   If yes, how many? NO

Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY15 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.*

Name of Germplasm/Cultivar	**Grain Class**	**FHB Resistance** (S, MS, MR, R, where R represents your most resistant check)	**FHB Rating** (0-9)	**Year Released**

Add rows if needed.

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Abbreviations for Grain Classes

Barley - BAR
Durum - DUR
Hard Red Winter - HRW
Hard White Winter - HWW
Hard Red Spring - HRS
Soft Red Winter - SRW
Soft White Winter - SWW

Publications, Conference Papers, and Presentations

Refer to the FY15-FPR_Instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY15 grant. If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

Jiang, C., Zhang, C. K., Wu, C. L., Hou, R., Wang, C. F., and Xu, J. -R. 2016. Exogenous and intracellular cAMP regulated gene expression and cellular differentiation associated with DON production in *Fusarium graminearum*. Environmental Microbiology. DOI: 10.1111/1462-2920.13279.
Status: Published
Acknowledgement of Federal Support: Not

Gao, X. L., Jin, Q., Jiang, C., Li, Y., Li, C., Liu, H. Q., Kang, Z. S., and Xu, J. -R. 2016. FgPrp4 kinase is important for spliceosome B-complex activation and splicing efficiency in *Fusarium graminearum*. PLoS Genetics. 12(4): e1005973. doi:10.1371/journal.pgen.10059733.
Status: Published
Acknowledgement of Federal Support: Yes

Guo, L., Zhao, G. Y., Xu, J. –R. Kistler, H. C., Gao, L. X., and Ma, L. J. 2016. Compartmentalized gene regulatory network of the pathogenic fungus *Fusarium graminearum*. New Phytologist. 10.1111/nph.13912.
Status: Published
Acknowledgement of Federal Support: Yes

Liu, H. Q., Wang, Q., He, Y., Chen, L. F., Hao, C., Jiang, C., Li, Y., Dai, Y. F., Kang, Z., and Xu, J. -R. 2016. Genome-wide A-to-I RNA editing in fungi independent of ADAR enzymes. Genome Research. 26: 499-509.
Status: Published
Acknowledgement of Federal Support: Yes.

Zhao, S., Zhang, S., Li, C. H., Hao, C., Liu, H., Xu, J.-R., and Jin, Q. 2016. FgSsn3 kinase, a component of the mediator complex, is important for sexual reproduction and pathogenesis in *Fusarium graminearum*. Nature Scientific Reports. 6: DOI:10.1038/srep22333
Status: Published
Acknowledgement of Federal Support: No

Jiang, C., Xu, J. -R., and Li, H. Q. 2016. Distinct cell cycle regulation during saprophytic and pathogenic growth in fungal pathogens. Current Genetics. 62: 185-189.
Status: Published
Acknowledgement of Federal Support: No

Li, C. H., Melesse, M., Zhang, S., Hao, C., Wang, C., Zhang, HC, Hall, M. C., and **Xu, J.-R**. 2015. *FgCDC14* regulates cytokinesis, morphogenesis, and pathogenesis in *Fusarium graminearum*. Molecular Microbiology. 98: 770-786.
Status: Published
Acknowledgement of Federal Support: Yes

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.
Status: Published
Acknowledgement of Federal Support: Yes

Books or other non-periodical, one-time publications. None

Other publications, conference papers and presentations.

Genetic network regulating DON biosynthesis in the wheat scab fungus *Fusarium graminearum*. Keynote presentation. The First Chinese Mycotoxin Congress, June 28-30, 2016. Beijing, China.
Status: Presented
Acknowledgement of Federal Support: Yes

Stage-specific RNA editing in filamentous fungi. Invited presentation at the 13th European Conference on Fungal Genetics. Paris, France. April 3 – April 7, 2016.
Status: Presented
Acknowledgement of Federal Support: Yes

RNA editing in *Neurospora crassa*. The 2016 Neurospora Genetics Conference. Asilomar, California. March 10-13, 2016.
Status: Presented
Acknowledgement of Federal Support: Yes

Genome wide RNA editing in filamentous fungi. Invited presentation at the XXIII Plant and Animal Genome Conference. January 8-12, 2016. San Diego, California, USA.
Status: Presented
Acknowledgement of Federal Support: Yes

A-to-I RNA editing occurs specifically during sexual reproduction in *Fusarium graminearum*. Invited presentation at Rutgers University. January 7, 2016. New Brunswick, NJ, USA.
Status: Presented
Acknowledgement of Federal Support: Yes