Cover Page

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<thead>
<tr>
<th>Principle Investigator (PI):</th>
<th>Jin-Rong Xu</th>
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<tbody>
<tr>
<td>Institution:</td>
<td>Purdue University</td>
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<tr>
<td>E-mail:</td>
<td><a href="mailto:jinrong@purdue.edu">jinrong@purdue.edu</a></td>
</tr>
<tr>
<td>Phone:</td>
<td>765-494-6918</td>
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<tr>
<td>Fiscal Year:</td>
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<tr>
<td>USDA-ARS Agreement ID:</td>
<td>59-0200-3-009</td>
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<tr>
<td>USDA-ARS Agreement Title:</td>
<td>Exploring Novel Approaches to Reduce the Impact of Fusarium Head Blight and DON.</td>
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<td>FY15 USDA-ARS Award Amount:</td>
<td>$47,241</td>
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<td>Recipient Organization:</td>
<td>Purdue University</td>
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<td>AG Sponsored Program Services</td>
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<td>615 W. State Street</td>
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<td>West Lafayette, IN 47907</td>
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USWBSI Individual Project(s)

<table>
<thead>
<tr>
<th>USWBSI Research Category*</th>
<th>Project Title</th>
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<tr>
<td>PBG</td>
<td>Different Roles of Two Beta-Tubulins in Fungicide Resistance and DON Production.</td>
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FY15 Total ARS Award Amount $47,241

Principal Investigator

Date 7/15/2016

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* 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 *TUB2* increased the expression of *TUB1*. 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 provided them with 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 prepare 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.

<table>
<thead>
<tr>
<th>Name of Germplasm/Cultivar</th>
<th>Grain Class</th>
<th>FHB Resistance (S, MS, MR, R, where R represents your most resistant check)</th>
<th>FHB Rating (0-9)</th>
<th>Year Released</th>
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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.

Status: Published
Acknowledgement of Federal Support: Not

Status: Published
Acknowledgement of Federal Support: Yes

Status: Published
Acknowledgement of Federal Support: Yes

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

Status: Published
Acknowledgement of Federal Support: No

(Form – FPR15)
Status: Published
Acknowledgement of Federal Support: Yes

Status: Published
Acknowledgement of Federal Support: Yes

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

**Other publications, conference papers and presentations.**

Status: Presented
Acknowledgement of Federal Support: Yes

Status: Presented
Acknowledgement of Federal Support: Yes

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

Status: Presented
Acknowledgement of Federal Support: Yes