USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY16 Final Performance Report
Due date: July 28, 2017

Cover Page

<table>
<thead>
<tr>
<th>Principle Investigator (PI):</th>
<th>JinRong Xu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institution:</td>
<td>Purdue University</td>
</tr>
<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>
</tr>
<tr>
<td>Fiscal Year:</td>
<td>2016</td>
</tr>
<tr>
<td>USDA-ARS Agreement ID:</td>
<td>59-0200-3-009</td>
</tr>
<tr>
<td>USDA-ARS Agreement Title:</td>
<td>Exploring Novel Approaches to Reduce the Impact of Fusarium Head Blight and DON.</td>
</tr>
<tr>
<td>FY16 USDA-ARS Award Amount:</td>
<td>$ 50,259</td>
</tr>
<tr>
<td>Recipient Organization:</td>
<td>Purdue University</td>
</tr>
<tr>
<td></td>
<td>AG Sponsed Program Services</td>
</tr>
<tr>
<td></td>
<td>615 W. State Street</td>
</tr>
<tr>
<td></td>
<td>West Lafayette, IN 47907</td>
</tr>
<tr>
<td>DUNS Number:</td>
<td>07-205-1394</td>
</tr>
<tr>
<td>EIN:</td>
<td>35-6002041</td>
</tr>
<tr>
<td>Recipient Identifying Number or Account Number:</td>
<td>106616</td>
</tr>
<tr>
<td>Project/Grant Reporting Period:</td>
<td>7/1/16 - 6/30/17</td>
</tr>
<tr>
<td>Reporting Period End Date:</td>
<td>06/30/17</td>
</tr>
</tbody>
</table>

USWBSI Individual Project(s)

<table>
<thead>
<tr>
<th>USWBSI Research Category*</th>
<th>Project Title</th>
<th>ARS Award Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBG</td>
<td>Distinct Regulatory Functions of the TRI6 and TRI10 Genes in DON Biosynthesis.</td>
<td>$ 50,259</td>
</tr>
<tr>
<td></td>
<td><strong>FY16 Total ARS Award Amount</strong></td>
<td><strong>$ 50,259</strong></td>
</tr>
</tbody>
</table>

* 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

7/28/2017
Principal Investigator
Date
Project 1: Distinct Regulatory Functions of the TRI6 and TRI10 Genes in DON Biosynthesis.

1. What are the major goals and objectives of the project?

The goal of this study is to characterize regulatory networks involved in the regulation of DON biosynthesis and plant infection in *Fusarium graminearum*. It has three specific objectives:

- Objective 1 is to determine the functional relationship between TRI6 and TRI10.
- Objective 2 is to further characterize the functions of TRI10 in DON biosynthesis.
- Objective 3 is to determine the regulation of TRI6 and TRI10 by PAC1 and AreA.

2. What was accomplished under these goals? Address items 1-4) below for each goal or objective.

OBJECTIVE 1: Determine the functional relationship between TRI6 and TRI10.

1) Major activities: The interaction between Tri6 and Tri10 was assayed by co-immunoprecipitation assays with epitope tagged constructs. We also generated TRI10-3xFALG transformants and used the affinity purification approach to identify Tri10-interacting proteins. Tri6 was one of the Tri10-interacting proteins identified by MS-MS analysis. In addition, the *tri6 tri10* double mutant was generated to identify genes commonly regulated by Tri6 and Tri10. Furthermore, we conducted strand-specific RNA-seq analysis with the *tri5, tri6*, and *tri10* mutants. Strand-specific RNA-seq allowed us to identify anti-sense transcripts and A-to-I RNA editing. Several TRI genes were found to produce antisense transcripts.

2) Specific objectives: We aimed to further characterize the Tri6-Tri10 interaction and characterize genes co-regulated by TRI6 and TRI10 by RNA-seq analysis.

3) Significant results: Although we failed to detect their interaction by co-IP, Tri6 was identified by MS-MS analysis as one of the Tri10-interacting proteins, indicating that they weakly interacted with each other in vivo. Interestingly, antisense transcripts of TRI6 were found to be rare in the wild type but became the major majority of its transcripts in the *tri10* mutant, showing a regulatory role of TRI10 on TRI6. Furthermore, we found that only antisense transcripts of TRI5 (no more sense transcripts) were observed in the *tri6* or *tri10* mutant.

4) Key outcomes or other achievements: The TRI genes responsible for DON biosynthesis are regulated by the Tri6 and Tri10 transcription factors. However, the mechanism responsible for the co-regulation of TRI genes by Tri6 and Tri10 not well characterized. Our results showed the functional relationship between Tri6 and Tri10 and the importance of antisense transcripts in the regulation of DON production. Deletion of TRI6 or TRI10 eliminated the transcription of TRI5 but resulted in the expression of its antisense transcripts. Deletion of TRI10 almost blocked the transcription of TRI6 sense
transcripts but significantly increased the expression of its antisense transcripts, indicate a regulatory role of TRI10 on TRI6 expression. Earlier studies with regular RNA-seq, microarray analysis, and qRT-PCR assays failed to distinguish sense and antisense transcripts and failed to detect the regulation of TRI6 by TRI10.

**OBJECTIVE 2:** Further characterize the functions of TRI10 in DON biosynthesis.

1) **Major activities:** The putative AGGCCXXC/ACA Tri10-binding site was identified. The putative Tri10-binding site in the promoter of the TRI5 gene was functionally characterized. In addition, the subcellular localization of Tri10-GFP was examined under different growth and DON-inducing conditions. We also examined the colocalization of Tri10 and Tri4 in the toxisomes.

2) **Specific objectives:** One aim is to identify the Tri10-binding sites in the TRI genes. Another aim is to characterize the possible non-transcription factor function of Tri10 in the toxisome.

3) **Significant results:** We have identified and characterized the putative Tri10-binding site. Localization of Tri10 to the toxisome and enhanced formation of bulbous hyphae (even in the tri6 mutant) were only observed when TRI10 was expressed with a strong constitutive promoter. Expression of TRI10-GFP with its native promoter had no effect on the formation of bulbous hyphae associated with DON production in the wild type or tri6 mutant. Co-localization of Tri10 with Tri4 was only observed in some of the toxisomes, and they appeared to be localized to the opposite sides of the toxisomes. Interestingly, RNA-seq analysis with the TRI10 overexpression strain showed that three genes right next to the major TRI gene cluster, FGSG_03546, FGSG_03547, and FGSG_3548 were also up-regulated when it was cultured under DON-inducing conditions. These genes may be also involved in DON production.

4) **Key outcomes or other achievements:** Our results showed Tri10 has dual functions as a transcription factor and a component of the toxisome. As a transcription factor, Tri10 regulates the transcription of TRI6, TRI5, and other TRI genes. As a toxisome component, localization of Tri10 to the toxisome was only observed when TRI10-GFP was over-expressed with a strong promoter. In some of the toxisomes, Tri10 and Tri4 appeared to localize to opposite sides of the toxisome. One other achievement is that we have conducted affinity purification with several genes that are known to regulate DON biosynthesis, including the Gpmk1 and Mgv1. Their interacting proteins may be involved in the regulatory network controlling TRI gene expression in F. graminearum.

**OBJECTIVE 3:** Determine the regulation of TRI6 and TRI10 by PAC1 and AreA.

1) **Major activities:** We have generated the TRI6 mutant allele deleted of the two PacC binding sites in its promoter. When it was transformed into the tri6 deletion mutant, this mutant TRI6 allele failed to complement the tri6 mutant in DON production at acidic pH. For the TRI10 gene, we also generated mutant alleles deleted of individual PacC binding
sites in its promoter and transformed them into the tri10 mutant. The resulting transformants were assayed for DON production under different pH conditions. In addition, we have generated and transformed the AREA$^{S874A}$-GFP construct into the TRI10-3xFLAG transformant. Total proteins isolated from the resulting transformants were used for co-immunoprecipitation assays.

2) **Specific objectives:** We aimed to use site-directly mutagenesis approach to determine the role of conserved PacC-binding sites in the TRI6 and TRI10 promoters. The other aim is to determine relationship between the phosphorylation of AreA by PKA and its interaction with Tri10 in the regulation of DON production.

3) **Significant results:** We found that the PacC-binding sites in the TRI6 promoter were essential for the TRI6 function. These results indicated that pH may directly regulate the expression of TRI6, a key transcription factor for DON biosynthesis. For TRI10, deletion of individual PacC binding sites had no significant effect on its function in the regulation of DON biosynthesis. In co-immunoprecipitation assays, the S874A mutation in AreA had no significant effects on its interaction with Tri10, suggesting that the interaction of AreA with Tri10 is not regulated by the cAMP-PKA pathway.

4) **Key outcomes or other achievements:** The cAMP-PKA pathway, pH signaling, and the PacC, AreA, and Tri10 transcription factors are known to regulate TRI gene expression and DON biosynthesis. However, the relationships between cAMP or pH signaling and these well-conserved transcription factors are not clear. Our results showed that TRI6 but not TRI10 expression was directly regulated by the binding of the PacC transcription factor to its promoter, which may explain why DON production favors acidic conditions. Our results also showed that phosphorylation of AreA by PKA had no significant impact on the AreA-Tri10 interaction.

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

One PhD graduate student and one postdoc research associate have been involved in this project. In addition, this project provided training of Dr. Jianghua Wang, a visiting professor from Shanghai Academy of Agricultural Sciences. He returned to China in May, 2017 to run a lab for mycotoxin detection at his home institute.

This project provided participating young investigators training in routine molecular biology techniques, fungal genetics, and cell biology. The PhD student and postdoc involved in this project also were trained to present a poster at the Fungal Genetics Conference. In addition, one undergraduate student hired by this project was trained to prepare media and fungal cultures.
4. **How have the results been disseminated to communities of interest?**

Targeted audience of this project mainly are scientists studying with Fusarium pathogens, mycotoxins, and disease resistance. The principal investigator presented results from this project as the keynote presentation on trichothecene regulation in a keynote presentation at a mycological society meeting. The student and postdoc in the lab presented a poster at the fungal genetics conference attended by plant pathologists and fungal geneticists. In addition, the PI attended the 2016 scab forum and APS meeting and exchanged data generated in this study with scientists of similar research interest.
Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY16 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 FY16 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 FY16 award period?
   YES

   If yes, how many?
   ONE

3. Have any post docs who worked for you during the FY16 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?
   YES (partially supported)

   If yes, how many?
   ONE

4. Have any post docs who worked for you during the FY16 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?
   NO

   If yes, how many?
**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 FY16 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

(Form – FPR16)
Publications, Conference Papers, and Presentations

Instructions: Refer to the FY16-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY16 grant. Only include citations for publications submitted or presentations given during your award period (7/1/16 - 6/30/17). 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: Yes


Status: Published
Acknowledgement of Federal Support: Yes


Status: Published
Acknowledgement of Federal Support: Yes

Other publications, conference papers and presentations.


Status: Invited keynote presentation presented
Acknowledgement of Federal Support: YES (talk),


Status: Abstract Published and Poster Presented

(Form – FPR16)