USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY18 Performance Report
Due date: July 12, 2019

Cover Page

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<tr>
<th>Principle Investigator (PI):</th>
<th>Jin-Rong Xu</th>
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<tr>
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<td>Fiscal Year:</td>
<td>2018</td>
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<tr>
<td>USDA-ARS Agreement ID:</td>
<td>59-0206-7-007</td>
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<tr>
<td>USDA-ARS Agreement Title:</td>
<td>Distinct Regulatory Functions of the TRI6 and TRI10 Genes in DON Biosynthesis.</td>
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<tr>
<td>FY18 USDA-ARS Award Amount:</td>
<td>$ 51,555</td>
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<td>Recipient Organization:</td>
<td>Purdue University</td>
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<td>6/1/18 - 5/31/19</td>
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USWBSI Individual Project(s)

<table>
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<tr>
<th>USWBSI Research Category*</th>
<th>Project Title</th>
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<tr>
<td>PBG</td>
<td>Epigenetic Regulation of DON Biosynthesis in Fusarium graminearum.</td>
<td>$ 51,555</td>
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FY18 Total ARS Award Amount  $ 51,555

Principal Investigator

Date  7/12/2019

* 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
    NWFW – Northern Soft Winter Wheat Region
    SWW – Southern Soft Red Winter Wheat Region
Project 1: Epigenetic Regulation of DON Biosynthesis in Fusarium graminearum.

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

The trichothecene mycotoxin deoxynivalenol (DON) produced by *Fusarium graminearum* also is an important virulence factor. The TRI genes responsible for DON biosynthesis are regulated by two transcription factors, Tri6 and Tri10. The goal of this study is to characterize the regulation of DON production by antisense transcripts of TRI5 or TRI6 and histone acetylation via PKA. Objective 1 aims to characterize the regulation of TRI6 sense and antisense transcripts by TRI10, which is important to understand their functional relationship. Objective 2 is to further characterize the regulation of TRI5 expression and related LncRNA by Tri6 and Tri10. Objective 3 aims to characterize the relationship between PKA and Sas3 on H3 acetylation and DON biosynthesis. This study fits the research area of PBG on developing new strategies for reducing impact of FHB and mycotoxin contamination. Proposed experiments aim to characterize the epigenetic control of DON biosynthesis in *F. graminearum*. Reducing or eliminating DON biosynthesis can be used as a novel approach to control FHB or avoid mycotoxin contamination.

2. What was accomplished under these goals?

**Objective 1** aims to characterize the regulation of sense and antisense transcripts of TRI6 by Tri10.

1) major activities:
   Tri6 was confirmed to bind to its own promoter and suppresses its own expression. Expression of the TRI6\(^{\Delta P10}\) allele deleted of the putative Tri10-binding site failed to complement the tri6 mutant in DON production. In the TRI6\(^{\Delta P10}\) transformants, the sense transcripts of TRI6 were significantly reduced in the absence of the Tri10-binding site. We also transformed the TRI6\(^{\Delta P10}\) construct into the tri10 mutant. The antisense transcript of TRI6 was still detectable in the resulting transformant although its expression was reduced. Overexpression of the TRI6 antisense transcript with the RP27 promoter suppressed DON biosynthesis, confirming its suppressive role. We also showed that overexpression TRI10 with the RP27 promoter increased the expression of TRI6 sense transcripts. The TRI6\(^{\Delta CT100}\) allele deleted of the C-terminal 100 bp of TRI6 was generated and transformed into the tri6 tri10 double mutant.

2) specific objectives are to characterize the regulation of sense and antisense transcripts of TRI6 by Tri10.

3) significant results
   We showed that binding of Tri10 to the Tri10-binding site is important for TRI6 sense transcripts. The negative self-regulation of Tri6 on its own transcription is likely relieved by binding of Tri10 to the TRI6 promoter. However, the Tri10-binding site in the promoter of TRI6 was not essential for the expression of its antisense transcripts.
4) key outcomes or other achievements
   Binding of Tri6 to its own promoter represses its expression. The negative self-regulation of Tri6 on its own transcription is likely relieved by binding of Tri10 to the TRI6 promoter. The Tri6-binding and Tri10-binding sites are adjacent to each other on the TRI6 promoter region. The interaction of Tri6 with Tri10 was confirmed by yeast two-hybrid and immuno-coprecipitation assays.

Objective 2 aims to further characterize the regulation of TRI5 expression by Tri6 and Tri10.

1) major activities
   Both the GTGAATGTTCGTGA and TGKHRGGCCT sequences in the TRI5 promoter region were shown to be important for TRI5 expression. Deletion of the Tri6-binding site reduced the expression of TRI5 sense transcripts but increased the expression of the LncRNA located in its promoter region. We also showed that the Tri10-binding site was essential for TRI5 expression because the TRI5\textsuperscript{AT10B} allele failed to complement the tri5 deletion mutant. When the TRI5 promoter was replaced with the TRI12 promoter, the expression of this LncRNA was not detectable but TRI5 expression and DON production were increased. Overexpression of this LncRNA with a TrpC promoter inserted in situ behind the Tri6-binding site of TRI5 suppressed DON production. In addition, we generated and characterized the PRP27-TRI6-GFP transformants. Although we failed to observe Tri6-GFP signals, DON production and sense transcripts of TRI5 were slightly increased by overexpression of TRI6.

2) specific objectives are to further characterize the regulation of TRI5 expression by Tri6 and Tri10

3) significant results
   Our results showed that the LncRNA located in the promoter region of TRI5 plays a negative role in regulating TRI5 expression and DON production. Interestingly, the Tri10-binding site is in the LncRNA region of TRI5 promoter. Binding of Tri10 to this region likely reduces the expression of the LncRNA.

4) key outcomes or other achievements
   Our results showed that the LncRNA located in the promoter region of TRI5 plays a negative role in regulating TRI5 expression and DON production. Although the underlying mechanism is not clear, both Tri6 and Tri10 are involved in regulating the expression of this LncRNA. Stimulating the expression of this LncRNA can be used as a strategy to reduce DON production by F. graminearum.

Objective 3 is to characterize the functional relationship between PKA and Sas3 on H3 acetylation and DON biosynthesis.

1) major activities
   In the preliminary study, S332 and S333 of Sas3 were identified as the putative PKA phosphorylation sites. We generated the SAS3\textsuperscript{S332A S333A} allele and transformed it into the...
sas3 deletion mutant. The resulting transformants were normal in DON production and TRI gene expression. These results indicate that phosphorylation of Sas3 at S332 and S333 by PKA is not directly involved in the regulation of DON biosynthesis by the cAMP-PKA pathway in F. graminearum.

Because Sas3 is a component of the NuA3 histone acetylase (HAT) complex and the sas3 mutant was defective in DON production, pathogenesis, and H3K14 acetylation, we have identified several components of the NuA3 and NuA4 complexes in F. graminearum, including the ING2 ortholog. Deletion of ING2 significantly reduced growth rate and DON production. The ing2 mutant was blocked in sexual and asexual reproduction. In addition, we functionally characterized all the components of the Set3 histone deacetylase (HDAC) complex in F. graminearum.

2) specific objectives are to characterize the functional relationship between PKA and Sas3 on H3 acetylation and DON biosynthesis. This objective was proposed because we found that S332 and S333 of Sas3 were phosphorylated by PKA.

3) significant results
   Our site-directed mutagenesis results showed that the phosphorylation of Sas3 as S332 and S333 by PKA may be important for other processes but not for DON production. However, like Sas3, the ING2 ortholog and other Nu3A/Nu4A HAT complex were found to be important for regulating DON biosynthesis, growth, and reproduction.

4) key outcomes or other achievements
   The cAMP-PKA pathway plays a critical role in regulating DON biosynthesis and Sas3 histone acetyltransferase is phosphorylated by PKA in F. graminearum. The phosphorylation of Sas3 as S332 and S333 by PKA may be important for other processes but not for DON production.

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

This project has provided training opportunities for one PhD student on DON measurement and infection assays with F. graminearum. This student also was able to master various molecular techniques related to RNA and DNA during this project. In addition, a visiting PhD student and a visiting scholar participated in this project also learned how to work with this important fungal pathogen and regulation of DON biosynthesis.

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

Results from this project were presented at professional meetings attended by the PI and PhD student. The PI also presented some of the results at the 2019 Fusarium workshop, which was attended by over 30 participants at Kansas State University.
Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY18 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 FY18 award period?
   None

   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 FY18 award period?
   None.

   If yes, how many?

3. Have any post docs who worked for you during the FY18 award period and were supported by funding from your USWBSI grant taken faculty positions with universities? Yes

   If yes, how many? 1

   Dr. Yanyan Wang was partially supported by funding from USWBSI. She is an assistant professor at Institute of Microbiology, Chinese Academy of Sciences.

4. Have any post docs who worked for you during the FY18 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?
**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 FY18 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

**NOTE:** 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

Instructions: Refer to the FY18-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY18 grant. Only include citations for publications submitted or presentations given during your award period (6/1/18 - 5/31/19). If you did not have any publications or presentations, state ‘Nothing to Report’ directly above the Journal publications section.

NOTE: Directly below each reference/citation, you must indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in publication/presentation.

Journal publications.


Status: Published
Acknowledgement of Federal Support: Yes


Status: Published
Acknowledgement of Federal Support: Yes


Status: Published
Acknowledgement of Federal Support: Yes


Status: Published
Acknowledgement of Federal Support: Yes
Acknowledgement of Federal Support: Yes

**Books or other non-periodical, one-time publications.**

Status: Submitted
Acknowledgement of Federal Support: Yes

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

(Form – PR18)