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

Cover Page

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Fiscal Year:	2018
USDA-ARS Agreement ID:	59-0206-7-007
USDA-ARS Agreement Title:	Distinct Regulatory Functions of the TRI6 and TRI10 Genes in DON Biosynthesis.
FY18 USDA-ARS Award Amount:	$ 51,555
Recipient Organization:	Purdue University AG Spnsored Program Services 615 W. State Street West Lafauette, IN 47907
DUNS Number:	07-205-1394
EIN:	35-6002041
Recipient Identifying Number or Account Number:	F9002798602006
Project/Grant Reporting Period:	6/1/18 - 5/31/19
Reporting Period End Date:	05/31/19

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Epigenetic Regulation of DON Biosynthesis in Fusarium graminearum.	$ 51,555
	FY18 Total ARS Award Amount	$ 51,555

7/12/2019

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: *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 $TRI5^{\Delta T10B}$ 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 P_{RP27}-*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 $SAS3^{S332A\ S333A}$ 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 fount 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.

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

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.

Jiang, C., Cao, S., Wang, Z., Xu, H., Liang, J., Liu, H., Wang, G. H., Ding, M., Gong, C., Feng, C. J., Hao, C. F., and Xu, J. -R. 2019. Plant infection involves an expanded subfamily of GPCR genes in the wheat scab fungus *Fusarium graminearum.* Nature Microbiology. 10.1038/s41564-019-0468-8.
Status: Published
Acknowledgement of Federal Support: Yes

Hao, C. F., Yin, J. R., Sun, M., Qang, Q. H., Liang, J., Liu, H. Q., Bian, Z. Y., and Xu, J. -R. 2019. The meiosis-specific APC activator *FgAMA1* is dispensable for meiosis but important for ascosporogenesis in *Fusarium graminearum*. Molecular Microbiology. 111 (5): 1245-1262. Doi: 10.1111/mmi.14219.
Status: Published
Acknowledgement of Federal Support: Yes

Wang, Q. H., Liu, H., Xu, H., Hei, R., Zhang, S., Jiang, C., and Xu, J. -R. 2019. Independent losses and duplications of autophagy-related genes in fungal tree of life. Environmental Microbiology. 21: 226-243. (Journal cover) doi: 10.1111/1462-2920.14451.
Status: Published
Acknowledgement of Federal Support: Yes

Chen, D., Wu, C., Hao, C., Bian, Z., and Xu, J. -R. 2018. Sexual specific functions of Tub1 beta-tubulins require stage-specific RNA processing and expression in *Fusarium graminearum*. Environmental Microbiology. 20 (11): 4009-4021. doi: 10.1111/1462-2920.14441.
Status: Published
Acknowledgement of Federal Support: Yes

Li, C. Q, Zhang, Y. H., Wang, H., Chen, L. F., Zhang, J., Sun, M. L., Xu, J. -R., and Wang, C. F. 2018. The *PKR1* regulatory subunit of protein kinase A (PKA) is involved in regulating growth, sexual and asexual development, and pathogenesis in *Fusarium graminearum*. Molecular Plant Pathology. 19 (4): 909-921. doi: 10.1111/mpp.12576.

Status: Published

Acknowledgement of Federal Support: Yes

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

Jiang, C., Ren, J., and Xu, J. -R. 2019. Cellular signaling in Fusarium. Invited book chapter on *Fusarium* genomics. Edited by Nadia Ponts, Christian Barreau, and Marie Foulongne. Caister Academic Press. (Invited book chapter, In press)

Status: Submitted

Acknowledgement of Federal Support: Yes

Other publications, conference papers and presentations.