USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Final Performance Report
Due date: July 24, 2020

Cover Page

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Fiscal Year:	2019
USDA-ARS Agreement ID:	59-0206-7-007
USDA-ARS Agreement Title:	Distinct Regulatory Functions of the *TRI6* and *TRI10* Genes in DON Biosynthesis
FY19 USDA-ARS Award Amount:	$ 63,183
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:	6/1/19 - 5/31/20
Reporting Period End Date:	5/31/2020

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Epigenetic Regulation of DON Biosynthesis in *Fusarium graminearum*	$ 63,183
	FY19 Total ARS Award Amount	**$ 63,183**

7/22/2020

Principal Investigator Date

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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

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Project 1: *Epigenetic Regulation of DON Biosynthesis in Fusarium graminearum*

1. What are the major goals and objectives of the research 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 or objectives? *(For each major goal/objective, address items a-b) below.)*

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

a) What were the major activities?

The Tri6 transcription factor 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 transformed into the *tri6 tri10* double mutant. The *tri6 tri10 TRI6*$^{\Delta CT100}$ transformants were defective in DON production.

b) What were the significant results?

We showed that binding of Tri10 to the Tri10-binding site is important for *TRI6* sense transcripts. The negative self-regulation of the *TRI6* gene 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.

c) List 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 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. The interacting regions between Tri6 and Tri10 were identified.

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

a) What were the major activities?

Both the GTGAATGTTCGTGA and TGKHRGGCCT sequences in the promoter region of the *TRI5* gene 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 in DON biosynthesis. 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 the *TRI5* gene were slightly increased by overexpression of *TRI6*.

b) What were the 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.

c) List 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.

a) What were the 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*. A manuscript on ING2 has been submitted to PLoS Genetics (under revision).

b) What were the 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.

c) List 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. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

Yes but only slightly. We are trying to write publications on the importance of TRI5 antisense in DON biosynthesis. Some of the experiments need to be repeated to obtain publication quality images. We are catching up with these experiments.

4. 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 (Derica Tavares from Brazil) and a visiting scholar (from Institute of Microbiology, Academy of Sciences, China) participated in this project also learned how to work with this important fungal pathogen and regulation of DON biosynthesis.

5. 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 FY19 award period (6/1/19 - 5/31/20). 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 FY19 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 FY19 award period?**
   None

   If yes, how many?

3. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   Yes.

   If yes, how many? One. Dr. Yanyan Wang was partially supported by funding from USWBSI. She is now an assistant professor at Institute of Microbiology, Chinese Academy of Sciences.

4. **Have any post docs who worked for you during the FY19 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?

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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 FY19 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

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Publications, Conference Papers, and Presentations

Instructions: Refer to the FY19-FPR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY19 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (6/1/19 - 5/31/20**) should be included. If you did not publish/submit or present anything, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each citation, you **must** indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in the publication/ presentation. See example below for a poster presentation with an abstract:

> De Wolf, E., D. Shah, P. Paul, L. Madden, S. Crawford, D. Hane, S. Canty, R. Dill-Macky, D. Van Sanford, K. Imhoff and D. Miller. 2019. "Impact of Prediction Tools for Fusarium Head Blight in the US, 2009-2019." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum*, Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY. p. 12.
> Status: Abstract Published and Poster Presented
> Acknowledgement of Federal Support: YES (Abstract and Poster)

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

Jiang, C., Hei, R., Yang, Y., Zhang, S., Wang, Q. H., Wang, W., Zhang, Q., Yan, M., Zhu, G. R., Huang, P. P., Liu, H. Q., and Xu, J. -R. The orphan secretory protein Osp24 of *Fusarium graminearum* modulates host immunity by mediating the proteasomal degradation of TaSnRK1. Nature Communication. In press.
Status: Accepted (in press)
Acknowledgement of Federal Support: Yes

Jiang, H., Xia, A., Ye, M., Ren, J., Liu, H., Wang, Q., Wu, C., Lu, P., Xu, J. -R., and Jiang, C. 2020. Opposing functions of Fng1 and the Rpd3 HDAC complex in H4 acetylation in *Fusarium graminearum.* PLoS Genetics. Under revision.
Status: Submitted (under revision)
Acknowledgement of Federal Support: Yes

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

Other publications, conference papers and presentations.