USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report
Due date: July 31, 2018

Cover Page

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Fiscal Year:	2017
USDA-ARS Agreement ID:	59-0206-7-007
USDA-ARS Agreement Title:	Distinct Regulatory Functions of the TRI6 and TRI10 Genes in DON Biosynthesis.
FY17 USDA-ARS Award Amount:	$ 50,162
Recipient Organization:	Purdue University AG Spnsored Program Services 615 W. State Street West Lafauette, IN 47907
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Project/Grant Reporting Period:	6/1/17 - 5/31/18
Reporting Period End Date:	05/31/18

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Distinct Regulatory Functions of the TRI6 and TRI10 Genes in DON Biosynthesis.	$ 50,162
	FY17 Total ARS Award Amount	$ 50,162

7/31/2018

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

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PI: Xu, Jin-Rong
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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

Major activities: The interaction between Tri6 and Tri10 was assayed by co-immunoprecipitation (co-IP) and bimolecular fluorescence complementation (BiFC) assays. In addition to pulldown assays with *TRI10*-3xFLAG transformants, we generated TRI6-S-tag transformants. Unfortunately, affinity purification with the resulting transformants failed to identify Tri10 in proteins co-purified with Tri6. Strand-specific RNA-seq analysis resulted in the identification of antisense transcripts of *TRI5* in the *tri6* and *tri10* mutants and antisense transcripts of *TRI6*. RNA-seq analysis with the *tri6, tri10,* and *tri6 tri10* mutants also lead to the identification of genes specifically or co-regulated by Tri6 and Tri10.

Specific objectives: We aimed to determine the functional relationship between *TRI6* and *TRI10* and genes regulated by these two transcription factors.

Significant results: .Our results showed that Tri6 only weakly interacted with Tri10 in *F. graminearum* and most of the genes co-regulated by Tri6 and Tri10 are related to DON biosynthesis. We also found that TRI10 regulates the expression of TRI6 because antisense transcripts of *TRI6* became abundant in the *tri10* mutant. Furthermore, *TRI5* had only antisense transcripts in the *tri6* or *tri10* mutant. Putative promoter elements responsible for the antisense transcripts of *TRI5* and *TRI6* were identified.

Key outcomes or other achievements: Two transcription factors, Tri6 and Tri10, regulates the expression of *TRI* genes that are responsible for DON biosynthesis. However, the molecular mechanism involved in the co-regulation of *TRI* genes by Tri6 and Tri10 not well characterized. Our results showed that Tri6 and Tri10 may transiently form heterodimers to regulate the expression of *TRI* genes. Results from our study also showed that *TRI5* and *TRI6* produced antisense transcripts, indicating epigenetic control of *TRI5* and *TRI6* expression.

OBJECTIVE 2

Major activities: The Tri10-binding sequence AGGCCNNMCA was identified and functionally characterized for its regulatory role in *TRI5* expression. Site-directly mutagenesis was used to determine the importance of the AGGCC nucleotides. In addition, we showed that Tri10-GFP localized to the peri-nuclear membranes under DON

inducing conditions. In comparison with Tri4 and Tri1, Tri10 often localized to the opposite side of putative toxisomes.

Specific objectives: One aim is to identify the Tri10-binding site and characterize its functions as a transcriptional regulator on *TRI* gene expression. Another aim is to determine the possible function of Tri10 in the toxisome formation.

Significant results: We showed that over-expression of *TRI10*-GFP stimulated *TRI* gene expression and the formation of bulbous hyphae associated with DON production. Deletion of *TRI6* blocked DON accumulation but not the formation of bulbous hyphae in transformants overexpressing *TRI10*-GFP. Localization of Tri10-GFP to the toxisomes was observed in some but not all the toxisomes and its localization patterns differed from that of Tri4 and Tri1. Deletion of three genes co-regulated by *TRI10* with the *TRI* genes had no obvious effects on growth or DON biosynthesis.

Key outcomes or other achievements: Results from this project further showed that Tri10 has dual functions in DON production in *F. graminearum*. As a transcription factor, Tri10 could direct regulate the transcription of *TRI5* and other *TRI* genes but it also could form heterodimers with Tri6. As a toxisome component, Tri10 may facilitate its formation or movement during DON production. However, not all the toxisomes had Tri10-GFP signals in transformants overexpressing *TRI10*-GFP, and localization of Tri10 to toxisomes was not observed in transformants expressing the *TRI10*-GFP fusion construct with its native promoter. The *TRI* gene cluster may contain additional components because three neighboring genes were co-regulated by *TRI10* in *F. graminearum*. However, deletion of these three genes had no detectable phenotypes under conditions assayed.

OBJECTIVE 3

Major activities: We generated the *TRI6* and *TRI10* mutant alleles deleted of the conserved PacC-binding sites in their promoter and transformed them into the *tri6* and *tri10* deletion mutants, respectively. The resulting transformants expressing the *TRI6* mutant allele were defective in DON production, indicating the importance of the PacC-binding sites. The same deletion approach was used to show the importance of the putative AreA-binding sites in the *TRI6* promoter. We also compared the interaction of Tri10 with the wild-type AreA and AreA with the S874A or S874D mutation by co-IP assays. Co-IP assays also were used to determine the AreA-Tri10 interaction in the *cpk1* mutant.

Specific objectives: Specific aims are to determine the role of conserved PacC-binding sites in the *TRI6* and *TRI10* promoters and to determine the relationship between the phosphorylation of AreA by PKA and its interaction with Tri10 in regulating DON biosynthesis.

Significant results: Our results showed that the conserved PacC-binding sites in the *TRI6* promoters were important for its functions in regulating DON biosynthesis. It is possible that pH may regulate DON biosynthesis by directly regulating *TRI6* expression. The predicted AreA-binding sites also were important for *TRI6* functions. However, phosphorylation of AreA at S874 had no effects on its interaction with Tri10, which may be important for regulating DON biosynthesis via the cAMP-PKA pathway.

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 PacC and AreA may directly regulate the expression of *TRI6* by binding to its promoters. Because the AreA-Tri10 interaction was not affected by mutations at S874 and *CPK1* deletion, the regulation of DON biosynthesis by the cAMP-PKA pathway may not involve the phosphorylation of AreA by PKA.

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. The PhD student is continuing the epigenetic control TRI gene expression. The postdoc research associate, Sherry Zhang, left Purdue in February. She accepted a faculty position in Northwest Agricultural and Forestry University. The PhD student and postdoc research associate were trained in routine molecular biology techniques, fungal genetics, cell biology, and plant pathology. The PhD student and postdoc involved in this project also were trained to present posters at meetings. This project also provided training opportunities for one undergraduate student helpers.

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 speaker at the Asian Conference on Plant Pathology and the Fifth International Conference on Biotic Plant Interactions in 2017. Related data also were presented at the 2018 European Fungal Genetics Conference attended by plant pathologists and fungal geneticists. In addition, the PI attended the 2017 scab forum 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 FY17 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?**

   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 FY17 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.*

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 FY17-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17 grant. Only include citations for publications submitted or presentations given during your award period (6/1/17 - 5/31/18). 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.

Sun, M., Zhang, Y., Wang, Q., Wu, C., Jiang, C., and Xu, J. -R. 2018. The tri-snRNP specific protein FgSnu66 is functionally related to FgPrp4 kinase in *Fusarium graminearum*. Molecular Microbiology. In Press.
Status: Accepted
Acknowledgement of Federal Support: Yes

Gao, X., Jin, Q., Ju Zhang, Song, C., Wang, J. H., and Xu, J. -R. 2018. Phosphorylation by Prp4 releases the self-inhibition of FgPrp31 in *Fusarium graminearum*. Current Genetics. In press.
Status: Accepted
Acknowledgement of Federal Support: Yes

Tang, G. F., Chen, Y., Xu, J. -R., Kistler, H. C., and Ma, Z. H. 2018. The fungal myosin I is essential for *Fusarium* toxisome formation. PLoS Pathogens. doi/10.1371/journal.ppat.1006827.
Status: Published
Acknowledgement of Federal Support: No

Zhang, Y. M., Gao, X. L., Sun, M., Liu, H., and **Xu, J. –R**. 2017. The *FgSRP1* SR-protein gene is important for plant infection and mRNA processing in *Fusarium graminearum.* Environmental Microbiology. doi: 10.1111/1462-2920.13844.
Status: Published
Acknowledgement of Federal Support: Yes

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

Jiang, C., Ren, J., and Xu, J. –R. 2018. Cellular signaling in Fusarium. Invited book chapter on Fusarium genomics. Edited by Nadia Ponts, Christian Barreau, and Marie Foulongne. Caister Academic Press.
Status: In press (invited book chapter)
Acknowledgement of Federal Support: YES

Other publications, conference papers and presentations.

Epigenetic control of mycotoxin production. Invited presentation at the BARD workshop on Nutritional factors modulating plant and fruit susceptibility to pathogens. February 25, 2018. Haifa, Israel.
Status: Invited presentation (presented) and abstract published.
Acknowledgement of Federal Support: YES (talk),

Regulation of DON biosynthesis in the wheat scab fungus *Fusarium graminearum*. Invited presentation at the first APEC Conference on Mycotoxin Prevention and Control in Food and Feed Commodities in Asia-Pacific Region. Oct. 25-27, 2017. Beijing, China.
Status: Invited presentation (presented) and abstract published
Acknowledgement of Federal Support: YES (talk),

A-to-I RNA editing and Ascosporogenesis in *Fusarium graminearum*. Invited keynote presentation at The Asian Conference on Plant Pathology. Sept. 13-16, 2017. Jeju, Korea.
Status: Invited presentation (presented)
Acknowledgement of Federal Support: YES (talk),

RNA editing and Ascosporogenesis in fungal pathogens. Invited keynote presentation at The 5th International Conference on Biotic Plant Interactions. August 17-21, Xiamen, China.
Status: Invited presentation (presented) and abstract published.
Acknowledgement of Federal Support: YES (talk),