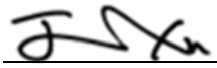
USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY16 Final Performance Report
Due date: July 28, 2017

Cover Page

Principle Investigator (PI):	JinRong Xu
Institution:	Purdue University
E-mail:	jinrong@purdue.edu
Phone:	765-494-6918
Fiscal Year:	2016
USDA-ARS Agreement ID:	59-0200-3-009
USDA-ARS Agreement Title:	Exploring Novel Approaches to Reduce the Impact of Fusarium Head Blight and DON.
FY16 USDA-ARS Award Amount:	$ 50,259
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:	106616
Project/Grant Reporting Period:	7/1/16 - 6/30/17
Reporting Period End Date:	06/30/17

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.	$ 50,259
	FY16 Total ARS Award Amount	**$ 50,259**



7/28/2017

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: *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.*

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 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.

Li, C. Q, Zhang, Y. H., Wang, H., Chen, L. F., Zhang, J., Sun, M. L., Xu, J. -R., and Wang, C. F. 2017. The *PKR1* regulatory subunit of PKA is involved in regulating growth, sexual and asexual development, and pathogenesis in *Fusarium graminearum*. Molecular Plant Pathology. doi: 10.1111/mpp.12576.
Status: Published
Acknowledgement of Federal Support: Yes

Yin, T., Zhang, Q., Wang, J., Liu, H., Wang, C. F., Xu, J. -R., and Jiang, C. 2017. The cyclase-associated protein FgCap1 has both PKA-dependent and -independent functions during DON production and plant infection n in *Fusarium graminearum.* Mol. Plant Pathology. DOI: 10.1111/mpp.12540.
Status: Published
Acknowledgement of Federal Support: Yes

Jiang, C., Zhang, C. K., Wu, C. L., Hou, R., Wang, C. F., and Xu, J. -R. 2016. Exogenous and intracellular cAMP regulated gene expression and cellular differentiation associated with DON production in *Fusarium graminearum*. Environmental Microbiology. 18: 3689-3701.
Status: Published
Acknowledgement of Federal Support: Yes

Other publications, conference papers and presentations.

J. -R. Xu. 2016. Genetic network regulating DON biosynthesis in the wheat scab fungus *Fusarium graminearum*. Invited keynote presentation at the First Chinese Mycotoxin Congress. June 28-30, 2016. Beijing, China.
Status: Invited keynote presentation presented
Acknowledgement of Federal Support: YES (talk),

Jiang, C., Yin, T., Zhang, Q., Wang, J. H., Liu, H., Wang, C.F., Xu, J. -R. Abstract 397W. The cyclase-associated protein FgCap1 has both PKA-dependent and independent functions during DON production and plant infection in *Fusarium graminearum.* Abstract presented at the 29th Fungal Genetics Conference, March 14-19, 2017. Asilomar Conference Center, Pacific Grove, CA. USA.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster).