USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY20 Annual Performance Progress Report
Due date: August 31, 2021

Cover Page

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Fiscal Year:	2020
USDA-ARS Agreement ID:	59-0206-0-168
USDA-ARS Agreement Title:	Mechanisms of Ammonium Sencing and Ammonium Suppression of DON Biosynthesis
FY20 USDA-ARS Award Amount:	$ 70,737
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:	17000690
Project/Grant Reporting Period:	6/1/20 - 5/31/21
Reporting Period End Date:	5/31/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Mechanisms of Ammonium Sensing and Ammonium Suppression of DON Biosynthesis	$ 70,737
	FY20 Total ARS Award Amount	**$ 70,737**

8/29/2021

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R- Research
S – Service (DON Testing Labs)
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: *Mechanisms of Ammonium Sensing and Ammonium Suppression of DON Biosynthesis*

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

The goal of this study is to understand how ammonium sensing leads to the suppression of DON production. Objective 1 aims to identify and characterize the amino acid sequences of Mep2-CT responsible for ammonium suppression of DON production. The roles of Mep2 in regulating Are1 and genes responsible for the uptake and utilization of arginine or putrescine also will be determined. Objective 2 aims to characterize the interaction and functional relationship between Mep2 and Ras2 and the roles of cAMP-PKA in ammonium repression. Objective 3 will determine the roles of Are1 in *TRI* gene expression and Mep2 functions.

2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address these three items below.)*

Objective 1

a) What were the major activities?

The $Mep2^{CT3}$, $Mep2^{N3}$, and $Mep2^{C3}$ chimeric alleles in which the CT of Mep2 is replaced with different regions of Mep3 were generated and transformed into the *mep2* deletion mutant. Only the $Mep2^{C3}$ allele could compliment the defects of the *mep2* mutant in growth and DON production, indicating the importance of the first half of Mep2's C-terminal region. Therefore, amino acid residues that are conserved in Mep2 or Mep2 orthologs but differ between Mep2 in this region are likely responsible for functional specificity of Mep2 as an ammonium sensor.

We also conducted RNA-seq analysis and qRT-PCR assays and showed that some of the candidate genes involved in arginine or putrescine uptake and metabolism, including the *FgCAR1*, *FgDUR3*, and *FgAGP2* genes are regulated by Mep2 at 50 mM ammonium conditions. In addition, we characterized the defects of the *mep1 mep2* and *mep2 mep3* double mutants. Whereas the *mep1 mep2* double mutant had the same phenotype with the mep2 mutant, the *mep2 mep3* double mutant had more severe defects in germ tube growth than the *mep2* mutant at low ammonium concentration. Interestingly, whereas all the single mutants were normal in sexual reproduction, the *mep2 mep3* double mutant had a defect in ascospore discharge.

b) What were the significant results?

Our results showed that the first half of Mep2's cytoplasmic tail region plays a critical role in its specific function as an ammonium sensor. Sequence alignment showed that several amino acid residues that are well conserved in Mep2 or Mep2 orthologs but differ between Mep2 and Mep3 in this region are likely responsible the functional specificity. We also showed that MEP2 is involved in regulating the expression of candidate genes related to arginine or putrescine uptake and metabolism. Arginine and putrescine are known to induce DON production. Furthermore, we showed that

MEP2 and *MEP3* had overlapping functions during germ tube growth and sexual reproduction.

c) List key outcomes or other achievements.

Identified the region and amino acid residues that are likely responsible the functional specificity of Mep2 as an ammonium sensor.

Determine the role of Mep2 in the regulation of candidate genes involved in the uptake or metabolism of arginine and putrescine, two plant compounds that are known to induce DON production.

Identified the overlapping functions between Mep2 and Mep3 during germ tube growth and ascospore discharge, two important steps in the infection cycle of *F. graminearum*.

Objective 2

a) What were the major activities?

The *RAS2*^{DA} and *RAS2*^{DN} alleles carrying dominant active and dominant negative mutations were generated and transformed into the *mep2* deletion mutant. Whereas expressing the *RAS2*^{DN} allele had no effect, transformants expressing *RAS2*^{DA} were partially rescued in the defects for the *mep2* deletion mutants in growth on low ammonium medium and ammonium suppression of DON biosynthesis. These data showed that Ras2 plays a critical role in intracellular signaling downstream from Mep2. However, our preliminary data suggested that the direct interaction between Ras2 and Mep2-CT could not be detected in yeast two-hybrid assays or BiFC assays. Currently, we are attempting to assay their interactions in vivo by co-IP assays but their interactions may be too transient at the membrane or require other co-factors for facilitation.

We also assayed the expression levels of the *NIA1, NII1, TRI5,* and *TRI6* genes in the *mep2* mutant and showed that *MEP2* is involved in the regulation of their expression under high ammonium conditions. Furthermore, we assayed PKA activities and the expression and activation of Gpmk1 MAPK in the *mep2* and *mep2 mep3* mutant.

b) What were the significant results?

Our data showed that Ras2 plays a critical role in intracellular signaling downstream from the putative ammonium sensor Mep2 for regulating DON production. Our results also showed that *MEP2* is required for ammonium suppression of genes involved in nitrate or nitrite utilization and DON biosynthesis. Because Ras proteins are known to function upstream from the cAMP-PKA and MAPK signal transduction pathways, our results also indicate that Ras2 likely functions downstream from Mep2 to activate these two pathways in response to nitrogen starvation signals in *F. graminearum*.

c) List key outcomes or other achievements.

Demonstrated the role of Ras2 in intracellular signaling downstream from Mep2 ammonium sensor for regulating DON production.

Showed the importance of Mep2 in ammonium suppression of genes involved in nitrate or nitrite utilization and DON biosynthesis.

Preliminarily characterized the signaling relationship between Ras2 and cAMP-PKA or MAPK pathways downstream from Mep2 ammonium sensor.

Objective 3

a) What were the major activities?

We had no success in obtaining Are1-Flag or Are1-S-tag transformants that expressed abundant Are1 proteins for western blot detection, likely due to its low abundance and protein instability. Therefore, it is not possible to conduct ChIP-seq or phosphoproteomics with these transformants.

Nevertheless, both TRI6 and TRI5 have multiple putative AreA binding (HGATAR) sites. The function of predicted AreA-binding sites (HGATAR) in the promoter regions of *TRI5* and *TRI6* will be investigated by site-directed mutagenesis. Are1 also has several predicted PKA and MAPK phosphorylation sites that can be characterized by alanine scan mutations.

b) What were the significant results?

As we stated in the proposal, objective 1 and objective 2 are the main objectives to characterize the roles of Mep2 as an ammonium sensor and its functional relationship with Ras2 signaling and its downstream PKA/MAP pathways. For objective 3, due its low expression level or instability, we found that it is impossible to conduct ChIP-seq and phosphoproteomics assays with Are1.

c) List key outcomes or other achievements.

Due its low expression level or instability, we found that it is impossible to conduct ChIP-seq and phosphoproteomics assays with Are1. Therefore, it may be more effective to use the candidate approach to characterize predicted or putative Are1-binding sites and PKA or MAPK phosphorylation sites.

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

Purdue had a restriction on lab assess that was not lifted until in the summer of 2021. Graduate students working on this project could not work on some of the experiments timely due to the campus de-densification and lab restrictions. All these restrictions have now been lifted. (However, it takes some time to regain the research momentum and intensity in the lab)

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

One MS student, two PhD students, and a visiting scholar involved in various aspects of this project were trained in various molecular techniques, including western blot analysis, site-directed mutagenesis, and RNA-seq data analysis. They also were trained to present their data with PPT presentation in virtual lab meetings.

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

Results from our studies with the wheat scab fungus have been published in referred scientific journals and presented at professional meetings (two on-line meetings in 2020)

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (6/1/20 - 5/31/21). 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?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? Penelope Vu, a MS students involved in this project will defend her thesis in the fall semester in 2021.

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?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? Zhuyuna Bian, a PhD student, will defend her thesis in this fall semester.

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 ☒No ☒ Not Applicable
   If yes, how many? Click to enter number here.

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?**
   ☐Yes ☐No ☒ Not Applicable
   If yes, how many? Click to enter number here.

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 FY20 award period (6/1/20 - 5/31/21). 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	FHB Rating (0-9)	Year Released
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
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Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Publications, Conference Papers, and Presentations

Instructions: Refer to the PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (6/1/20 - 5/31/21**) 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:

Z.J. Winn, R. Acharya, J. Lyerly, G. Brown-Guedira, C. Cowger, C. Griffey, J. Fitzgerald, R.E. Mason and J.P. Murphy. 2020. "Mapping of Fusarium Head Blight Resistance in NC13-20076 Soft Red Winter Wheat." In: S. Canty, A. Hoffstetter, and R. Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum (*p. 12.), Virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

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. 16 (11): e1009185. (* co-corresponding author)
Status: Published
Acknowledgement of Federal Support: No

Yin, J., Hao, C., Wang, W., Wang, G. H., Xiang, P., Xu, J. -R**.,** and Zhang, X. 2020. FgPal1 regulates morphogenesis and pathogenesis in *Fusarium graminearum*. Environmental Microbiology. 22(12), 5373–5386.
Status: Published
Acknowledgement of Federal Support: YES

Zhang, Y.M., Dai, Y.F., Huang, Y., Lu, P., Xu, H.F., **Xu, J. -R**., and Liu, H. Q. 2020. The SR-protein FgSrp2 regulates vegetative growth, sexual reproduction and pre-mRNA processing by interacting with FgSrp1 *in Fusarium graminearum*. Current Genetics. 66 (3):607-619.
Status: Published
Acknowledgement of Federal Support: No

Wang, H., Li, C. L., Tian, N., Zhang, J., **Xu, J. -R.**, and Wang, C. F. 2019. Stage-specific functional relationships between Tub1 and Tub2 beta-tubulins in the wheat scab fungus *Fusarium*

graminearum. Fungal Genetics and Biology. 132: 103251. doi.org/10.1016/j.fgb.2019.103251.
Status: Published
Acknowledgement of Federal Support: YES

Liang, J., Fu, X. H., Hao, C. F., Bian, Z., Xu, J. -R., and Wang, G. H. 2021. *FgBUD14* is important for ascosporogenesis and involves both stage-specific alternative splicing and RNA editing during sexual reproduction. Environmental Microbiology. doi.org/10.1111/1462-2920.15446.
Status: Published
Acknowledgement of funding from USWBSI: YES

Sun, M. L., Luan, Q., Chen, Y. T., Wang, W., Dong, Y. R., Bian, Z. Y., Chen, L. F., Hao, C., Xu, J. -R*., and Liu, H. Q*. 2021. Stage-specific regulation of purine metabolism during infectious growth and sexual reproduction in *Fusarium graminearum*. New Phytologist. doi: 10.1111/nph.17170. (*Co-corresponding author)
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. 2020. An orphan protein of *Fusarium graminearum* modulates host immunity by mediating proteasomal degradation of TaSnRK1. Nature Communication. 11:4382. doi.org/10.1038/s41467-020-18240.
Status: Published
Acknowledgement of Federal Support: YES

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

None.

Other publications, conference papers and presentations.

JinRong Xu, 2020. Orphan proteins of *Fusarium graminearum* important for wheat infection. Invited presentation at the 2020 annual USWBS forum
Status: Presented
Acknowledgement of Federal Support: YES

JinRong Xu, 2020. RNA editing during sexual reproduction in filamentous ascomycetes. Plenary presentation for the section on Fungal Biology, Genomics and Engineering (NEN-PL3-1) at the International Union of Microbiological Societies Congress. South Korea. Nov. 16-20, 2020.
Status: Presented
Acknowledgement of Federal Support: YES