USDA-ARS | U.S. Wheat and Barley Scab Initiative

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-2-157
USDA-ARS Agreement Title:	Regulation of Plant Infection and DON Biosynthesis in Fusarium graminearum
Principle Investigator (PI):	Jin-Rong Xu
Institution:	Purdue University
Institution UEI:	YRXVL4JYCEF5
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$53,295
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Period of Performance:	May 1, 2022 – April 30, 2024
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Signal recognition by GPCRs during plant infection in Fusarium graminearum	$53,295
	FY22 Total ARS Award Amount	$53,295

I am submitting this report as an: ☒ Annual Report

I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.

Principal Investigator Signature

7/25/2023

Date Report Submitted

‡ BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
EC-HQ – Executive Committee-Headquarters
FST-R – Food Safety & Toxicology (Research)
FST-S – Food Safety & Toxicology (Service)
GDER – Gene Discovery & Engineering Resistance
HWW-CP – Hard Winter Wheat Coordinated Project
MGMT – FHB Management
MGMT-IM – FHB Management – Integrated Management Coordinated Project
PBG – Pathogen Biology & Genetics
TSCI – Transformational Science
VDHR – Variety Development & Uniform Nurseries
NWW –Northern Soft Winter Wheat Region
SPR – Spring Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: Signal recognition by GPCRs during plant infection in Fusarium graminearum

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

G-protein coupled receptors (GPCRs) are important targets of pharmaceutical compounds, accounting over 30% of currently available drugs. In comparison with saprophytic fungi, GPCRs are expanded in plant pathogenic fungi, likely for recognizing specific plant signals at different infection stages. *Fusarium graminearum* has 105 GPCR genes, including six GIV (GPCR important for virulence) genes important for plant infection. This study aims to further characterize the roles of these six *GIV* GPCRs in fungal pathogenesis and develop approaches to identify plant compounds (ligands) recognized by them. It consists of three objectives. Objective 1 is to characterize the functional relationships among these *GIV* GPCR genes. Objective 2 is to screen for anther compounds stimulatory to Gpmk1 activation via Giv1. Objective 3 is to develop a yeast reporter system to screen for anther compounds recognized by Giv1 and other GIV GPCRs.

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

a) What were the major activities?

Objective 1. We have generated the *giv1 giv2*, *giv2 giv3*, and *giv5 giv6* double mutants and the *giv1 giv2 giv3* and *giv4 giv5 giv6,* triple mutants. In comparison with the single mutants, the *giv2 giv3* double mutant was only slightly reduced in virulence. The *giv1 giv2* double mutant was reduced over 30% in virulence compared to the *giv1* mutant. In comparison with the *giv5 giv6* double mutant, the *giv4 giv5 giv6* triple mutant was further reduced in virulence but not as significantly as the *giv1* or *giv1 giv2* mutant. Preliminary data showed that the *giv1 giv2 giv3* trouble mutant was more significantly reduced in virulence than the other single or double mutants but still had disease index over one. We have also generated constructs to generate *giv1 giv2 giv3* triple mutants by CRISPR in the wild type and other *giv* mutant background.

Objective 2. We have generated the *FST12*-GFP transformants. However, GFP signals were not observed in the nucleus in over a dozen *FST12*-GFP transformants. Based on our experience with the *TRI6* and *TRI10* that regulate *TRI* gene expression for DON biosynthesis, transcription factor genes may be expressed at relative low level and difficult to observe their GFP signals. To overexpress with a strong constitutive promoter, we have generated the P_{RP30}-*FST12*-GFP construct in which the FST12-GFP is under the control of RP30 promoter. Transformants expressing the P_{RP30}-*FST12*-GFP construct will be generated and characterized.

Objective 3. We have generated the PFuz1-Mel1 and PFuz1-LacZ yeast transformant (STE2 STE3). Treatments with alpha pheromone could induce their expression and detection of Mel1 and LacZ activities, indicating that these are suitable reporter constructs. However, the blue signals were relatively weak and modifications with approaches used in yeast studies (such as error-prone PCR) may be important. In addition, we have generated the GIV-Ste2 chimeric alleles and cloned into yeast expression vector. All these GIV-Ste2 chimeric constructs have the N-terminal tail and extracellular domains of GIV genes and the C-terminal cytoplasmic tail and three intracellular loops of Ste2. Yeast transformants expressing P_{Fuz1}-GFP and three of the Giv-Ste2 constructs have been generated.

b) What were the significant results?

Objective 1. We have generated mutants deleted of two or three *GIV* genes with different combinations, including the *giv1 giv2* double mutant and *giv2 giv5 giv6* triple mutants. Some of the resulting double or triple mutants were further reduced in virulence, confirming that these closely related GIV GPCR genes have overlapping functions during plant infection. In addition, we are developing approaches to generate mutants deleted of more than three *GIV* genes.

Objective 2. The RP30 promoter was developed to constitutively express genes in *F. graminearum*. Comparative analysis showed that the RP30 promoter is stronger than RP27 and other promoters for gene overexpression in this important wheat pathogen.

Objective 3. The Mel1 and LacZ reporter constructs were generated and tested. Although the blue signals were relatively weak and further modifications are necessary, our data showed that these reporter constructs are suitable for detecting wheat head compounds recognized by individual *GIV* genes in the yeast system. In addition, we have generated three of the planned GIV-Ste2 chimeric constructs.

c) List key outcomes or other achievements.

Objective 1

- Generated double and triple mutants deleted of different *GIV* GPCR genes.
- Preliminary characterization of the defects of the double and triple *GIV* mutants.
- Developing approaches to generate mutants deleted of more than three *GIV* GPCR genes.

Objective 2

- Generated the *FST12*-GFP transformants.
- Developed the RP30 promoter for overexpressing FST12-GFP in *F. graminearum*.

Objective 3

- Generated the PFuz1-Mel1 and PFuz1-LacZ reporter constructs.
- Assayed activities of new reporter genes in yeast transformants for screening.
- Developed the *GIV*-Ste2 chimeric alleles and cloned into yeast expression vector.
- Generated yeast transformants expressing P_{Fuz1}-GFP and three *GIV*-Ste2 constructs.

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

Two PhD students were responsible for all the experiments related to project. They were trained in basic molecular techniques and fungal genetics, including yeast vector modifications, fungal transformation, qRT-PCR, and RNA-seq data analysis. They were also trained in preparing posters and Powerpoint presentations as well as manuscripts for publications.

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

Results from out studies with *Fusarium graminearum* have been published in several referred scientific journals and a book chapter in 2022. I also presented yeast reporter strain development and preliminary testing results in seminars on GPCRs at three different universities and a research conference.

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your FHB work that were a result of funding from your FY22 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period** should be included.

Did you publish/submit or present anything during this award period May 1, 2022 – April 30, 2023?

☑ Yes, I've included the citation reference in listing(s) below.

☐ No, I have nothing to report.

Journal publications as a result of FY22 award

List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like.

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published [include DOI#]; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

1) Ding, M. Y., Cao, S. L., Xu, D., Xia, A., Wang, Z., Wang, W., Duan, K., Wu, C., Wang, Q., Liang, J., Wang, D., Liu, H., Xu, J. R.* and Jiang, C.*. 2023. A non-pheromone GPCR specifically regulates meiosis and ascosporogenesis via a conserved MAP kinase pathway in *Fusarium graminearum.* Submitted to PNAS. (Under revision) (*Co-corresponding authors) Acknowledged federal support: yes.
2) Xin, K. Y., Zhang, Y., Fan, L., Qi, Z. M., Feng, C. J., Xu, J. R., and Liu, H. Q. 2023. Experimental evidence for the functional importance and adaptive advantage of A-to-I RNA editing in fungi. PNAS. 120: e2219029120). doi.org/10.1073/pnas.2219029120. Acknowledged federal support: yes.
3) Gong, C., Xu, D. Y., Sun, D. Y., Kang, J., Wang, W., Xu, J. R.,* and Zhang, X*. 2022. FgSnt1 of the Set3 HDAC complex plays a key role in mediating the regulation of histone acetylation by the cAMP-PKA pathway in *Fusarium graminearum*. PLoS Genetics. doi.org/10.1371/journal.pgen.1010510. (*Co-corresponding authors) Acknowledged federal support: yes.
4) Hu, Y., Hou, R., Wang, Z.Y., Zhang, W. W., and Xu, J. R. 2022. Nitrogen repression of DON biosynthesis is mediated by Mep2 ammonium permease in *Fusarium graminearum*. Environmental Microbiology. 4: 5392-5407. doi.org/10.1111/1462-2920.16233. Acknowledged federal support: yes.
5) Feng, C.J., Cao, X., Du, Y., Chen, Y.T., Xin, K.Y., Zou, J.W., Jin, Q.J., Xu, J.R., and Liu, H.Q. 2022. Uncovering cis-regulatory elements important for A-to-I RNA editing in fungi. mBio. 14;e0187222. doi: 10.1128/mbio.01872-22. Acknowledged federal support: yes.

Books or other non-periodical, one-time publications as a result of FY22 award

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (book, thesis, or dissertation, other); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Other publications, conference papers and presentations as a result of FY22 award

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication.

Wang, Z.Y., Zhang, X., Jiang, C., and Xu, J. R. 2022. Regulation of plant infection processes by MAP kinase pathways in ascomycetous pathogens. Pages 211-226. In the Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research. Vol. V: Fungal Associations. Ed. B. Scott and C. Mesarich. Springer Nature, Switzerland. doi.org/10.1007/978-3-031-16503-0_8. ISBN: 9783031165023. Acknowledged federal support: yes.