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

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Novel approaches to combat FHB and mycotoxin contamination
Principle Investigator (PI):	Guixia Hao
Institution:	USDA-Agricultural Research Service
Institution UEI:	N/A
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$119,846
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Period of Performance:	May 1, 2022 - April 30, 2023
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Characterization and Expression of Plant Transporters to Reduce FHB and Mycotoxins	$59,846
PBG	Explore RNAi to Control FHB and Mycotoxin Contamination	$60,000
	FY22 Total ARS Award Amount	$119,846

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.

Guixia Hao _____ 6/26/23 _____

Principal Investigator Signature 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: Characterization and Expression of Plant Transporters to Reduce FHB and Mycotoxins

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

   The goal of this project is to identify plant transporters and utilize it to reduce FHB and mycotoxin contamination.

   The objectives of this proposal are:
   - Identification of transporters responsible for 3-ADON excretion using transgenic Arabidopsis expressing FgTri101.
   - Generation of transgenic wheat expressing FgTri101 and determine if transgenic wheat expressing FgTri101 can excrete 3-ADON.
   - Stacking FgTri101 and the Arabidopsis transporter in transgenic wheat and evaluate transgenic lines for DON detoxification and resistance to FHB.

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 treated the transgenic Arabidopsis plants expressing FgTri101 with DON for 24 h. The TRI101 transgenic plants without DON treatment served as controls.
   - We isolated RNA from treated and control plants, confirmed RNA quality and used them for RNA Seq.
   - We obtained and analyzed the RNA seq data to identify Arabidopsis transporters that are upregulated by DON treatment.

   Objective 2
   - We cloned FgTri101 from *F. graminearum* and introduced it into pAHC17 vector.
   - We confirmed pAHC17-Tri101 construct and used it for generating transgenic wheat plants.
   - We generated 5 transgenic lines, performed PCR screening, and examined *FgTRI101* expression in leaves and heads by RT-PCR
   - Propagate seeds and obtained T1 and T2 seeds for analysis.
   - Performed DON sensitivity and DON to 3-ADON assays using wheat seedlings.

   b) What were the significant results?

   Objective 1
   - By transcriptomic analysis, three Arabidopsis transporter candidates were identified, and their functions are investigation.

   Objective 2
   - Four wheat transgenic lines expressing *FgTRI101* were confirmed. One line exhibited high gene expression in wheat leaves and heads.
   - FgTri101 transgenic seedlings showed DON tolerance and longer root growth.
   - Surprisingly, 3-ADON was not detected consistently in FgTri101 transgenic wheat lines.

c) List key outcomes or other achievements.

Although we demonstrated that Tri101 transgenic Arabidopsis converted DON to 3-ADON and excreted 3-ADON to the medium, we could not detect 3-ADON consistently in Tri101 transgenic wheat. Previous studies reported that no 3-ADON were detected in Tri101 transgenic rice, barley, and wheat. We speculate that 3-ADON may be unstable in monocot plants.

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

One ORISE fellow, Gabdiel Yulfo-Soto, has been trained in molecular biology including DNA isolation, RCR and RT-PCR, screening transgenic plants, root growth and DON to 3-ADON conversion assays, inoculation and scoring of FHB assays. Gabdiel recently was offered a permanent tech position in another USDA/ARS location.

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

Nothing to report.

Project 2: Explore RNAi to Control FHB and Mycotoxin Contamination

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

The goal of this project is to develop an endophytic fungal RNAi delivery platform to reduce FHB and mycotoxin contamination.

The objectives of this proposal are:

- **Objective 1:** Design and generate *F. graminearum* RNAi mutants targeting genes that are essential for its pathogenesis, and trichothecene and zearalenone biosynthesis, and determine their effects on toxin production and FHB severity.
- **Objective 2**: Build, evaluate, and optimize the *S. zeae*-mediated RNAi delivery system. We will generate *S. zeae* GFP-RNAi strains, examine RNAi molecule production and the transferring of RNAi signals from *S. zeae* to plants and *F. graminearum*, and determine gene silencing efficacy.

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 amplified a fragment targeting *TRI5* gene and cloned into the pDONR/Zeo vector. We constructed TRI5 RNAi silencing vector pSGATE1-TRI5 through LR reaction.
- We transformed *F. graminearum* protoplasts with pSGATE1-TRI5 silencing vector and obtained three *F. graminearum* RNAi mutants targeting *TRI5*. We isolated RNA from these mutants and performed gene expression studies using RT-PCR.
- We cultured *F. graminearum* TRI5 RNAi mutants in agmatine media and measured DON production in these mutants.
- We introduced pSGATE1-TRI5 to *S. zeae* strain to generate *S. zeae* TRI5 RNAi strain.
- We performed RT-PCR to examine dsRNA production in *S. zeae* TRI5 RNAi strains.
- We performed wheat seeds treatment with *S. zeae* TRI5 RNAi strain and examined disease and mycotoxin reduction efficacy.

Objective 2:

- We isolated total RNA and sRNA from *S. zeae* strains expressing pSGATE1-GFP RNAi construct.
- We performed qPCR to detect dsRNA and Northern blot to detect sRNA.
- We performed plate and liquid medium assays to check if *S. zeae* GFP RNAi strains can reduce GFP signal expressed by a *F. graminearum* GFP strain.

b) What were the significant results?

Objective 1

- We obtained three *F. graminearum* TRI5 RNAi mutants and confirmed the significant reduction of *TRI5* expression and toxin in the RNAi mutants.
- We confirmed *TRI5* dsRNA production in *S. zeae* TRI5 RNAi strains and showed these strains reduced toxin produced by *F. graminearum* in medium.

- Preliminary data showed that wheat seeds treated with *S. zeae* TRI5 RNAi strain reduced FHB and mycotoxin.

Objective 2

- We found that *S. zeae* GFP RNAi strains can produce dsRNA but did not produce detectable sRNA.
- We confirmed *S. zeae* GFP RNAi strains significantly reduced GFP signal produced by *F. graminearum* GFP strain.

c) List key outcomes or other achievements.

We build an endophytic fungal RNAi delivery platform successfully and need further investigation for application.

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

Two ORISE fellows, Karen Yin and Nick Rhodes, have been trained in molecular biology including DNA and RNA isolation, RCR and RT-PCR, fungal mutant generation. They were also trained in toxin analysis, fungal inoculation and scoring of FHB assays. Karen has taken a Scientist position in Beijing University, China.

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

Poster presentation at the 2022 National Fusarium Head Blight Forum, Dec. 7-11, 2022. Tampa, FL

Oral presentation at GDER-PBG virtual conference, April 27, 2023

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).

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.

Hao, G., McCormick, S., Yin, G., and Vaughan M. M. (2022). Development of an endophytic fungal RNAi delivery platform to control Fusarium Head Blight and mycotoxin contamination. Proceedings of the 2022 National Fusarium Head Blight Forum. Tampa, FL. December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Hao, G. 2023. "Progress on endophytic fungal RNAi delivery to control Fusarium head blight and mycotoxin". GDER-PBG Virtual conference, April 27, 2023
Status: Talk given
Acknowledgement of Federal Support: YES