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:	Developing New Technologies for Improving Resistance to Fusarium Head Blight
Principle Investigator (PI):	Steve Scofield
Institution:	USDA-Agricultural Research Service
Institution UEI:	N/A
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$121,341
PI Mailing Address:	Crop Production and Pest Control Research Unit 915 Mitch Daniles Blvd West Lafayette, IN 47907
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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	Mitigate FHB in Wheat by Knockdown of Defense Repressors	$61,254
PBG	Spherical nucleic acid nanomaterials as fungicide and FHB resistance-promoting agents	$60,087
	FY22 Total ARS Award Amount	$121,341

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.

Steven R Scofield

July 24, 2023

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: Mitigate FHB in Wheat by Knockdown of Defense Repressors

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1. **What are the major goals and objectives of the research project?**
   The major goal of this project is to target the knockdown of *NPR3* and *NPR4*, two genes that based on studies in *Arabidopsis thaliana* encode proteins that repress the defense activator NPR1. NPR1, which is the receptor for salicylic acid, a signaling metabolite in plant defense, was previously shown to promote resistance against *Fusarium graminearum* in Arabidopsis and wheat. Thus, by knocking down the expression of *NPR3* and *NPR4*, it is expected that in response to *Fusarium graminearum* infection the activation of NPR1 signaling will be faster and stronger, resulting in enhanced resistance to *F. graminearum.*
   Three aims were proposed:
   1. Develop RNAi lines to reduce wheat *WhNPR3* and *WhNPR4* expression
   2. Identify mutations in *WhNPR3* and *WhNPR4* that can be utilized as non-GMO alleles for enhancing FHB resistance in wheat under aim 3
   3. Characterize response to *F. graminearum* infection in *WhNPR3* and *WhNPR4* knockdown lines identified under aims 1 and 2.

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?**
   - Two RNAi constructs WhNPR3-RNAi and WhNPR4-RNAi have been made in which a linker separated sense and an anti-sense fragments of WhNPR3 and WhNPR4, respectively, were cloned such that the recombinant construct results in a hair-pin loop structure that when processed by Dicer-like RNAse will generate target specific small RNA that when utilized by the RISC complex will promote turnover of the target gene transcript. These RNAi constructs have been delivered to the USWBSI-supported central wheat transformation lab at Kansas State University for transforming wheat. Transgenic wheat plants are expected in spring 2024.

   - Nonsense and missense mutations have been identified in the homeologs for WhNPR3 and WhNPR4. Seeds for these mutants in the Kronos background were propagated. They were segregating for the mutant alleles. Efforts to identify plants that are homozygous for each mutation are ongoing.

   - To determine if *NPR3* and *NPR4* knockdown results in enhanced resistance against *F. graminearum* in Arabidopsis, disease severity was monitored in the *npr3* and *npr4* mutants as well as in the *npr3 npr4* double mutant.

   b) **What were the significant results?**
   In Arabidopsis, knockdown of either *NPR3* or *NPR4* resulted in enhanced resistance to *F. graminearum* in the leaf infection assays. The combined knockdown of both *NPR3* and *NPR4* resulted in leaf collapse without any disease symptoms in the *npr3 npr4* double mutant plants. This leaf collapse, which was observed within 2-3 days of fungal

inoculation, is an indication of a hypersensitive response (HR)-like phenomenon that normally accompanies an incompatible interaction in Arabidopsis against some pathogens. This is suggestive of a relatively strong defense response to *F. graminearum* in the *npr3 npr4* double mutant. This HR-like phenotype and its impact on fungal growth is being further investigated.

c) List key outcomes or other achievements.

The results of the Arabidopsis *npr3* and *npr4* mutants that display enhanced disease resistance *F. graminearum*, and in particular the double *npr3 npr4* mutant, which displays a HR-like response is very encouraging on the utility of knockdown of *NPR3* and *NPR4* gene function for promoting resistance against FHB in wheat.

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

Training: Two graduate students worked part-time assisting with this project. They gained training in plant-pathogen interaction, in particular assessing disease severity and molecular pathology, planning experiments, collecting, recording, and analyzing and interpreting data. A new postdoc spearheaded this project, while the other senior postdoc helped with training of the students as well as the new postdoc. The postdoc spearheading this project gained training in working with Fusarium graminearum interaction with Arabidopsis and wheat. In addition, he gained experience in molecular plant pathology. The senior postdoc received training towards his long-term goal in pursuing a future independent career in academics, including mentoring others, managing lab personnel, ensuring research-related compliance and reporting, and day-to-day function of a research lab.

Professional Development: This project contributed to the professional development of the graduate students and postdocs who participated in the weekly group meetings, weekly department seminars, the BioDiscovery Institute research talks and the FHB forum. They developed their presentation skills by preparing posters and/or talks arising out of their work. Co-PI Shah worked individually with the graduate students and postdocs, meeting with them biweekly, to help them prepare towards their long-term professional goals.

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

Results were disseminated to communities of interest in multiple ways:

- Poster presented at the Annual USWBSI National Fusarium Head Blight Forum in Tampa Bay, December 2022.
- Flash talk by graduate student at USWBSI National FHB Forum in Tampa Bay, December 2022
- Outcomes of this work were disseminated to undergraduates in an introductory biology class taught in Spring 2023 at the University of North Texas.

Project 2: Spherical nucleic acid nanomaterials as fungicide and FHB resistance-promoting agents

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1. **What are the major goals and objectives of the research project?**
   The goal of this project is to develop novel spherical nucleic acid (SNA) nanomaterial-based technology to control FHB. This project builds upon USWBSI-supported findings that demonstrate the utility of RNA-interference (RNAi)-based approaches in plants to: (i) knock down expression of *Fusarium graminearum* genes by a mechanism called host-induced gene silencing (HIGS), and (ii) knock down expression of wheat 'FHB susceptibility' genes for mitigating FHB. The specific goals of this project are to develop SNA nanomaterials as fungicides that selectively target *F. graminearum* growth, viability, and virulence, and as agents that promote plant resistance to FHB.
   Two objectives are being pursued:

   1. Identify candidate siRNA sequences that effectively silence *F. graminearum* genes associated with severity of FHB.
   2. Synthesize lipophilic SNA nanoparticles and evaluate their efficacy in silencing target gene expression to limit fungal growth and toxin accumulation, and enhancing FHB resistance in wheat.

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?
   - Synthesized liposomal and micellular siRNA for targeting three different *F. graminearum* genes and examined their effect on gene silencing in *F. graminearum*
   - Examined efficiency of fungal gene expression knockdown with different SNA formulations in the fungus cultivated in vitro.
   - Studied the affect of fungal gene knockdown on fungal growth.

   b) What were the significant results?
   - *SNA synthesis:* A lipid nanoparticle-based technology was utilized to develop double stranded spherical nucleic acids (SNAs) that incorporate siRNA for gene knockdown application. Two different forms of SNAs were synthesized, micellular and liposomal. The micellular ones consisted of a hydrophobically terminated siRNA that spontaneously assemble in small micelles, while liposomal SNAs consist of 100 nm liposomes core decorated with a shell of highly oriented siRNA. We previously confirmed using Confocal and epifluorescence micrographs that the nanomaterials were able to cross over the fungal cell wall, thus pointing to high-efficiency entry of the materials that enrich in fungal mycelia.

- *SNAs show an ability to knockdown of some fungal genes:* To assess potential efficacy of SNA-based fungicides, the fungus was treated with SNAs (micellular and liposomal) constructed with siRNA aimed at knocking down three fungal genes that are either essential for fungal growth or are virulence factors. The most effective knockdown in vitro was observed for a virulence gene. Silencing of two genes that are known to affect fungal growth/development was not as effective. We still need to examine knockdown under different culture conditions and further optimize the concentration of siRNA used in the SNA and the application process for each gene. Additional genes will be included for targeting by SNA in upcoming year.

c) List key outcomes or other achievements.

- Demonstrated rapid uptake of SNAs into *F. graminearum* for both liposomal and micelle formulations and observed gene knockdown for virulence factor-encoding gene.
- Trained a new postdoc in nanotechnology-based delivery systems, working with Fusarium graminearum and molecular plant pathology.

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

Training: A new postdoc who is spearheading this project was provided training in working with nanomaterials as a delivery system to target the knockdown of fungal genes. The postdoc also received training in working with *Fusarium graminearum*, and in molecular plant pathology.

Professional Development: This project contributed to the professional development of the postdoc who participated in the weekly group meetings, weekly Department of Biological Sciences seminars, the BioDiscovery Institute research talks and the FHB forum. He developed his presentation skills by preparing posters arising out of their work. Co-PI Shah worked individually with the postdoc, meeting with him biweekly to help him prepare towards his long-term professional goals.

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

- Poster presented at the Annual USWBSI National Fusarium Head Blight Forum in Tampa Bay, December 2022.
- Outcomes of this work were disseminated to undergraduates in an introductory biology class taught in Spring 2023 at the University of North Texas.

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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?

x 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.

Alam, S.T., Sarowar, S., Mondal, H.A., Makandar, R., Chowdhury, Z., Louis, J. and Shah, J. (2022) Opposing effects of *MYZUS PERSICAE- INDUCED LIPASE 1* and jasmonic acid influence the outcome of *Arabidopsis thaliana–Fusarium graminearum* interaction. Molecular Plant Pathology. 23, 1141-1153. https://doi.org/10.1111/mpp.13216

Acknowledgement of 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.

N/A

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.

Poster Presentations

Montoya, B., Mittal, I., Scofield, S., Shah, J., Meckes, B. (2022). Spherical Nucleic Acids for Fusarium graminearum gene regulation. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa, FL. December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf Acknowledgment of federal support: Yes

Mittal, I., Alam, S., Chabra, B., Shulaev, E., Mohan, V., Girija, A., Rawat, N., Dong, Y., Trick, H. N., Scofield, S., Shah, J. (2022). Targeting Susceptibility Genes in Wheat to Enhance Resistance Against Fusarium Head Blight. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa, FL. December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf Acknowledgment of federal support: Yes

Flash talk

Targeting Susceptibility Genes in Wheat to Enhance Resistance Against Fusarium Head Blight. Flash talk by graduate student Isha Mittal, US Wheat and Barley Scab Initiative. Tampa, FL; December 2022. *Acknowledgment of federal support: Yes*