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

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

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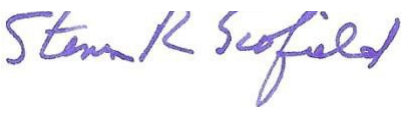
Phone:	765-494-3674
Fiscal Year:	2021
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Molecular-genetic Approaches to Mitigate Fusarium Head Blight Disease in Wheat
FY20 USDA-ARS Award Amount:	$125,156
Recipient Organization:	USDA-ARS Crop Prod. & Pest Control Research 915 West State Street, West Lafayette, IN 47907-2054
DUNS Number:	N/A
EIN:	N/A
Project/Grant Period:	5/1/21 - 4/30/22
Reporting Period End Date:	4/30/2022

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	RNA-Interference Targeting of Fungal Genes for Enhancing FHB Resistance	$42,433
GDER	Wheat Variants Deficient in a FHB Susceptibility Factor	$40,391
TSCI	Spherical Nucleic Acid Nanomaterials as Fungicide and FHB Resistance-promoting Agents	$42,332
	FY21 Total ARS Award Amount	$125,156

I am submitting this report as an: ☒ Annual Report ☐ Final 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.



July25, 2022

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: RNA-Interference Targeting of Fungal Genes for Enhancing FHB Resistance

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

The goal of this project is to transiently knockdown expression of *Fusarium graminearum* effector genes that are required for virulence when the fungus infects wheat. RNA-interference (RNAi)-based host-induced gene silencing (HIGS) is the approach that was used to knockdown expression of fungal genes that encode secretory proteins. Two fungal genes were targeted: (i) FGL1, which encodes a lipase, and (ii) FgNahG, which encodes a salicylate hydroxylase. In Arabidopsis, silencing of these two genes enhances resistance to *F. graminearum* infection.

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?

Transgenic wheat lines that contained FGL1-RNAi and FgNahG-RNAi constructs were evaluated for response to *Fg*.

At different times during the course of this project, training and professional development opportunities were provided to a research scientist, and a graduate student.

b) What were the significant results?

Multiple wheat FGL1-RNAi and FgNahG-RNAi lines were tested for FHB severity. As in Arabidopsis, the wheat FGL1-RNAi lines exhibited enhanced resistance against FHB. However, this effect was gradually diminished in subsequent generations. In case of the wheat FgNahG-RNAi lines, despite expression of the constructs we did not observe significant differences in FHB severity compared to the control non-transformed genotype, presumably due to redundancy with other salicylate hydroxylases in *Fusarium graminearum*.

c) List key outcomes or other achievements.

This study provided proof-of-concept that silencing of some fungal pathogenicity genes can be transiently targeted for controlling growth of *Fusarium graminearum* in planta. Based on these outcomes, we are now pursuing the development of lipid-based nanoparticles as a mechanism to deliver small interfering (si) RNA to knockdown fungal gene expression. If successful, this will facilitate the development of siRNA-based pathogen-specific fungicide.

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

Training: The research scientist who led this project received training in molecular biology, plant physiology and pathology.

The graduate student who worked part-time on this project received training in plant genetics, molecular biology, pathology, physiology. She learnt first-hand on how to work with wheat and *Fusarium graminearum*, plan experiments, collect, record, analyze and interpret data.

Professional Development: This project contributed to the professional development of the research scientist and graduate student who participated in the weekly group meetings, department seminars, the BioDiscovery Institute research talks and the FHB forum. The Co-PI has worked individually with the research scientist and the graduate students, meeting with them biweekly, to help them prepare towards achieving their long-term professional goals. The research scientist is now a certified infectious disease specialist at HealthTrackRx, a molecular diagnostic company based in Denton, Texas.

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

Results were disseminated to communities of interest in multiple ways:

- Posters presented: at the Annual USWBSI Forum in 2021
- Oral presentations: at the BioDiscovery Institute retreat (2022).
- Outcomes of this work were disseminated to undergraduates in an introductory biology class taught in Spring 2022 at the University of North Texas.
- Links to publications resulting from the prior funding associated with this project were made available to the FHB community via the USWBSI website.

Project 2: Wheat Variants Deficient in a FHB Susceptibility Factor

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1. **What are the major goals and objectives of the research project?**
   The goal of this project is to knockdown function of a wheat lipoxygenase-encoding gene that contributes to susceptibility to FHB. TILLING was utilized to identify nonsense mutations at this locus, with the purpose of providing a non-GMO genetic material that can be integrated into wheat breeding programs.

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?
   Molecular makers were developed and utilized to identify hexaploid wheat that are homozygous for the mutant *Lpx3* alleles. The impact of mutations in Lpx3 homeologs on FHB disease severity and DON content was characterized for TILLING lines that contain mutations in the *Lpx3* homeologs on chromosomes 4A, 4B and 4D. Double mutant plants were created that contained mutations in two Lpx3 homeologs.

   Training and professional development opportunities were provided to a graduate student and a postdoc.

   b) What were the significant results?
   Strongest resistance that was accompanied by reduction in DON content was observed in lines containing non-sense mutations in the Lpx3 homeolog on Chromosome 4A. Reduced disease severity and DON accumulation was also observed in mutations at the *Lpx3* loci in Chromosome 4B.

   c) List key outcomes or other achievements.
   A good source of resistance that also limits DON accumulation is provided by knockdown of mutants that contain nonsense alleles at the L*px3* locus on chromosome 4A and 4B.

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

   Training: The graduate student working on this project received training in plant genetics, molecular biology, pathology, physiology. She learnt first-hand on how to work with wheat and *Fusarium graminearum*, plan experiments, collect, record, analyze and interpret data. In addition, as part of this project, she became proficient with lipid physiology and RNAseq analysis. A postdoc was actively involved in training the graduate student in the lab in the area of molecular biology and physiology.

The 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. He also gained expertise in RNAseq analysis and bioinformatic analysis of wheat genome data.

Professional Development: This project contributed to the professional development of the graduate student and postdoc 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 and working on an upcoming manuscript. The Co-PI worked individually with the graduate student and postdoc, 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:

- Posters presented: at the Annual USWBSI Forum in 2021, at the International Conference of Arabidopsis Research (2021)
- Oral presentations: at the annual conference of the American Society of Plant Biologists (2021), at the BioDiscovery Institute retreat (2022).
- Outcomes of this work were disseminated to undergraduates in an introductory biology class taught in Spring 2022 at the University of North Texas.

Project 3: 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 recent findings, including those supported by the USWBSI, 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.

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 and examined uptake of the structures into *F. graminearum* across multiple time points
- Examined initial efficiency of fungal gene expression knockdown with different SNA formulations in the fungus cultivated in vitro.

b) What were the significant results?

SNA synthesis: Following our strategy of creating nanomaterials to generate potential fungicides for *F. graminearum,* we were able to successfully synthesize 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.

Nanoparticle Uptake into Fungal Mycelia Occurs with SNAs: Since material entry is requisite for gene regulation activity, we demonstrated that SNAs enter fungal mycelia after short incubation periods (as little as 20 min), suggesting active enrichment. Confocal and epifluorescence micrographs showed that the nanomaterials were able to cross over the fungal cell wall. Overall, these results point to high-efficiency entry of the materials that enrich in fungal mycelia, which could lead to effective gene silencing.

SNAs show an ability to knockdown 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 FgFGL1, a gene responsible for lipid metabolism that contributes to fungal virulence. FGL1 expression was induced by inclusion of wheat germ oil in the media. In preliminary studies, the inclusion of SNA resulted in reduced accumulation

of FGL1 transcript. While this result is promising, 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. It is possible that culture conditions and time to evaluate knockdown impact the amount observed. Continued studies will examine these key tenets as we seek to understand the mechanisms by which this occurs.

c) List key outcomes or other achievements.

- Demonstrated rapid uptake of SNAs into *F. graminearum* for both liposomal and micelle formulations
- Observed preliminary gene knockdown of candidate virulence genes
- Trained a MS student in nanotechnology synthesis, fungal cell culture, and molecular biology

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

The MS student was provided the following training by Co-PI Meckes and in molecular biology and fungal biology be a graduate student in Co-PI Shah's lab. This student was provided with the following training developmental opportunities

Training: The student took courses relevant to the proposal in computational methods and nanotechnology characterization and fabrication. He received one-on-one training on techniques intended to characterize nanomaterials that include; liposomal nanoparticle synthesis, dynamic light scattering evaluation of nanoparticle sizes, high-performance liquid chromatography, spherical nucleic acid assembly, and fluorescence microscopy. The student also received training in culturing Fusarium graminearum in Co-PI Shah's lab. He received weekly feedback from Co-PI Meckes.

Professional Development: The student attended seminars hosted by the Department of Biomedical Engineering and ones hosted by the BioDiscovery Institute at the University of North Texas. He also attended the National FHB Forum (virtual) and presented his research as a poster. This meeting is focused on FHB related research providing broader understanding of how *Fusarium graminearum* affects crops and the underpinnings of resistance.

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

Results of this work have been disseminated at the 2021 National FHB Forum and at annual retreat of the BioDiscovery Institute at the University of North Texas.

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 FY21 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?

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

☐ No, I have nothing to report.

Journal publications as a result of FY21 grant 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 FY21 grant 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 FY21 grant award

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

Montoya, B., Mittal, I., Shah, J., Meckes, B. (2021) *Development of Biocompatible siRNA Nanoparticles to Mitigate FHB in Wheat* (pp. 50). US Wheat and Barley Scab Initiative. Poster presented. *Proceedings of the 2021 National Fusarium Head Blight Forum*. https://scabusa.org/forum/2021/2021NFHBForumProceedings.pdf

Mittal, I., Alam, S., Chabra, B., Shulaev, E., Mohan, V., Dong, Y., Scofield, S., Rawat, N., Shah, J. (2021) *Knockdown of Lpx3 Function in Wheat Enhances FHB Resistance and Lowers DON Content* (pp. 49). US Wheat and Barley Scab Initiative. Poster presented. *Proceedings of the 2021 National Fusarium Head Blight Forum*. Published: https://scabusa.org/forum/2021/2021NFHBForumProceedings.pdf

Mittal, I., Alam, S., Chabra, B., Shulaev, E., Mohan, V., Rawat, N., Shah, J. (2021) Targeting Wheat Genes Associated with Susceptibility *to Fusarium graminearum* for Enhancing FHB Resistance. Poster presented, virtual.

Mittal, I., Alam, S., Chabra, B., Shulaev, E., Mohan, V., Rawat, N., Shah, J. (2021) 9-lipoxygenase as a susceptibility factor in Arabidopsis and wheat interaction with *Fusarium graminearum*. International Conference on Arabidopsis Research -2021, Poster presented, virtual. https://southern.aspb.org/meetings/