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

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-0-138
USDA-ARS Agreement Title:	Developing Native and Induce Fusarium Head Blight (FHB) Resistance Solutions in Wheat
Principle Investigator (PI):	Mohsen Mohammadi
Institution:	Purdue University
Institution UEI:	YRXVL4JYCEF5
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$182,282
PI Mailing Address:	Purdue University, Department of Agronomy 915 W. State Street, West Lafayette, IN 47907
PI E-mail:	mohamm20@purdue.edu
PI Phone:	765-496-6851
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
VDHR-NWW	Conventional and Marker-based Breeding to Improve Yield and FHB Resistance in Wheat	$85,383
TSCI	Biodegradable Nanomaterial-based Non-GMO RNAi Delivery for Controlling FHB Disease	$96,899
	FY22 Total ARS Award Amount	$182,282

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.

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: Conventional and Marker-based Breeding to Improve Yield and FHB Resistance in Wheat

1. **What are the major goals and objectives of the research project?**
   The overarching goal of this project is to continue breeding procedure and produce new genetic variations that harbor both yield and FHB resistance traits, and ultimately releasing high yielding varieties to growers. The detailed objectives are: producing new breeding crosses every year, generation advancement of breeding populations, and line testing locally and regionally.

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. Breeding crosses. During this grant cycle (fall 2022 and spring 2023) we have performed ~200 breeding crosse among high yielding lines and moderately resistant germplasm.

   F1 plants were grown in greenhouse during summer 2022. Seeds of F2 generation will be collected in August 2022. Large plot (or long row) F2 generations will be planted in fall 2022. That is one year from identification of high yielding lines to sowing of F2 generation in the field.

   Objective 2. Generation advancement. F1 plants from crosses of last year were grown in spring 2023 in the greenhouse to produce bulk of seed for F2 generation. 506 long rows (12 ft) of F2 families and 271 long rows of F3 families were grown in the field for selection among and within families. Lines were selected from headrows and were hand harvested.

   Objective 3. Line testing. Six Y1 testing trials were planted in row and column design, each accommodating 80 plots and ~70 test entries. One Y2 trial was conducted with 120 plots and ~40 advance lines selected that were selected from Y1s and sparse testing of last year. One elite trial was conducted in two locations (WL and VIN) with ~40 test entries and three replications. Several multi-state trials were re-formatted and know is referred to as BIG6 (6 states involved are IN, IL, OH, KY, MI, and NY). This test included 480 plots in a p-rep design where some entries.

   Objective 4. Scab testing of advanced lines. Two reps of FHB evaluation rows for each of the stage-3 and Elite lines were tested in scab nursery. We inoculated them with scabby corn kernels, and created a desirable environment for disease establishment by using misted irrigation post-inoculation. Incidence and severity were taken in a scale of 1-9. Rows were harvested by hand. They will be threshed, FDK will be measured, and grain samples will be sent to University of Minnesota for DON testing. This year was dry and incidence was very low. We also conducted the preliminary and advanced nurseries (P+NUWWSN FHB) under misted system. We also conducted the uniform eastern regional winter wheat nurseries (UERWWN) trial.

   b) **What were the significant results?**

   Breeding trials were successfully harvested. Variations in grain yield and FHB traits were observed during the season and harvest time. At this point (July 24) we have incorporated plot length and reported back cooperative trials such as BIG6.
   In addition, incidence and severity traits were measured from the scab nursery, which will be complemented with FDK and DON and then reported to the coordinator.

c) List key outcomes or other achievements.

The key outcome after we finalize data and share them with collaborators will be selection of higher and more stable yielding lines across multi-location. The ultimate outcome will be selection of candidates for variety release. The results will also identify lines that are resistant or moderately resistant to FHB across wider environments.

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

One postdoc and three graduate students not funded by this grant were educated essentials of field- based plant breeding during the peak of the field work. In particular, they learned management of segregating generations and how to handle them.

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

Dr. Mohammadi will be presenting a poster from the breeding activities in the National FHB Forum in the fall 2023.

Project 2: Biodegradable Nanomaterial-based Non-GMO RNAi Delivery for Controlling FHB Disease

---

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

Spray-Induced Gene Silencing (SIGS) can be used as a safe and robust method of plant protection for both pre-harvest (Koch et al., 2016; Mitter et al., 2017) and post-harvest strategy (Wang et al., 2016), which is especially also useful for FHB disease with continuous mycotoxin production after harvest. In this research, which is a collaboration between plant-fungal scientists and a nanotechnologist, the goal is to use principles of RNA interference (RNAi) (Agrawal et al., 2003) and host-induced gene silencing (HIGS) (Huang et al., 2006) to develop non-transgenic approaches for wheat resistance to FHB. In particular, this research aims to test the hypothesis that whether a spray product, containing double-stranded RNA tool(s), can control the FHB disease. Because the lifetime of RNA molecules in the environment is short, and they are degraded rapidly, we aim to leverage nanotechnology and incorporate it into silencing RNA tools for slow-release and possibly the penetration of RNA molecules to plant or fungal cells.

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. Design and development of nanostructures and integration designed dsRNA into core shell nanoparticles for spray application.

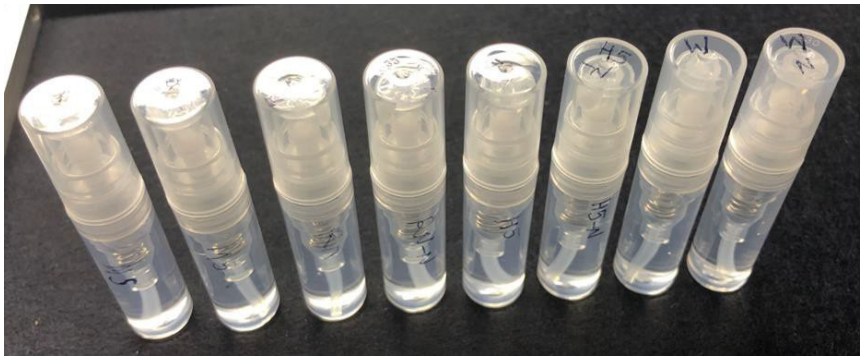
1a. Design and develop scalable production of core-shell dsRNA-chitosan nanostructures for controlled sustained release of dsRNA over the course of two weeks in the plant.

After the initial failure with Chitosan as nanocarrier, the team continued the project with the synthesis of graphene quantum dots (GQDs) nanoparticles as an effective alternative that has no anti-fungal properties. The Graphene Quantum Dots (GQDs) used in this study were prepared (**Figure 2a**) using the previously reported method with minor modifications through a bottom-up approach by direct pyrolysis of citric acid (CA). The size and shape of GQDs were confirmed using Transmission Electron Microscopy (TEM) and the corresponding TEM image is shown in **Figure 2b**. The presence of several nanosized black dots (indicated with yellow color arrows) in the TEM image confirms the successful synthesis of GQDs having a size of around 2-5 nm. Since the siRNA is a negatively charged molecule, to load the siRNA onto GQDs, it is necessary to understand the surface charge of as-synthesized GQDs. Therefore, the zeta potential of GQDs was collected using Zetasizer and observed that GQDs displayed (**Figure 2c**) a slightly negative charge (-1.71 mV). To make the surface charge of GQDs positive, branched Polyethyleneimine (bPEI) surface functionalization strategy was followed. The zeta potential collected (**Figure 2d**) after the surface functionalization of GQDs with bPEI exhibited a positive surface charge (+59.1 mV). These surface functionalized GQDs were further used for loading dsRNA through the ionic interaction mechanisms.

Citrate-derived carbon dots functionalized with branched PEIs (bPEIs) have been used to deliver plasmid DNA and siRNA to animal cells (Liu et al., 2012; Pierrat et al., 2015).

1b. Integration of designed dsRNA into core shell nanoparticles with uniform spray application process.

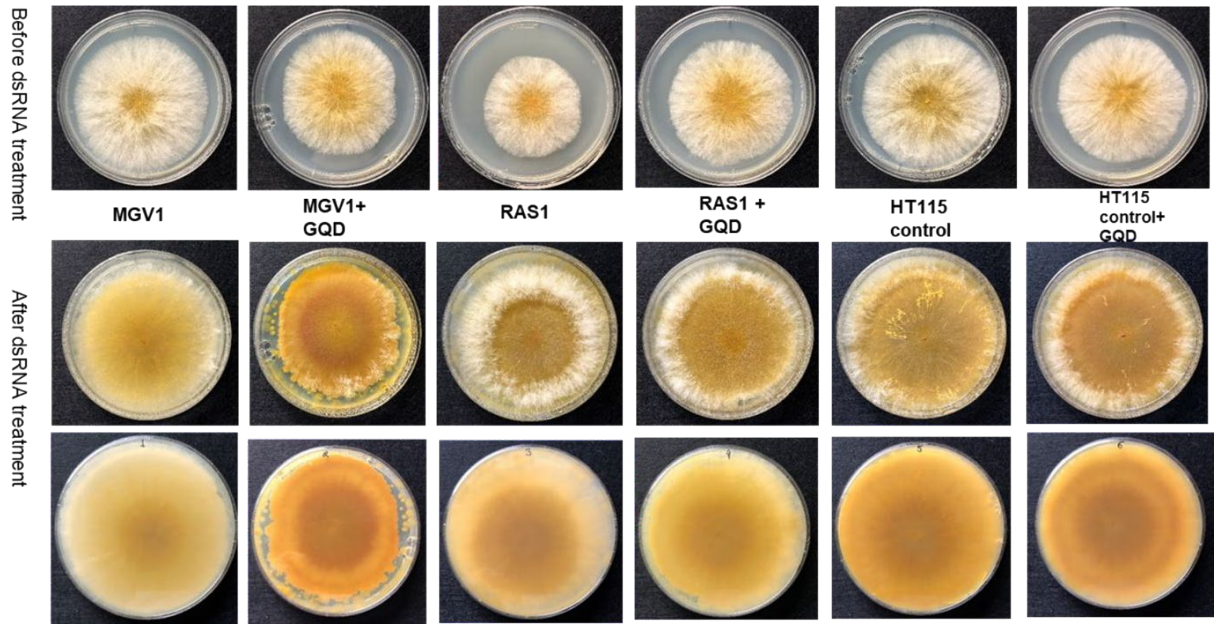
We have developed a protocol for the mixing of GQDs with dsRNAs along with the non-ionic surfactants silwet L-77, which allows successfully administering the dsRNA with functionalized GQDs onto plants using spray application using commercially available sprayer bottles.



Objective 2. Evaluate silencing of candidate genes in *F. graminearum* with siRNA oligos delivered by nanoparticles

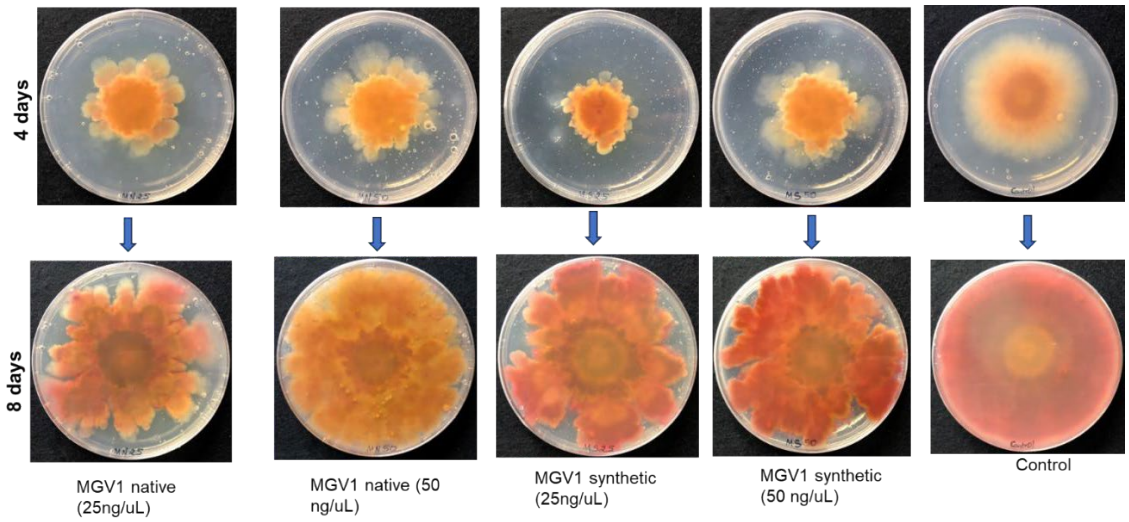
2a. Silencing of candidate genes with nanoparticle-coated siRNA oligos.

We designed an assay for the application of dsRNA rather than siRNA for the silencing of the Fusarium genes. For the confirmation of the effectiveness of the in-vivo produced dsRNAs while coating with GQDs, Fusarium were grown on PDA plates for four days. dsRNA for *MGV1* and *RAS1* (both native and synthetic dsRNAs) molecules were sprayed after four days. PDA plates were further incubated at 28 °C for four days. The resulting phenotypes were compared with the HT115 as well as water control.



Results show that the there is the inhibition of the mycelium growth in the plates while dsRNAs were mixed with the GQDs as compared to the control plates.

In the next step, Fungal mycelia were grown on potato dextrose agar (PDA) plates using the initial mycelium of 0.5 mm in length from the fresh plate culture. PDA plates were prepared by mixing two different concentrations (25 ng/uL as well as 50 ng/uL) of the respective dsRNA produced to see their effectiveness. The fusarium strain was incubated on potato dextrose agar (PDA) medium for 8 days (Lee et al. 2014). Results show that 25 ng/uL was effective as compared to the 50 ng/uL which also resonates with the previous studies. At higher concentrations of dsRNA, aggregation is likely to occur which decreases the silencing efficacy of formulations (Pierrat et al., 2015; Schwartz et al. 2020).



2b. Assays for siRNA delivery and effects of two or more siRNA oligos.

The co-PI JRX just received the backorder siRNAs materials. The work will be resumed immediately.

2c. Determine accumulative effects of simultaneously silencing of two or more genes.

The co-PI JRX just received the backorder siRNAs materials. The work will be resumed immediately.

Objective 3. Target gene prioritization, dsRNA design with cell-penetrating facilitation, and spraying and phenotyping FHB inoculated susceptible varieties by nanomaterial coated dsRNAs

3a. Prioritization of *Fg* genes, development and dsRNA design with cell-penetrating facilitation for applications on wheat.

The genes we selected for targeting in wheat-Fusarium interaction included *MGV1*, *RAS1*, *COT1*, *YCK1*, and *CAK1* genes that are important or essential for growth (Hou et al. 2002, Bluhm et al. 2007, Wang et al. 2011), *CID1* (Zhou et al., 2010), *Fgpal1* (Yin et al., 2020) *FgPp2A* & *FgPpg1* (Yu et al., 2014), and *FTL1* (Ding et al., 2009), that were all previously published by Xu laboratory. We started designing and producing dsRNA tools targeting five genes *MGV1*, *RAS1, COT1, YCK1 and FgPp2A.* To design RNAi constructs that could silence each target gene, we used the plant-specific dsRNA design pssRNAit server (Ahmed et al., 2020). A 500-700 bp fragment from the coding region was used for prediction of siRNA sites. Parameters were set to maximize the number of siRNA sites in 100-300 predicted region and minimize any off-target region. After identification of the most suitable region, we used the native stretch of DNA predicted pssRNAit to have greatest number of predicted siRNAs, and called it a

"native" segment. In addition, we used each of the predicted siRNA in tandem one after another in a hypothetical manner that does not exist in nature. We named this hypothetical tandem a "synthetic" segment. We then synthesized both native and synthetic segments for each gene by commercial DNA synthesis providers. For each of the Fusarium genes *MGV1* and *RAS1* and the wheat *phytoene desaturase* (*PDS*) gene we produced a native and a synthetic segment and cloned them in the double T7 promoter vector L4440 (Addgene 1654). The recombinant L4440 was transformed into the mutant strain HT115 (DE3) by a standard transformation procedure. This strain lacks RNase III, which is a dsRNA degradation enzyme. Therefore, the dsRNA produced in this bacterium will not be degraded. The L4440 also has T7 RNA polymerase-mediated transcription promoter which is induced by isopropyl β-d-1-thiogalactopyranoside (IPTG). For induction and large-scale production of dsRNA, the procedure developed by Lisa Timmons (Carnegie Institution of Washington), with some modifications based on Zhang et al (2019) and Ahn et al., (2019) was followed. Recent production resulted in a bulk of native and synthetic dsRNA with high yields.

3b. Treating FHB inoculated susceptible varieties by nanomaterial coated dsRNA design, and performing FHB phenotyping to characterize the efficacy of non-GMO gene silencing strategy.

We were able to develop the same strategy using a marker gene that is commonly used for the most silencing applications in wheat. Similar to the approach taken for *MGV1* and *RAS1*, we used phytoene desaturase (*PDS*) and designed native and synthetic constructs. We are also collaborating with co-PI RR group to test the efficacy of dsRNAs coated with graphene quantum dots (GQD) nanoparticles. A Graduate student in RR lab and Binod Gyawali in MM lab are working on the preparation of formulation for the graphene quantum dots (GQD). Various concentrations of GQD in response to the concentrations of produced dsRNA are yet be investigated. It will help to decipher the best combination of dsRNA with the GQDs for the phenotypic study.

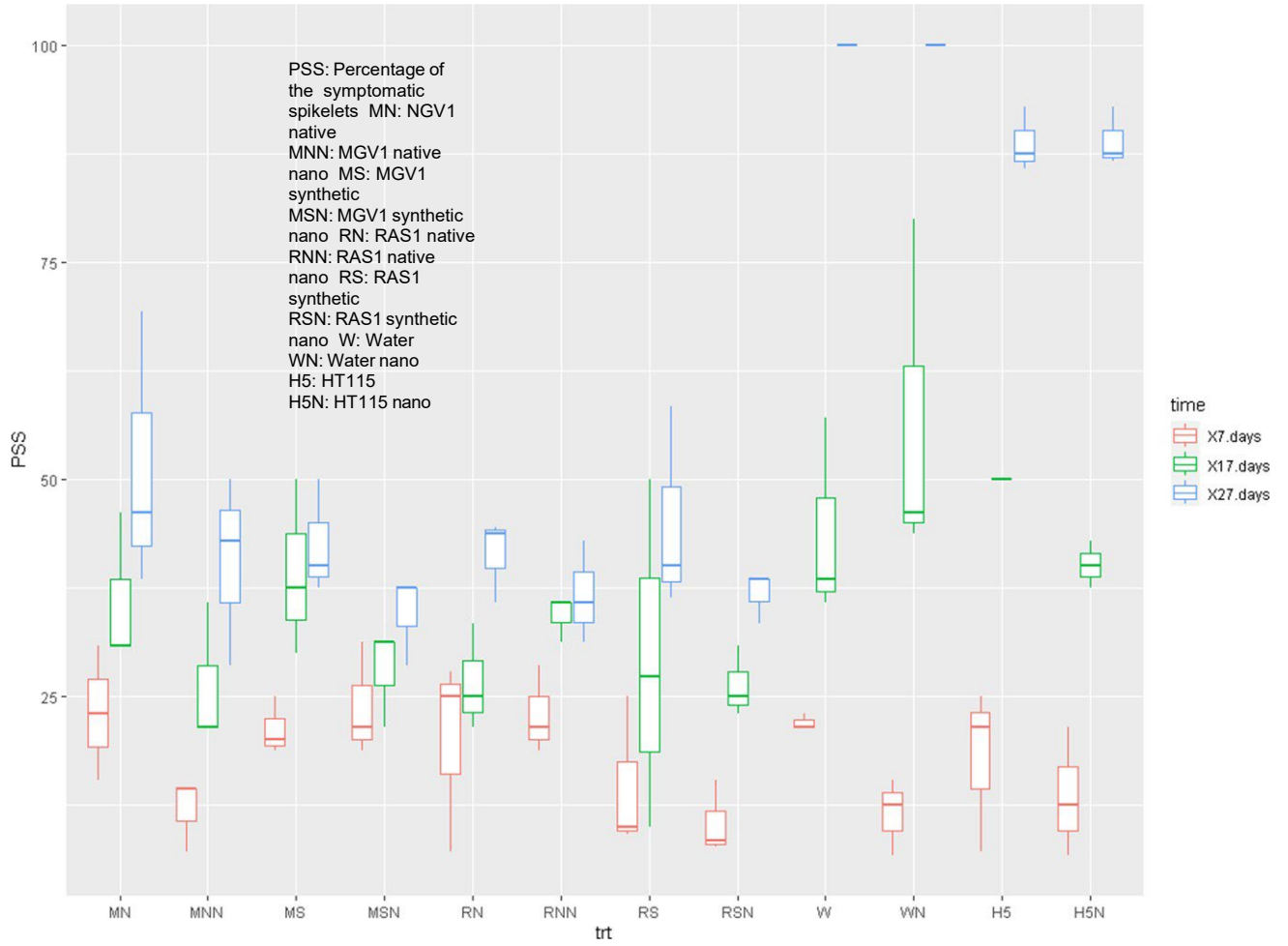
Inoculation of healthy plants with fusarium followed by dsRNA spray for the control of fusarium spread within the spikes

We also tested the effectiveness of the GQDs coated dsRNAs on the wheat heads.

Photos were taken 21 days after the first fusarium inoculation. Results show that *RAS1* dsRNAs-GQDs reduced the spread of fusarium in the wheat spikes as compared to control.

Experiments were done in the greenhouse under controlled conditions. We used two susceptible spring growth habit wheat varieties 'Gilat' (An Israeli accession) and 'AL105' (which is a line derived from the cross of parents 'Yecora' and 'Penny') to examine whether dsRNA-GQD conjugate application is able to control the disease establishment or spread. The field collected *F. graminearum* strains from Indiana was used for the preparation of inoculum. For greenhouse assay, spores were diluted to (5×10^4 spores/mL) (Lai et al. 2020).

The pathogenicity assay of Fg strain was carried out with wheat cultivars 'Gilat' and 'AL105'. Wheat spikes at flowering stages were inoculated with 10 µL droplet of macroconidia suspension (5×10^4 spores/mL) (Lai et al. 2020). The inoculated spikes were covered with sealed polyethylene bags for 3 days. Visually infected spikelets were scored at 7, 17 and 27 days after inoculation (dai). The percentages of infected spikelets were calculated as percentage of symptomatic spikelets (PSS) (Su et al. 2019). For each variable, two major treatments were used as naked dsRNA as well as nanomaterials coated dsRNA and will be used as plant spraying (Wang and Jin 2017; Koch et al. 2019). Each treatment includes a minimum of five replicates under all conditions.



Boxplot showing the percentage of symptomatic spikelets (y-axis) in response to the dsRNA treatment for two genes *MGV1* and *RAS1* having both native and synthetic fragments. Two treatments, dsRNA with or without GQDs were used in all the conditions. Data showed a reduction in FHB severity in GQDs-mediated dsRNA treatment compared to control treatments. The percentage of infected spikelets (PSS) were notably lower in GQDs-mediated dsRNA-treated plants, indicating effective suppression of *F. graminearum* pathogenicity.

b) What were the significant results?

Obj1. We are continuously working on the synthesis of the GQDs which were found effective as compared to Chitosan. The process has shown successful results and we will further continue the efforts on using this nanomaterial on plants. **Obj2**. We tested GQDs to coat dsRNAs for *F. graminearum* treatments. We have seen successful results during the spray of the dsRNA or making the plate culture mixing with the dsRNA as part of the media. **Obj3**. A plant-specific prediction server (pssRNAit - Ahmed et al., 2020) was used to design native and synthetic dsRNA silencing tools. Both native and synthetic tools were synthesized. Compared to the high cost of in-vitro transcription, we were able to source L4440 vector coupled with the HT115 mutant to produce large scale sRNA in-vivo for three genes i.e., wheat PDS and Fg *MGV1* and *RAS1.* We were also able to reduce the Fusarium spray on the wheat heads after the application of the naked as well as GQDs coated dsRNAs

c) List key outcomes or other achievements.

Obj1. RR team is continuously synthesizing GQDs, surface modification and verification as per the requirement of the MM group.
Obj2. Graphene Quantum Dots (GQDs) were found to be effective as compared to other nanoparticles. Results showed that RAS1-dsRNA and MGV1-dsRNA were able to reduce disease spread on plate culture while simultaneously silencing the respective genes.
Obj3. The production of dsRNA targeting *MGV1* and *RAS1* was optimized in mutant strait HT115 by using inducible double T7 promoter that will be used on Fg-inoculated susceptible wheat plants in the fall greenhouse season. Results showed that RAS1-dsRNA and MGV1-dsRNA were able to reduce disease spread on plate culture as well as on wheat spikes. However, RAS1-dsRNA-GQDs and MGV1-dsRNA-GQDs treatments could effectively restrict the spread of disease on wheat rachis.

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

Obj1. Devendra Sarnaik is a graduate student and Sachin Kadian was a post-doctoral associate working on the project (RR research group). Venkat Kasi, a graduate student recently started working on this project.
Obj2. A MS student, Penelope Vu, was trained in this project (JRZ research group).
Obj3. Binod Gyawali, a graduate student, is working on his dissertation thesis on Obj3. He is actively involved in dsRNA design, cloning, and production of dsRNA for testing (MM research group).

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

The team is working on publishing the recent results in a peer reviewed journal. Results will also be presented in the 2023 USWBSI annual meeting in Cincinnati Ohio.

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.

Gyawali B, Nepal N, Rahimi R, Xu J-R, and Mohammadi M. (2023). Application of *RAS1* and *MGV1* dsRNA with Graphene Quantum Dots Nanocarriers Reduces the Spread of *Fusarium graminearu*m in Wheat Rachis. Canadian Society of Plant Biologists. June 18-21, Quebec City, Canada.

Gyawali B, Nepal N, Vu P, Kadian S, Rahimi R, Xu JR, and Mohammadi M. (2022). Application of dsRNA coated with graphene quantum dots offers a promising strategy for silencing fungi and plant's genes. Proceedings of the 2022 National FHB Forum; Tampa, FL. Dec 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf

Gyawali B and Mohammadi M. (2022). Soft Red Winter Wheat Germplasm with Fhb7 Transferred from *Thinopyrum elongatum*. National Association of Plant Breeders Annual Meeting. August 8-11, Ames, Iowa. Poster Presentation.

Mohammadi M. (2022). NorGrains Genomic Selection Pipeline at Purdue & Undergraduate Engagement in Applied Research. National Association of Plant Breeders Annual Meeting. August 8-11, Ames, Iowa. Poster Presentation.

Steigenga N and Mohammadi M. (2022). Genomic Selection: Marker Set Optimization Improves Prediction Accuracy. Proceedings of the 2022 National FHB Forum; Tampa, FL. Dec 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf

Mohammadi M. (2022). NorGrains & BIG6 Genomic Selection Pipeline at Purdue Graduate & Undergraduate Education in Plant Breeding Research. Proceedings of the 2022 National FHB Forum; Tampa, FL. Dec 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf

Gyawali B and Mohammadi M. (2022). Releasing lines pyramided with Fhb1 and Fhb7. Proceedings of the 2022 National FHB Forum; Tampa, FL. Dec 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf