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

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-2-134
USDA-ARS Agreement Title:	Barley Exosome Cataloging by Proteomics and RNAseq
Principle Investigator (PI):	John McLaughlin
Institution:	Rutgers The State University of New Jersey
Institution UEI:	M1LVPE5GLSD9
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$72,675
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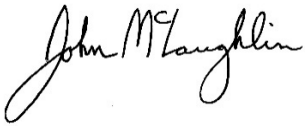
Period of Performance:	May 1, 2022 – April 30, 2024
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Exosome Mediated Protection against FHB	$72,675
	FY22 Total ARS Award Amount	$72,675

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.



07/26/2023

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: Exosome Mediated Protection against FHB

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

Host-induced gene silencing (HIGS) has been shown to offer protection against a wide range of pests and pathogens. The literature contains >100 examples of the use of HIGS targeting pathogenic viruses, insects, fungi, and nematodes. Although there is a wide range of effectiveness among these examples, it has become clear that understanding cross-kingdom communication between plants and pathogens can offer important tools for plant pathologists as they work to increase crop resistance to pathogens like *Fusarium graminearum*. One understudied aspect of cross-kingdom communication is the delivery of anti-pathogenic RNA and protein between the plant and pathogen. Plants and fungi produce exosomes that contain sRNAs and proteins that are thought to play a role in modulating plant-fungal interactions. However, it is not well understood if crops like wheat and barley uses exosomes to transfer extracellular proteins and sRNAs to *F.g*. and how those exosomes may impact fungal growth.

The aim of this research is to better understand if barley exosomes contain RNA and protein that impact *F.g*. To achieve this goal, we isolate apoplastic exosomes from mock inoculated and *F. g.* infected/trichothecene exposed barley seedlings and characterize the contents by high-throughput analysis of exosomal proteins and sRNAs. Global analysis of exosome cargo will provide a catalog of candidate genes that can be tested for their role in pathogenicity. This work will provide novel insights into how barley controls Fusarium infection and identify new proteins and sRNAs that can be used to improve resistance to Fusarium head blight (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?

We grow barley in the greenhouse to isolate leaf tissue for apoplastic fluid collection. Barley is treated to mock and F.g./DON treatment along a time course to permit barley response to fungi/trichothecene. We vacuum infuse the leaf tissue with vesicle isolation buffer and then perform low RPM spins to isolate apoplastic fluid. From that apoplastic fluid, we isolate exosomes using ultracentrifugation and density gradient purification for RNA and protein analysis.

b) What were the significant results?

We identified an Arabidopsis TET8 antibody, a marker for exosomes, that binds a barley TET8-orthologous protein isolated from our barley exosome total protein preparations. We used super resolution microscopy to image and quantify the exosome heterogeneity (~50-100 nM vesicles) from our isolated and purified samples. In addition, proteomic analysis (LC-MS/MS by

spectral count) revealed enrichment of specific exosome proteins, including the syntaxin, PENETRATION 1 (PEN1) in the purified apoplastic preparations compared to the raw exosome preparations (not purified via density gradient methods). Gene Ontology (GO) tools identified enrichment of proteins for vesicle mediated transport, endomembrane system, GTPase activity. Specific proteins of potential interest identified in the enriched exosome fraction included small Heat Shock Proteins, Annexins, GAPDH enzymes, and GSH enzymes. The apoplastic fluid has also been analyzed by proteomics. Barley leaf apoplastic fluid (48 hours post *F.g*. infection) becomes enriched with Pathogenesis-Related (PR) proteins: chitinases, nsLTPs, thaumatin-like proteins, Bowman–Birk-type trypsin inhibitors, among others. We are currently comparing the apoplastic fluid and exosome findings. RNA is also being compared between what is contained in the apoplastic vs the exosome.

c) List key outcomes or other achievements.

Our key achievement has been to obtain high quality apoplastic fluid for exosome isolation. This allows us excellent starting material for RNA and protein isolation (both apoplastic fluid and exosome analysis). Proteomics has revealed barley PR proteins which are induced by *F.g*. in both the apoplastic and exosome samples. We have also developed expertise in super resolution microscopy to visualize isolated exosomes via training from ONI using the Nanoimager. This provides a useful tool to analyze the heterogeneity of exosomes isolated from barley. RNA analysis has also revealed accumulation of long-noncoding RNAs in barley apoplastic fluid. We are in the process of comparing these RNA to what is found in exosomes.

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

During this research period I have mentored four undergraduates in the laboratory. One of the students, Ms. Silvia Juarez Rojas (Aresty program during the 2022 Spring Semester and the Koury Walker First-Generation Summer Research Scholarship), has worked with me to isolate barley exosomes using ultracentrifugation and downstream purification methods. She has learned how to isolate exosomes from barley, to grow Fusarium to produce spores, learned to count spores using flow cytometry, learned how to perform the detached leaf assay for fungal growth assays using F.g. tagged with GFP, and to quantify fungal grow using Image J/Fiji. This summer, Ms. Aysha Ponna, who is in the Project SUPER (Science for Undergraduates: a Program for Excellence in Research at Rutgers), is helping me to isolate and analyze apoplastic fluid from barley.

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

This work was presented at the 2022 National Fusarium Head Blight Forum and I will present an update at the upcoming 2023 FHB Forum in 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.

John E. McLaughlin and Nilgun E. Tumer. (2022). Measuring the Impact of Barley Apoplastic Exosomes on FHB. Proceedings of the National Fusarium Head Blight Forum; Tampa, FL. December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf

Sebastian Gallon, John E. McLaughlin, and Nilgun E. Tumer. (2022). Trichothecenes impact chloroplast protein homeostasis and stress responses. Proceedings of the National Fusarium Head Blight Forum; Tampa, FL. December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf