USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY20 Annual Performance Progress Report
Due date: August 31, 2021

Cover Page

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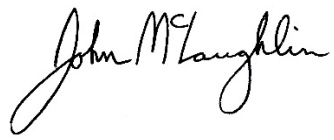
Phone:	848-932-6359
Fiscal Year:	2020
USDA-ARS Agreement ID:	59-0206-0-148
USDA-ARS Agreement Title:	Exosome Mediated Protection against FHB
FY20 USDA-ARS Award Amount:	$ 48,450
Recipient Organization:	Rutgers, The State University of New Jersey Division of Grant and Contract Accounting ASB 111, 3 Rutgers Plaza New Brunswick, NJ 08901·8559
DUNS Number:	00-191-2864
EIN:	22-6001086
Recipient Identifying Number or Account Number:	828314/127088
Project/Grant Reporting Period:	6/1/20 - 5/31/21
Reporting Period End Date:	5/31/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Exosome Mediated Protection against FHB	$ 48,450
	FY20 Total ARS Award Amount	**$ 48,450**



08/30/2021

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R- Research
S – Service (DON Testing Labs)
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Soft Winter Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: *Exosome Mediated Protection against FHB*

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

   Plant and fungal exosomes are a proposed to play a significant role in cross-kingdom communication which impact plant-fungal interactions. Evidence that exosomes deliver molecules that impact disease have been reported in the literature, mainly from the use of model species like Arabidopsis. This current research is investigating barley exosomes and their role in the barley-*Fusarium graminearum* interaction. The major goals include isolating barley exosomes free from contaminating debris using a combination of ultracentrifugation and density gradient fractionation from control and *F. graminearum* infected barley. The contents of the isolated barley exosomes are being cataloged with proteomics (spectral counting) and RNAseq. In addition, exosomes from barley will be tested against *F. graminearum* using *in vitro* assays.

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 have used both ultracentrifugation and tangential flow filtration to isolate exosomes from barley. Both methods have shown that we can isolate exosomes not only from apoplastic fluid and ground barley leaf tissue, but also from the remaining vesicle isolation buffer used to infuse barley leave tissue. This is significant because different classes of exosomes may be discovered using these methods. Using density gradient fractionation, we have purified plant exosomes from debris and are in the process of cataloging the contents using proteomics with expertise from the Center for Advanced Proteomics Research (CAPR) here at Rutgers. The next step is to prepare sRNA from exosome samples for RNAseq analysis.

   b) What were the significant results?

   We screened several exosome-specific antibodies and found that antibodies raised against the Arabidopsis protein tetraspanin-8 (gift from Hailing Jin, University of California, Riverside) associated with isolated barley exosome proteins using Western analysis. We have also characterized the exosomes using super resolution microscopy (NanoImager from ONI). Following exosome movement in buffer allows measurement of exosome size characteristics using nanoparticle tracking analysis (NTA) which is based on the mathematics associated with the Brownian motion of single particles in solution.

c) List key outcomes or other achievements.

Exosomes stained with the fluorescent lipophlic dye DiOC6 can be visualized in the resulting OptiPrep density gradient, giving higher confidence in the isolation procedure.

Exosome contents are being cataloged and this information will be presented at the USWBSI national meeting in December.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

The research was impacted somewhat by delays in obtaining certain supplies. This is less of a problem now.

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

This grant has provide undergraduate training opportunities for two students at Rutgers University. One student, Silvia Rojas is in the Aresty Research Assistant (RA) Program (https://aresty.rutgers.edu/programs/research-assistant-program). The other student, Vivyan Abdo, is working on the project for research credits.

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

The results will be presented as a poster at the USWBSI national meeting in December.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (6/1/20 - 5/31/21). The term "support" below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student's stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY19 award period?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? Click to enter number here.

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY19 award period?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? Click to enter number here.

3. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? Click to enter number here.

4. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? Click to enter number here.

Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY20 award period (6/1/20 - 5/31/21). All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

NOTE: Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.

Name of Germplasm/Cultivar	Grain Class	FHB Resistance	FHB Rating (0-9)	Year Released
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
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Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Publications, Conference Papers, and Presentations

Instructions: Refer to the PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (6/1/20 - 5/31/21**) should be included. If you did not publish/submit or present anything, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each citation, you **must** indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in the publication/ presentation. See example below for a poster presentation with an abstract:

Z.J. Winn, R. Acharya, J. Lyerly, G. Brown-Guedira, C. Cowger, C. Griffey, J. Fitzgerald, R.E. Mason and J.P. Murphy. 2020. "Mapping of Fusarium Head Blight Resistance in NC13-20076 Soft Red Winter Wheat." In: S. Canty, A. Hoffstetter, and R. Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum (*p. 12.), Virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report