Project FY22-GD-013: Exosome Mediated Protection against FHB

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

The **primary goal** of this research project was **to isolate exosomes from both mock-inoculated and *Fusarium graminearum* (F.g.) infected barley seedlings, and then characterize their contents through high-throughput analysis of exosomal proteins and small RNAs (sRNAs)**. This work aimed to:

- **Provide a catalog of candidate proteins and genes** that could be quickly tested for their role in pathogenicity.
- **Offer novel insights into how barley responded to and impacted *Fusarium* infection**.
- **Identify new proteins and sRNAs that could be used to improve resistance to FHB**.

This project directly addressed **research priority 3 of the GDER**, which emphasized the **utilization of new technologies to develop effective FHB resistance and/or reduce deoxynivalenol (DON) contamination**.

The **specific objectives** of the project were:

- **To isolate high-quality exosome preparations** from mock-inoculated and *F. graminearum*-inoculated barley seedlings, and **to determine if these exosomes possessed antifungal activity**.
- **To use proteomics tools to characterize the exosomal proteins** present in both mock-inoculated and *F. graminearum*-infected barley seedlings.
- **To characterize small RNAs in exosomes** isolated from mock-inoculated and *F. graminearum*-inoculated barley seedlings using **small RNA sequencing (sRNA-Seq)**. This objective also included integrating the genomics dataset with the proteomics data to functionally identify the key regulatory modules involved in *F. graminearum* defense.

2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address these three items below.)*

What were the major activities?

Apoplastic fluid from both barley leaf and floral tissue, following a time course schedule were isolated for exosome purification via differential centrifugation and density gradient (Optiprep) purification. Proteomic analysis of both leaf and floral tissue was successfully performed. RNA work is ongoing.

What were the significant results?

Identified proteins (*F.g* infected vs mock) isolated from exosomes, as determined by quantitative proteomic analysis using the programs Normalyzer and OmiLoupe, included:

1. Germin-like proteins (GLPs), which have a strong correlation to plant stress responses and disease resistance, and have been shown to be induced by pathogens like leaf blast in rice, with transgenic rice overexpressing GLP showing increased resistance.

2. Dirigent proteins (DIRs), known for their role in strengthening and synthesizing lignin, and increasing resistance to powdery mildew in wheat and barley, were also upregulated in barley exosomes in *F.g.* treated plants vs mock.
3. Other identified proteins included several sterol carrier proteins (SCP) domain-containing protein, chymotrypsin inhibitor-2, RGH1 (a gene involved in powdery mildew resistance in barley), peptidylprolyl isomerase (involved in stress defense and protein folding), and basic secretory proteases.
4. Laccase enzymes were substantially more abundant in exosomes of floral tissue infected with *F.g.* compared to the mock control.
5. Numerous uncharacterized proteins were identified in higher abundance in barley exosomes due to *F.g.* treatment.

These results indicate that the contents of barley exosomes do significantly change in response to *F.g.* infection and those proteins may impact disease progression.

List key outcomes or other achievements.

High school, undergraduate, and graduate students were trained during the period funded by this grant. One MS degree was earned due to this grant. Using the skills and information gained from this grant, a Busch Biomedical Grant was applied for: Optimizing the Chromium X Platform to Resolve RNA Complexities of Exosomes in Plant and Microbial Models with Biomedical Impacts. The goal is to use single cell genomic methods to characterize RNA content on a single exosome level to better define exosome heterogeneity.

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

This grant provided training for the following students:
Henry Cantor (high school student in Liberty Science Center Partners in Science summer program), Aresty Research Center undergraduate students Liam Cosgrove, Gabriella Miliano, and Preya Rumit Shah, and a graduate student, Sam Mellow who earned his Master of Science degree. Thesis title: Investigating the Role of Heat Shock Proteins and Extracellular Vesicles in Plant Resistance to Fusarium Head Blight. Sam was a member of the Graduate Program in Cell and Developmental Biology at Rutgers University and Dr. McLaughlin served as his thesis advisor.

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

Yes, the results have been presented at the GDER and PBG Joint Mid-year Meeting (2024) and during MS Thesis presentation at Rutgers University. A manuscript of the results is being prepared for peer review.