Project 1: Exosome Mediated Protection against FHB

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

The overall goal of our work is to discover plant genes and pathways that are impacted by *Fusarium graminearum* and trichothecenes to both understand and engineer enhanced resistance to FHB. In this research project we have focused on two areas:

Plants and fungi produce exosomes that contain sRNAs and proteins that play a role in modulating plant-fungal interactions. However, it is not well understood if barley uses exosomes to transfer extracellular proteins and sRNAs to *F. graminearum* and how those exosomes my impact fungal growth. The primary goal of this research is to isolate leaf apoplastic fluid and exosomes from mock inoculated and *F. g.* infected barley seedlings and characterize the contents by high-throughput analysis of exosome proteins and sRNAs. Global analysis of exosome cargo has provided a catalog of candidate proteins that can be later tested for their role in pathogenicity. This work has provided 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).

We are also investigating the impact of trichothecenes on the chloroplast. Previously we have shown that trichothecenes induce the chloroplast unfolded protein response in *Chlamydomonas* which leads to a cascade response that repairs damaged chloroplasts, including the induction of VIPP1/2 and HSP22E/F. We are interested in translating that research to higher plants. Work involving *Arabidopsis* and wheat is underway to explore the impact of trichothecenes on chloroplast stress (chloroplast-related heat shock protein induction).

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

What were the major activities?

Isolation and characterization of apoplast fluid from barley leaves and exosomes from that purified apoplast fluid. Working to isolate exosomes/other small vesicles from barley floral tissue.

What were the significant results?

We achieved high quality apoplastic fluid and exosome isolation from barley leaves (mock vs *F.g.* treated) using methods modified from the literature.

We identified specific heat shock proteins which are upregulated by DON when infused into wheat tissue. One specific wheat gene is an ortholog to the HSP that is upregulated in response to chloroplast stress in *Chlamydomonas*. We are looking at time course/DON dose exposure gene expression data to better understand this

process and the potential use of this gene to protect small grain cereals from DON-associated damage.

List key outcomes or other achievements.

Barley apoplast fluid proteome shows increase in antifungal proteins post *F.g.* infection. Using vacuum infused barley floral tissue, we successfully isolated apoplastic fluid from entire barley heads (floral tissue). The next step is to isolate and analyze exosomes/vesicles from this material.

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

A master's student, Mr. Sam Mellow, is working on this project. We have also involved several student undergraduate researchers on this project, including Ms. Aysha Ponna and Ms. Silvia Rojas Juarez. One high school student, Henry Cantor who is associated with the Liberty Science Center summer program, has photographed chloroplast damage due to DON using confocal microscopy.

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

We presented a poster of these results at the 2023 National Fusarium Head Blight Forum.

5. What do you plan to do during the next reporting period to accomplish the goals and objectives?

We are also attempting to isolate exosomes from barley floral tissue to compare the results to the leaf exosomes results. This information will be added to the manuscript that we are writing for publication.