Project Abstract

Project Title:	Exosome Mediated Protection against FHB	
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Understanding plant-fungal interactions in the wheat/barley-*Fusarium graminearum* pathosystem has the potential to identify novel mechanisms of resistance. Accumulating evidence suggests that functional molecules packaged into exosomes play a role in cell-to-cell and cross-kingdom interactions. Exosomes are a class of extracellular vesicles (EVs) which have been shown to contain a wide variety of molecules, including sRNAs and proteins. In animals, exosomes have been shown to act as vehicles for delivering RNA and modulating cell protein production. In plant-fungal interactions, bidirectional sRNA delivery has been shown to play a role in altering immune responses. Recent breakthroughs show that plants secrete exosome-like EVs that enable the transfer of sRNAs through the fungal pathogen cell wall to silence fungal genes that are critical for pathogenicity.

Barley produces sRNAs and proteins that play important roles in modulating the barley-*F. graminearum* interaction. However, it is not known whether barley uses exosome-like vesicles to transfer extracellular proteins and sRNAs into *F. graminearum*. One of the essential research areas in this field is the characterization of exosomal cargo which includes proteins and nucleic acids. Barley exosomes were found to be associated with powdery mildew fungus in infected leaves, but their cargo has not been characterized. In our laboratory, we have isolated exosomes from barley leaves and spikes at flowering using both ultracentrifugation and tangential flow filtration. These crude EV preparations were subjected to further purification using a discontinuous iodixanol (Optiprep) density gradient but substantial contamination remained.

Our updated specific objectives for this continuing project are:

1) Use of specific protease and RNase protection treatments to remove protein and RNA contamination from exosome preparations isolated from mock inoculated, trichothecene treated, and *F. graminearum* inoculated barley seedlings.
2) Characterize exosomal sRNAs and proteins from control and treated (trichothecene and fungal spore application) barley seedlings using RNAseq/proteomics tools.
3) Develop fluorescently labeled antibodies which recognize exosome-marker proteins such as Tet8 for high quality imaging and tracking of barley exosomes.

This project addresses the following FY 22 research priority of GDER: Priority 1) Identify native and induced wheat and barley gene variants that improve FHB resistance and/or reduce DON accumulation. 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).