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PROJECT 1 ABSTRACT
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Exosomes are extracellular vesicles (EVs) that play a pivotal role in cell-to-cell communication and host-pathogen interactions. They have emerged as vehicles for delivering RNA and modulating recipient cell protein production in animals. Despite the promising use of exosomes in mammalian systems, exosome-mediated delivery of small RNAs (sRNAs) and proteins has not been explored against *Fusarium graminearum* in barley or in wheat. Recent breakthroughs indicated that plants secrete exosome-like EVs to transfer sRNAs into fungal pathogens to silence fungal genes that are critical for pathogenicity, suggesting that plants have adapted exosome-mediated delivery of antifungal RNAs as part of their immune response against fungi.

Barley produces sRNAs that likely 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 characterization of exosomal cargo which includes non-random proteins and nucleic acids. Exosomes were found to be associated with powdery mildew fungus in barley leaves, but their cargo has not been characterized. The primary goal of this application is to isolate exosomes from mock inoculated and *F. graminearum* infected 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 quickly 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). Our specific objectives are:

1) Isolate high-quality exosome preparations from mock inoculated and *F. graminearum* inoculated barley seedlings.
2) Use proteomics tools to characterize exosomal proteins in mock inoculated and *F. graminearum* inoculated barley seedlings.
3) Characterize small RNAs in exosomes isolated from mock inoculated and *F. graminearum* inoculated barley seedlings using small RNA sequencing (sRNA-Seq).

Exosome-based delivery of plant proteins and RNAs that target *F. graminearum* genes can serve as new generation of fungicides against FHB and could circumvent the technical limitation of transformation and the public concern against GMOs. This project addresses the following FY 20-21 research priority of GDER: Priority 3) Utilize new technologies to develop effective FHB resistance and/or reduced DON contamination.