PI: Phil Bregitzer **PI's E-mail: Phil.Bregitzer@ARS.USDA.GOV**

Project ID: FY16-BA-005 **ARS Agreement #: N/A**

Research Category: **BAR-CP** **Duration of Award: 1 Year**

Project Title: Small RNAs in Response to RNAi Down Regulation in *F. graminearum*.

PROJECT 1 ABSTRACT

(1 Page Limit)

Host Induced Gene Silencing (HIGS) has great potential for introducing FHB resistance into commercial-quality plants. Designing vectors for efficient HIGS requires investigating fundamental questions of steps in the process. Previous works describe the capacity for HIGS to regulate key genes, effect pathogens, and even the potential for pyramiding multiple genes. HIGS can be induced by expressing inverted repeats (IRs) that are homologous to the targeted gene, and which produce double-stranded (ds) RNA that is processed into various small RNA (sRNA) species. Exactly what species are produced, and in what quantity, is critical. However, data on dsRNA processing of IRs targeting fungal genes is scarce. No data are available that examine the effect of IR length or on the order of IRs in pyramided loci on sRNA generation

To identify and characterize useful HIGS construct components for barley transformation, this research will model construct efficacy via transformation of *Fusarium graminearum.* Multiple constructs containing single and pyramided IRs of various lengths and in various orders, and which target various positions of targeted genes, will be assembled. The construct backbone will support insertion into specific genomic locations via homologous recombination to allow for direct and highly controlled comparisons of IR effects. Targets will include *TRI5*, *TRI6*, and *LAEA*, genes that affect mycotoxin production. Two other candidate fungal pathogenicity genes with easily quantifiable phenotypes will be investigated. Examining the silencing and sRNA profile resulting from IRs targeting these genes will facilitate understanding how to apply HIGS against FHB. Constructs varying for order, composition, and spacers between IRs will be evaluated. Transgenic strains with contrasting levels of target gene(s) suppression (as determined by qRT-PCR) will be selected for RNAseq analysis to determine the sRNA profile in response to IR expression.

Our objectives are:

1. Construct and transform *Fg* with fungal vectors with variable IRs and different HR sites, targeting single genes as well as multiple genes.
2. Characterize transformants for their sRNA profile resulting from IR expression.
3. Explore novel candidate pathogenicity factors for FHB and for pyramiding IR traits.

Constructing vectors, transforming, and phenotyping *Fg* will be accomplished FY16. In FY17 we will investigate novel genes against FHB and explore pyramiding traits. Understanding the mechanisms governing RNAi will facilitate developing useful FHB resistance in barley. Both growers and consumers will benefit from the full realization of genetic engineering for commercial needs, e.g. engineering FHB resistance in barley, to produce safer and better barley.