

PI: Guihua Bai**PI's E-mail:** guihua.bai@usda.gov**Project ID:** FY20-BA-031**ARS Agreement #:** N/A**Research Category:** GDER**Duration of Award:** 1 Year**Project Title:** Function Analysis of *Fhb1* using BSMV-mediated CRISPR/Cas9 Gene Editing System**PROJECT 2 ABSTRACT**

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Fusarium head blight (FHB) is a destructive wheat disease worldwide. Growing FHB resistant cultivars is one of the most effective approach for the disease control. Among the quantitative trait loci (QTLs) for FHB resistance reported to date, *Fhb1* is the only one showing a consistent major effect on FHB resistance in different genetic backgrounds and environments. Although *Fhb1* has been cloned recently, it remains unsettled if *Fhb1* resistance is determined by a pore-forming toxin (*TaPFT*) or a putative histidine-rich calcium binding protein (*TaHRC*), and gain- or loss-of-function of *TaHRC* if it is *TaHRC*. Further determination of *Fhb1* identity and its action mode is essential for breeders to select right strategy to effectively use *Fhb1* in improving FHB resistance. The objectives of this proposal are to determine if 1) gain-of-function mutation in *TaHRC* contributes to *Fhb1* resistance, 2) *TaPFT* contributes to *Fhb1* resistance, 3) any interaction between *TaHRC* and *TaPFT*, and 4) new genes for other *TaHRC* interacting proteins in the downstream of *Fhb1* regulatory network may contribute to *Fhb1* resistance. We will use BSMV-mediated CRISPR/Cas9 gene editing system to knock out *TaHRC-R* and *TaPFT* separately in the wheat line, Ning7840 that carries both genes. We will cross the Cas9-overexpressed (Cas9-OE) 'Bobwhite' that developed from our previous GDER project to 'Ning7840' to obtain the Cas9-OE 'Ning7840' lines and independently edit *TaHRC* and *TaPFT* in these lines. The protein-protein interactions between *TaHRC* and *TaPFT*, and between *TaHRC* and other proteins will be investigated by screening wheat cDNA library using Yeast Two-hybrid (Y2H) system. In previous studies, we cloned *TaHRC* and validated loss-of-function mutation of *TaHRC* reduced FHB severity. We also successfully developed a BSMV-mediated CRISPR/Cas9 gene editing system and successfully used the system to knock out the *TaHRC-S* allele in Cas9-OE 'Everest' lines in the previous GDER project. Successful completion of this project will settle the dispute on the gene for *Fhb1* and its resistance mechanisms and provide breeders with the new strategies for how to use *Fhb1* in developing new FHB resistant wheat cultivars and new FHB resistance genes, which eventually will benefit wheat farmers.