Project FY22-GD-008: Develop a New Transgene Free Editing System for Gene Function Validation and Breeding

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

The major goals of the project are to develop an efficient nanoparticle-mediated gene delivery system for genome editing in wheat, and to use gene editing system to improve FHB resistance and mitigate mycotoxin accumulation in wheat.

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

What were the major activities?

- Fine mapping defined a QTL for FHB resistance in 4BS interval where 16 high confidence candidate genes are annotated. Among the candidate genes, *Rht1* and *5-alpha* were selected based on RNA-Seq data as candidates for function validation by gene editing. The Knockout mutants of both genes were generated and *Rht1* mutants was evaluated for FHB resistance in one greenhouse experiment.
- To validate the *Fhb7*'s function, near-isogenic lines, *Fhb7*-overexpressing lines and *Fhb7*-gene-edited lines were evaluated for FHB resistance.
- NP-mediated CRISPR/Cas editing for TaHRC was conducted using the regrown seedlings by treating 1200 embryos of imbibed dry 'Bobwhite' seeds with Cas9 and TaHRC gRNA for protocol optimization, and the derived plants were screened for mutation using the high-throughput NGS-based mutant screening protocol.
- Sequenced 9 previous identified putative M1 plants from last cycle of screening.

What were the significant results?

- Phenotyping of *Fhb7* near-isogenic lines, overexpressing lines and gene-edited lines in repeated greenhouse experiments confirmed that *Fhb7* contributes major gene resistance to FHB and glutathione S-transferase (*GST*) is the causal gene for *Fhb7*.
- One season greenhouse data showed that plants that knocked out of *Rht1* dwarf allele showed increased plant height and FHB susceptibility, suggesting *Rht1* most likely contributes to FHB resistance to 4BS QTL.

List key outcomes or other achievements.

Confirmed the function of *GST* as the causal gene Fhb7 and settled the discrepancy on *Fhb7*'s functions on FHB resistance, which will facilitate further deployment of Fhb7 in US wheat cultivars.

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

One MS student (Ms. Julia Eilert), three post-doc (Dr. Yujiao Gao, Lanfei Zhao and Ruolin Bian) and one ARS scientist (Dr. Hongliang Wang) were trained in the projects during the past year.

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

The *Fhb7* function validation work has been published in Phytopathology journal recently.

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

- To determine the causal gene responsible for 4BS FHB resistance QTL, we will repeat phenotyping of edited *Rht-1* mutant lines for FHB resistance and start phenotyping of gene-edited mutants of 5-alpha gene for FHB resistance in greenhouses.
- Reexamine the NP-mediated CRISPR/Cas editing protocol using GFP to track the progress Cas-9 in the tissue.
- Increase the NP treatment temperature to 37 C to increase the editing efficiency because it was reported that 37 C increased editing efficiency.