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 e4icient nanoparticle-mediated gene delivery system for genome editing in wheat, and to use gene editing system to validate gene function on FHB resistance 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?
   - Conduct fine mapping of the QTL in 4BS
   - Conduct RNA-seq analysis
   - Conduct gene editing of two candidate genes in the QTL region
   - Clone the third candidates

   What were the significant results?
   - Finely mapped a major QTL for FHB resistance to ~1 Mb region on 4BS using a population with a Jagger mutant as a parent.
   - RNA-seq identified 3 candidate genes for FHB resistance in the finely mapped interval using
   - Gene editing knocked out two candidate genes in Fielder and one gene was phenotyped for one cycle in greenhouse to evaluate its functions on FHB resistance.
   - Cloned the third gene and it will be edited to evaluate its function.

   List key outcomes or other achievements.
   Identified the candidate genes for the QTL in 4BS using fine mapping and RNA-seq.

3. **What opportunities for training and professional development has the project provided?**
   Post-doc R Bian was trained for fine-mapping, RNA-seq and data analysis.

4. **How have the results been disseminated to communities of interest?**
   Once the final candidate is determined, the result will be released as a publication.

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?**
   2024-2025 Spring:
   1. Evaluate Rht1-knockout plants for FHB resistance and agronomic traits in the greenhouse conditions to confirm its function on those traits.
   2. Sequence to confirm the edited plants with sequence mutations in the second candidate gene and start first cycle of phenotyping for FHB resistance and plant height.
   3. Prepare construct and edit the third candidate gene.

2025 fall-2026 Spring:

1. Identify and confirm edited plants for third candidate gene and conduct phenotyping for FHB resistance to determine it is function on FHB resistance
2. Once the causal gene for the 4BS QTL is determined, it will be used to identify interacting genes using the yeast-two-hybrid system.
3. The causal sequence variation will be identified in the causal gene and markers will be designed and validated in a diversity panel to develop diagnostic markers for selecting the QTL in breeding.