Project FY22-GD-015: Manipulating a Conserved Susceptibility Factor for Developing FHB Resistant Wheat

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

Genetic resistance is the most sustainable approach for managing Fusarium Head Blight (FHB) in wheat and barley. As most of the resistance genes are sourced from un-adapted germplasm, linkage drag and variable penetrance in different genetic backgrounds limit their deployment in cultivars. The goal of this project is to characterize and manipulate a conserved susceptibility factor present in wheat cultivars for enhancing their FHB resistance. The project builds-up on three-years of work done by PI Rawat (Chhabra et al. 2021), which mapped a susceptibility factor in the peri-centromeric region of wheat chromosome 7AS. In this project, we will fine-map and isolate the susceptibility factor using Radiation Hybrid (RH) mapping. Collaborator Vijay Tiwari is an expert on RH mapping and will be vital in the SF-7AS fine mapping. After fine mapping we will perform VIGS, TILLING, and genome editing to validate the gene function and utilize the loss-of-function variants to enhance the FHB resistance in wheat cultivars. In the fourth year, PI will test the lines in field conditions for yield evaluation and FHB resistance.

Specific objectives of this project over a 4-year period are:

1. Fine mapping and isolation of 7AS susceptibility factor using RH mapping.
2. Validation of candidate genes using TILLING and Genome-editing.
3. Transfer of FHB resistant variants in wheat cultivars
4. Field Testing of the variant lines for yield evaluation.

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

What were the major activities?

Chinese Spring radiation panels were developed using 35kRad gamma radiation. As the deletion of the susceptibility factor is expected to provide resistance in homozygous condition, generation advancement was done for the irradiation panel. Considering R7 generation to have majority of deletions in homozygous state, we have been able to advance the population upto R6 generation so far. R6 generation with a total of 700 individuals has been developed. Work on characterizing the deletions in the targeted interval progress using genome-specific markers from Chhabra et al. and using GBS has been done. Phenotyping of the whole population at R6 generation was performed with 3 spikes per plant data points. We obtained ~20 lines that showed resistance response. Molecular analysis of the lines is being conducted to characterize the status of deletions in these lines. The deletions were still not in a homozygous state, so we advanced the generation and retrieved R7 plants. Phenotyping of homozygous deletions is in progress. Additionally, a panel of 700 irradiated lines was also developed in the HRW cultivar Jagger. The panel has been sequenced using GBS and is being currently analyzed for deletions in the chromosome 7A target interval. Phenotyping of the Jagger panel provided us with resistant deletion lines. Determination of homozygous events in our region of interest is being done.

We created a Radiation Hybrid panel using irradiated pollen from the Chinese Spring crossed with the Chinese Spring Deletion line 4. Genotyping of this panel identified some deletions in the region of interest. Phenotyping of these lines is in progress.

Additionally, we attempted conducting CRSIPR-Cas9 mediated gene editing to create homozygous deletions in the targetd regions. We have developed 11 CRISPR gene edits (6 in Chinese Spring and 5 in Bobwhite), and currently are genotyping the events to check for the presence of edits.

What were the significant results?

Development of populations and genotyping-based sequencing of the panels is the most significant result. Extensive phenotyping of the panels, identifying some resistant lines is also significant toward the progress of the project.

List key outcomes or other achievements.

Key outcomes include: Development of irradiated panels, and generation advancement up to R6, Phenotyping of the plants at this generation, as well as GBS of the deletion panel in Chinese spring and Jagger. Development of RH population and its genotyping was accomplished. This will allow us to narrow down the region.

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

The project has enabled the training of one PhD student and a Postdoctoral Researcher, as well as two undergraduate students and three high school students.

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

The results have been disseminated via oral and poster presentations at National FHB forum, Mid-Atlantic ASPB meeting, and University level presentations by the graduate student and PostDoc involved in the project.

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

The planned activities include: Robust phenotyping of the selected deletion lines from the Chinese Spring irradiated panel, 2- Genotyping of Jagger selected resistant lines for deletions, 3- Phenotyping of the Radiation Hybrid panel, 4- Genotyping of CRISPR-Cas edits to fine map the susceptibility gene region, 5-Phenotyping of the edited events in the region.