Project FY22-SP-009: Leveraging the Pangenome to Investigate Genetic Background Effects on the Fhb1 Locus

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

1) *Introgress a minimal Sumai 3 Fhb1 segment in elite germplasm.*
2) *Develop mapping populations to evaluate specific background effects.*
3) *Evaluate background gene expression effects on Fhb1.*

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

Objective 1:

What were the major activities?
Initial crosses to isolate FHB1 (from ND2710) in adapted germplasm (NDHRS13-0273, MT1621, MN15005-4) not carrying FHb1 occurred in Spring 2022, and BC_1 progeny were generated in Fall 2022. FHB1 heterozygous lines were crossed again in the Spring of 2023, generating BC_2. Crossing again occurred in the Fall of 2023 where 23 lines were FHB1 positive in the heterozygous state, generating BC_3. In the Spring of 2023, Lines were grown, and selfed with 129 being FHB1 positive. The originally-submitted adapted parent from SDSU ended up being carrier of FHb1, so non-carrier SD5103 will enter the crossing scheme 1 year behind the others with Initial F_1 generation in Spring 2023.

What were the significant results?
FHB1 genotyping works well to rapidly identify the lines that maintain heterozygous state after crossing and selfing.

List key outcomes or other achievements.
Backcrossing and selfing is progressing as expected with the expected segregation ratios.

Objective 2:

What were the major activities?
In Spring 2023, Vida was crossed to ND744, SD4343, and Lang to establish mapping populations that segregate for specific linkage blocks previously derived with Sumai3. In the Spring of 2024, these were selfed to generate F2s.

What were the significant results?
F1 lines were healthy and generated more than 20 seed per spike. There will be plenty of F2s to screen for further population development.

List key outcomes or other achievements.
NA

Objective 3:

What were the major activities?
In the Fall of 2023, a FHB greenhouse was established with the purpose of collecting RNA before and after inoculation. Eight HRSW lines were included, and spikelets were collected at 0 dpi, 5 dpi and 5 dpi of mock inoculated spikes. RNA from about half of the tissues was extracted and sent for sequencing. RNASeq reads from the Fall 2022 experiment were mapped from the to the Sumai3 genome and differentially expressed genes were identified.

What were the significant results?
So far RNA seq data from over 200 samples have been sequenced. Initial mapping of reads on the Sumai3 genome have identified ~30 differentially expressed genes that were implicated in a resistance response when the pathogen was applied. These will be validated in the next performance cycle with the new annotations.

List key outcomes or other achievements.
NA

3. What opportunities for training and professional development has the project provided?
The two students that are assisting in this project have learned several important skills in data science and bioinformatics in one-on-one training by the mentor.

4. How have the results been disseminated to communities of interest?
Through personal conversations and poster presentations at conferences

5. What do you plan to do during the next reporting period to accomplish the goals and objectives?
The three genomes will be published to graingenes along with their annotations under the Toronto agreement while the publication is being written. I expect it to be complete within the next year. The last greenhouse season will be conducted in the Fall of 2024 in the exact same manner as the 2023 season. Integration of multiple seasons helps reduce false positive associations.