Project FY22-SW-007: Genotyping Breeding Lines for FHB Resistance - Southern
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1.   What are the major goals and objectives of the research project?

This proposal supports collaborative genomics-assisted selection efforts by the Southern soft winter wheat programs. This project addresses the need to provide breeders with information about the genetic basis of resistance to FHB and other traits in locally adapted and newly introduced germplasm and to improve their ability to select for FHB resistance in cultivars using new breeding approaches. Specific objectives of this continuing research are: (1) to characterize entries in the Northern scab screening nurseries with markers linked to FHB QTL; (2) to characterize entries with diagnostic markers for genes having major effects on plant development and genes conferring resistance to other pests; (3) to implement genome-wide markers analyses to identify QTL associated with FHB resistance and perform genomic selection (GS); (4) to investigate new methodology for collecting genome-wide marker data; and (5) collaborate with complimentary projects to implement genomic selection in eastern soft winter wheat breeding programs.

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

What were the major activities?

In support of Objectives 1, 2 & 4, a pool of primers for amplicon sequencing targeting markers linked to FHB resistance QTL from Asian sources (Fhb1, QTL on chromosome 5A and 2D) as well as six QTL identified from soft red winter wheat sources Bess, NC-Neuse and Jamestown, was used to evaluate eastern germplasm. Markers linked to genes for plant growth and development, disease resistance genes, and quality traits are also included in the primer panel. Genomic DNA was isolated from entries in the 2023 SUWWSN and the marker pool evaluated on all entries. In addition, entries in other collaborative nurseries, including the Uniform Southern Winter Wheat Regional Nursery, Gulf Atlantic Wheat Nursery and advanced lines provided by the SunGrains breeding cooperative were evaluated. This included data for new assays that were added to the trait marker set.

In support of Objectives 3 & 5, tissue was received for 3936 breeding lines from collaborators in the Southern VDHR (North Carolina State University, Clemson, University of Georgia, Louisiana State University, University of Florida, University of Arkansas, Texas A&M).   An additional 1632 samples were received from University of Maryland and Virginia Tech. DNA was isolated, quantified, normalized and libraries prepared. Libraries were also prepared for entries in the Southern FHB nurseries as well as other collaborative tests targeted to this region. Pooled libraries were sent to Michigan State University for sequencing and SNP calling was completed during February 2023. Data were provided to Jeanette Lyerly, coordinator of the genomic selection and genome-wide association studies for the southern group.

In support of Objective 4, DNA for lines in the collaborative nurseries were genotyped using the ThermoFisher 5K AgriSeq mid-density genotyping platform for wheat. This platform was developed by the ARS genotyping labs and targets more than 200 markers associated with loci of agronomic importance in wheat as well as markers throughout the genome for use in genomic selection.

Advantages of the platform include very little missing data and ability to assess genome-wide and trait related markers with the same platform such that data for major loci are available to breeders at earlier generations. Analyses determined high reliability of major loci markers, ability to impute data in historical data sets, and genomic selection accuracy.

What were the significant results?

From Objectives 1 & 2, nursery reports were provided to cooperators and will be posted on the Plant Science Research Unit website. Furthermore, scripts were prepared that combine the output from different genotyping platforms and apply consistent calling criteria for automated generation of reports that are compliant with the USDA-ARS T3 database and are breeder friendly.

From Objectives 3 & 5, data were provided to collaborating programs to develop models for predictive breeding. Genomic estimated breeding values for FHB resistance traits were available to breeders in time for in field selection of lines

For Objective 4, our project collaborated with the Small Grains Genotyping Labs from the other growing regions and with commercial providers to develop approaches to obtain data for 3,000 to 5,000 DNA markers across the wheat genome. The 5K AgriSeq (ThemoFisher) and the 3K SNP array (Illumina) target markers associated with agronomically important genes while also providing the genomic information needed for prediction of line performance. For the 5K AgriSeq, each of the regional labs identified markers useful for their germplasm from joint analysis of DNA sequence data of wheat genes (exome capture). These same data were utilized for development of the 3K Illumina SNP platform by the genotyping lab at Fargo. Markers relevant across all wheat market classes and growing regions were included on both platforms. Our project has worked to ensure that markers associated with QTL associated with FHB resistance in eastern soft winter wheat can be assessed with these platforms.

List key outcomes or other achievements.

There is a need for genotyping solutions that provide repeatable data for genomic selection that are cost-effective and scalable. The new genotyping platforms developed and evaluated in conjunction with this project are available to all wheat breeders in the United States. It was determined that these platforms deliver data that is repeatable, has high information content and is amenable to storage in databases.

Collaborative improvement of the platforms is ongoing. The goal is continuous improvement of cost-effective genotyping approaches that are available to as many US programs as possible.

Our results from Objectives 3 & 5, overall, suggest that GS for FHB resistance can be utilized to streamline variety selection and evaluation. This effort includes genotyping thousands of selection candidates at earlier generations to allow for selection prior to entry into the FHB nurseries.

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

Post-doctoral scientist Dr. Luis Rivera-Burgos has gained experience with analysis of DNA sequence data, construction of linkage maps, and QTL analysis for FHB resistance. Post-doctoral scientist Dr. Zachary Winn has gained experience in preparation of script for automated analysis of data to generate breeder friendly genotyping reports.

Joy Horowitz was hired as a technician in the NCSU Department of Crop and Soil Sciences funded by this project. While providing valuable lab assistance, Joy is enrolled in classes at NCSU with the goal of obtaining skills in data science and a certificate in computer science. She is given opportunities to use her computer science training as part of this research.

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

Genotypic data are provided to the coordinators of the genomic selection project.
Genotypic data was provided to public and private wheat breeders via email in the form of marker reports on the nurseries.

Efforts to improve accessibility of genotyping data on the USDA-ARS T3 wheat database are ongoing.

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

Continued validation and improvement of newly developed mid-density genotyping platforms on breeder samples will be done. Changing genotyping platforms requires careful selection of markers that are reliable and repeatable. Also, reliable methods to translate between new and existing genotypic datasets are required. Developing strategies and tools to facilitate transition of breeding programs having historical data to these new genotyping platforms will help us accomplish project goals.

As a group, the four regional small grains genotyping labs are working with T3 to develop a system to format information for markers known to be associated with loci affecting traits. This work will improve data accessibility for all communities.