Project FY24-SW-003: 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 FHB resistance in cultivars using new breeding approaches.
Specific objectives of this continuing research are:
(1) to characterize entries in the Southern scab screening nurseries with markers for FHB QTL;
(2) to characterize entries with diagnostic markers for major loci affecting plant development and conferring resistance to other pests;
(3) to implement genome-wide markers analyses for GWAS for FHB resistance and to 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.)*

A broad genotyping effort was conducted to analyze wheat germplasm from all Southern and Mid-Atlantic wheat breeding programs using targeted and genome-wide marker approaches:

Amplicon sequencing for trait-linked markers (Objectives 1, 2 & 4):
A customized primer pool was developed to sequence amplicons targeting key QTLs for Fusarium head blight (FHB) resistance—Asian-derived Fhb1, chromosome 5A and 2D loci, and six QTLs from soft red winter wheat (Bess, NC-Neuse, Jamestown). Additional markers related to plant growth, disease resistance, and quality traits were also included. This panel was applied to genomic DNA from entries in the 2023 SUWWSN nursery, as well as diverse regional trials: the Uniform Southern Winter Wheat Regional Nursery, the Mason-Dixon and Gulf Atlantic Wheat tests, and advanced breeding lines. Newly published/developed trait assays were added and KASP assays were used for genotype markers that were not amenable to amplicon sequencing.

Genotyping-by-sequencing for genomic selection and GWAS (Objectives 3 & 5):
A total of 6504 breeding lines from the Southern VDHR region (including Georgia, Arkansas, Louisiana, Florida, North Carolina, South Carolina, Virginia and Maryland) were processed for GBS. DNA was extracted, quantified, normalized, and used to generate libraries—including from Southern FHB nurseries and related regional tests. Sequencing was performed at Michigan State

University; SNP calling was finalized in March 2024. These genotypic datasets were delivered to the genomic selection and GWAS coordinator for the Northern breeding group.

Mid-density targeted genotyping (Objective 4):
Collaborative nursery materials were genotyped using the updated ThermoFisher WHeat4K AgriSeq platform—designed by ARS genotyping labs to assay over 306 marker loc targeting 196 loci, in addition to genome-wide markers for genomic selection. Data for 182 genes were obtained and reported to the breeders during May. This platform proved reliable—yielding minimal missing data and supporting both major-loci tracking and genome-wide selection—thus providing breeders with early-generation access to critical genetic information.

What were the significant results?

Objectives 1 & 2: Nursery Reports and Data Integration

Nursery Reports: Comprehensive nursery reports were provided to cooperators and will be made accessible on the USDA-ARS Plant Science Research Unit website (once ARS allows updating). These reports offer valuable insights into genetic traits in wheat breeding lines.

Data Integration Scripts: Scripts were developed to harmonize outputs from various genotyping platforms, ensuring consistent variant calling. This standardization facilitates the automated and timely generation of breeder-friendly reports.

Objectives 3 & 5: Predictive Breeding and Genomic Selection

Predictive Models: Genotyping data were supplied to collaborating programs to develop models for predictive breeding. These models enable breeders to make informed selections based on genomic data. The GEBVs for Fusarium Head Blight (FHB) resistance traits could be available to breeders in time for in-field selection, enhancing the efficiency of breeding programs.

Objective 4: Development of Genotyping Platforms

Collaboration for Marker Development: The project continued collaborated with other regional Small Grains Genotyping Labs and commercial providers to develop approaches for obtaining data on 3,000 to 5,000 DNA markers across the wheat genome. Markers relevant across all wheat market classes and growing regions were included on both platforms, ensuring broad applicability. The project ensured that markers associated with QTLs for FHB resistance in eastern soft winter wheat are assessable with these platforms. These platforms are available to all breeding programs.

New Wheat4K SNP AgriSeq design: The Wheat AgriSeq design was streamlined to include 4,000 markers, including 306 target markers are associated with agronomically important genes and ~3600 genome-wide markers for predicting line performance.

List key outcomes or other achievements.

- Cost-Effective Genotyping Solutions: The newly developed genotyping platforms offer repeatable, high-information-content data that are cost-effective and scalable, addressing the need for efficient genomic selection tools.

- Data Accessibility: The platforms deliver data amenable to storage in databases, facilitating easy access and utilization by wheat breeders across the United States.
- Enhanced Breeding Efficiency: By providing timely genomic data and predictive models, the project has significantly enhanced the efficiency and effectiveness of wheat breeding programs.

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. When possible, these reports will be posted on the Plant Science Unit website; USDA-ARS currently has an embargo on updating websites.

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.