**Project 1:** A Double Haploid Initiative to Speed Development of FHB Resistant Soft Winter Wheat.

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

   **GOAL:** Expand the regional Double Haploid (DH) initiative to more quickly develop and release high-yielding varieties that contain an effective FHB resistance pyramid.

   **Objective 1.** Develop DH lines that combine multiple effective FHB resistance genes/QTL.

   **Objective 2.** Utilize marker assisted selection (MAS) to enrich topcross F1 populations for those genes/QTL.

   **Objective 3.** Share new DHs with all VDHR-SWW breeders after the initial culling such that each breeder evaluates about 1,300 new DHs each year.

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

   **What were the major activities?**

***Objective 1.*** *Develop DH lines combining FHB resistance QTL*

Six new DH populations containing a total of 1,182 DHs were received from Heartland Plant Innovations in October 2023. Three additional DH populations that were received in January 2023, which was another 127 DHs. Seed from these F1 crosses were developed during the Spring 2022 greenhouse crossing nursery. The 1,309 new DH lines representing nine pedigrees, ranging from 23 lines to 356 lines per population (mean of 145), were planted within the inoculated FHB nursery in November 2023.

In the Spring 2023 crossing nursery, another set of crosses were designated and created for DH production. Seed was harvested in April 2023 and then shipped to the USDA Plant Science Research Unit in Raleigh for DH production (to receive for November 2024 planting. The three crosses represented the following pedigrees: LA14234CBW-31 / NC15V25-20, SCGA14298-32-6 / NC16VT30-7-47, and 15VTK-1-101 / SCLA18WF0304-13. Seed quantity (>30) was sufficient for each cross for 150-200 DHs per population. Consistent across the three DH populations under development is the segregation of Hessian fly QTL H13 (most valuable for resistance against biotype L that is prevalent in Coastal Plain states) with multiple FHB resistance QTL (nine unique FHB QTL in total, with each cross having three or more QTL segregating in the population for durable resistance).

| Seed | Cross | Pedigree | QTL* |
|---|---|---|---|
| 73 | SC23W037 | LA14234CBW-31 / NC15V25-20 | H13, H9, Fhb1, F5ANi, F1BJ, Yr17, Lr18, Yr4BL, Sbm1 |
| 59 | SC23W185 | SCGA14298-32-6 / NC16VT30-7-47 | H13, F1AN, F4AN, F6AN, F3BB, Yr17, Lr18, Pm1a, Sbm1 |
| 57 | SC23W150 | 15VTK-1-101 / SCLA18WF0304-13 | H13, F3BM, F1BJ, F4AN, Yr17, Lr18, Sbm1 |

*Orange is a segregating QTL; green is a fixed favorable at the QTL.

***Objective 2.*** *Utilize MAS to advance DH lines populations with stacked resistance genes/QTL*

Instead of topcrossing $F_1$s to develop 3-way crosses, we are using a custom machine learning approach (see Winn, Lyerly, et al. 2022 Theor Appl Genet) to impute alleles at resistance QTL from genome-wide SNP data generated on DH lines and other breeding lines that reach preliminary yield testing (typically $F_{5:6}$ generation). This modified approach enables select DHs or $F_{5:6}$ lines carrying favorable combination (*i.e.*, stacks) of resistance alleles to be rapidly cycled back as parents that were evaluated in the inoculated FHB screening nursery. PopVar software (see Ballén-Taborda et al. 2024 Crop Science) is being implemented for selecting superior cross-combinations among recycled parents, which assists in deciding the several $F_1$ crosses chosen for DH production.

There were 63 South Carolina (Clemson University) DH lines evaluated in 2023-2024 preliminary yield trials. These 63 DHs were genotyped in fall 2023 and were run through the genomic prediction pipeline in winter 2023-2024 to generate genomic estimated breeding values (GEBVs) and major effect allele presence as described above. Of the 502 total breeding lines evaluated in the preliminary yield trial, 22 of the 63 DHs were in the top 20% (100 lines) yield category. Of these 22 DH lines, which represented all three DH populations (6, 7, and 9 DHs from each), 12 are predicted to have the resistance allele at Fhb1, three DHs are estimated to have Fhb1 and H13 in tandem, and eight have Fhb1 plus additional FHB resistance QTL(s). These DHs will be entered into advanced SunGrains multi-environment trials in 2024-2025.

***Objec&ve 3.*** *Share DHs with VDHR-SWW breeders to increase ROI of USWBSI funding*

Seed from most of the 217 DH lines, those with sufficient seed quantity, are currently being packaged to share with all seven of the VDHR-SWW breeders. These DH sets originating from the Clemson program will be given to SunGrains breeders at the 2024 summer meeting in August in NC. As appropriate, genomic estimated breeding values (GEBVs) and predicted QTL calls will be disseminated to the breeders via an established file sharing cloud storage system.

**What were the significant results?**

***Objective 1.*** *Develop DH lines combining FHB resistance QTL*

From the evaluation of 1,362 DHs grown in the 2023-2024 misted FHB headrow nursery, 217 (16% selection intensity) DH headrows were harvested and threshed in Spring 2024 to be entered into the wheat preliminary yield trial in 2024-2025. There were 652 F5 breeding lines that were selected in addition that were advanced using modified pedigreed selection. Approximately one-quarter of the 2024-2025 wheat preliminary yield trial entries will be DH lines, which is far greater than ever before in the Clemson program. All 217 lines will be genotyped in winter 2023-2024 to estimate their breeding values per se using genomic prediction and impute alleles at native FHB resistance QTL and alleles at other important trait loci. Based on pedigrees and segregating QTL within the DH populations, it is expected that the frequency of resistance QTL, such as Fhb1, F1B from Jamestown, and F4A from Neuse will be high enough to get two or more FHB resistance QTL combinations.

***Objective 2.*** *U:lize MAS to advance DH lines popula:ons with stacked resistance genes/QTL*

Of the 502 total breeding lines evaluated in the 2023-2024 preliminary yield trial, 22 of the 63 DHs included were in the top 20% (100 lines) yield category. For perspective, the DH lines made up only 12% of the trial entries yet 35% (22 of 63) of the DHs yielded in the top 20% of trial

entries. This finding is not coincidental but rather follows a longer term trend as DH lines make up exactly 50% (10 of 20) of SunGrains wheat cultivar releases in the past five years, despite a small minority of breeding lines across programs being DHs. Of the 22 high yielding DH lines, which represented all three DH populations evaluated (6, 7, and 9 DHs from each), 12 are predicted to have the resistance allele at Fhb1, three DHs are estimated to have Fhb1 and H13 in tandem, and eight have Fhb1 plus additional FHB resistance QTL(s). These DHs will be entered into advanced SunGrains multi-environment trials in 2024-2025, as well as into regional inoculated FHB nurseries coordinated by the VDHR-SWW.

***Objective 3.*** *Share DHs with VDHR-SWW breeders to increase ROI of USWBSI funding*
Seven of the 217 DH lines advanced from the spring 2024 inoculated FHB nursery were DHs shared by the LSU breeding program as part of this VDHR-SWW project. Doubled haploid lines that were developed prior to 2021 and advanced from headrows are currently in various stages of field testing. There are 38 DH lines specifically from the Clemson program in current yield trials, while the total number of DHs being evaluated in 2022-2023 advanced nurseries (USSRWWN, GAWN, SunWheat, and SunPre) totals 80. Importantly, 10 of the last 20 wheat cultivars released from SunGrains have been DH lines. Two DHs shared from this VDHR-SWW project will be entered by Clemson into the 2024-2025 Uniform Southern Soft Red Winter Wheat Nursery and given to SC Crop Improvement for foundation seed production.

**List key outcomes or other achievements.**

One SunGrains line, SCLANC11558-33, was increased as foundation seed by the South Carolina Crop Improvement Association for release. SCLANC11558-33 has been one of the highest yielding wheat entries in the South Georgia state variety trials for three consecutive years. The release documentation and report was drafted and will be submitted for approval by the Clemson University Variety Review and Release Committee. A manuscript describing this cultivar will be submitted to the Journal of Plant Registrations. SCLANC11558-33 is moderately resistant to scab because it has the FHB resistance allele at both F1B from Jamestown and F1A from Neuse.

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

This project does not directly fund personnel; however, graduate students, postdocs, and technicians in the program have all learned considerably about the DH production process and its value in the Clemson wheat breeding program. Utilizing this technology over the past several has expanded my ability and knowledge as a small grains breeder. Leveraging DH populations, select ones that were genotyped, for research applications has also enabled Clemson small grains program scientists to expand their germplasm evaluation and characterization of numerous important phenotypes for soft red winter wheat production.

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

The DH project probably does not get the notoriety or recognition that it deserves because it is a trusted approach that now is no longer a groundbreaking technology in wheat. In hindsight, more effort should have been given towards sharing stories about the exceptional gains made from the adoption of DH production and evaluation. However, advanced VDHR-SWW DH lines in final stages of testing and seed increase were highlighted during a Clemson University Wheat Field

Day held at the Pee Dee Research and Education Center in Florence, SC. Seed company representatives and growers in attendance were able to learn about promising future cultivar releases and their ability to withstand FHB pressure based on inoculated nursery reports and FHB resistance QTL they contain using visual signs in the field.

Like in previous years, the number of DH lines selected and harvested by the Clemson breeding program were submitted to the VDHR-SWW breeding programs to notify them that seed of the 217 DHs will be shared with them in summer 2024 for fall headrow planting. Pedigree information is shared with line designations while GEBVs and predicted marker data will be disseminated in winter 2024-2025 when genotyping, genomic predictions, and marker imputation using machine learning are completed.

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

As a result of the $1M USWBSI FY24 budget cut, the VDHR-SWW DH project was zeroed out and thus no new DH lines will be developed in the current budget cycle. As mentioned above, the 217 DHs that were selected and advanced in the 2023-2024 field season will still be genotyped and evaluated in yield trials in 2024-2025. The crosses sent in April 2023 to the USDA-ARS Plant Science Research Unit in Raleigh for DH production will be paid for using the FY23 NCE balance and thus be planted into the inoculated FHB nursery in November 2024. The negative impact of eliminating the DH project budget in FY24 will not be realized until the 2026-2027 field season when there will be no new DHs available to the eight VDHR-SWW breeding programs that develop soft winter wheat for 11 southern states. For perspective, this means there will be 4,000 fewer DHs in 2026-2027 field nurseries to evaluate for unique combinations of FHB resistance QTL, high yield, high test weight, foliar disease resistance, and Hessian fly resistance. The program will look into alternative methods to cover up this deficit by recycling shared VDHR-SWW DHs and potentially adopting rapid generation advancement of promising breeding lines.