Project FY22-SW-008: Expediting Development of Wheat with Improved FHB Resistance for the Coastal Plain

---

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

Objective 1. Phenotype screening of FHB resistance for advanced breeding lines.
Objective 2. Genetic screening to facilitate genomic and marker-assisted selection.
Objective 3. Prediction of progeny variance and transgressive segregation for FHB resistance.

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. Phenotype screening of FHB resistance for advanced breeding lines.

For the 2023-2024 FHB nursery, there were 510 samples that were harvested in June 2024 and processed in July 2025 for FDK analysis with the Vibe QM3 Grain Analyzer. The 510 samples were submitted to the VA Tech DON Testing Laboratory in two batches as done previously, prioritizing the Uniform Southern Scab Nursery (USSN) and Uniform Southern Soft Red Winter Wheat Nursery (USSRWWN). The entries of the Gulf Atlantic Wheat Nursery (GAWN) and SunWheat were analyzed for FDK and shipped to VA Tech immediately following. Each entry from these trials were replicated twice in the field for a total of 510 plots. As typical, PI Boyles ran statistics for all traits collected from the nursery, which included heading date, FHB rating, FDK, DON, and ratings for barley yellow dwarf virus, powdery mildew, and leaf rust. Results were shared with cooperating breeders periodically as new data became available for the different nurseries.

In addition to screening advanced wheat lines for cooperators and stakeholders, the Clemson breeding program also evaluated 1,370 doubled haploids (DHs) and 5,894 F_5 selections in the inoculated FHB nursery in spring 2024, albeit at a 50% reduced inoculum rate (20 grams of scabby corn per m^2). From this effort, 217 DHs and 652 F_5s were selected and processed for yield testing in 2024-2025. Small seed lots for the 217 DHs were prepared and shipped to the other seven VDHR-SWW breeding programs to evaluate them as headrows in their respective states for potential selection. In total, 1,519 DH seed packets were shared from the Clemson program.

The Clemson program graciously received isolates from Dr. Christina Cowger (ARS-Raleigh) in January 2025 (same as in prior project years) to begin preparing inoculum. This grain spawn inoculum was developed using the mung bean tea protocol as done previously. Two rounds of inoculum were developed and hand spread among all headrows approximately two weeks apart to account for maturity differences among diverse wheat lines. Mist-irrigation was deployed again in spring 2025 using the established Nelson sprinklers hooked up to a sand media filter to remove particulates and allow for fine misting. The headrow plots of advanced entries received 40 g/m^2 (two applications of 20 g/m^2 each) while headrows for 550 DHs and 6,000 F_5 breeding lines also received a lower inoculum application in spring 2025 (similar to spring 2024 as mentioned above) to purge susceptible entries but avoid severe infection to enable seed saving. Over 120 kg of grain spawn was applied to the nursery in spring 2025 2-3 weeks before heading.

For data collection, heading date was recorded for every FHB nursery headrow. Heavy disease pressure was observed in May, and PI Boyles along with research technicians Kimberly Baskins and William Caughman collected FHB field ratings to share with breeders for early selection decisions. A team of seven people in the Boyles program harvested approximately half of every FHB nursery headrow to collect grain for FDK and DON analysis. Sample processing immediately commenced after harvest was completed. Individual plots (*n*=498) of the USSN samples were imaged on the Vibe QM3 in July 2025 to estimate FDK prior to grinding grain to ship for DON analysis. Replicated grain samples for the USSRWWN, GAWN, and SunWheat entries will be processed and evaluated following the USSN.

Objective 2. Genetic screening to facilitate genomic and marker-assisted selection.
Of the 869 entries (217 DHs and 652 F_5s) in Clemson first-year yield trials, 816 lines were successfully genotyped using an established GBS protocol. The lines were grown out and tissue sampled in September 2024 and shipped to the Eastern Regional Small Grains Genotyping Lab (USDA-ARS Raleigh) for DNA extraction and library preparation. Sequencing was again outsourced to the Michigan State genomics core facility, and raw reads were provided to the ERSGGL in February 2025. The genomic prediction pipeline led by Jeanette Lyerly was performed between February and March to generate genomic estimated breeding values (GEBVs) to aid in selecting the best genotypes. These GEBVs, along with major effect QTL predictions using the established machine learning-based imputation protocol, were shared by Jeanette Lyerly on April 7, 2025. Because of this tremendous effort, the Clemson program was able to select lines with a higher frequency of important favorable alleles for FHB resistance, as well as known resistance alleles for Hessian fly, leaf rust, stripe rust, stem rust, powdery mildew, barley yellow dwarf virus, and soil borne mosaic virus. This prediction pipeline is working exceptionally well for the SunGrains breeding programs to advance lines with improved FHB resistance.

Objective 3. Prediction of progeny variance and transgressive segregation for FHB resistance.
Simulations were run on parents in December 2024 for the 2025 greenhouse crossing nursery, which was similar to the prior breeding cycle/project period. The robust SunGrains training population that is used for generating GEBVs for new breeding lines was also leveraged to estimate the best biparental crosses among the prospective parents, with the best crosses being determined by predicted progeny mean and genetic variance. As reported in the previous project report, the training population contained 1,411 lines, 29 locations (in 10 States), 16 years (2008-23). FHB traits were also estimated using historical data collected from VDHR-SWW inoculated FHB nurseries. Similarly, Jeanette Lyerly ran cross predictions on SunGrains wheat nurseries to understand what entries should be intercrossed to generate new segregating populations for advancement and selection. These nurseries included the GAWN, SunWheat and SunGrains preliminary wheat trial (SunPre).

The R package 'PopVar' (Mohammadi et al. 2015) was used again to predict the progeny performance of potential crosses. The specific parameters estimated by the program were progeny mean, genetic variance, inferior 10% of progeny mean, and superior 10% of progeny mean. Research on the utility of this approach was conducted by Clemson postdoctoral fellow Dr. Carolina Ballén-Taborda. Full details were published in Ballén-Taborda et al. 2024 Crop Science (doi:10.1002/csc2.21266).

What were the significant results?

Objective 1. Phenotype screening of FHB resistance for advanced breeding lines.

Final DON data were received from VA Tech on November 8, 2024. Unlike in 2023, the spring 2024 inoculated FHB nursery struggled to harbor heavy levels of scab incidence due to exceptionally cool, dry weather in Florence, SC. Despite below average disease pressure, DON levels ranged from 0-8.7 ppm, with the susceptible check 'SS 8641' having a mean DON of 5.4 ppm over the trials (only 1.5 in USSN where lower pressure was observed). There were two Clemson entries that were in the top three lowest overall DON levels over six reporting locations (**Table 1**), including the DH line SC19DH146-2 that has both Fhb1 and Jamestown 1B resistance. All eight SC entries in the 2023-2024 USSN had a lower DON level than the overall trial mean of 6.3 ppm to demonstrate good resistance progress in the Clemson breeding program.

Table 1. DON data of the most resistant experimental entries along with the standard checks from the 2023-2024 Uniform Southern Scab Nursery. There were two Clemson entries in the top five for DON.

Entry	Designation	SCF	TXC	KYX	LAW	LAA	VAW	Mean	*Rank*	GEBV
22	**LA14188C-28-3-1-4-1-2**	0.0	2.7	2.3	2.5	2.0	2.0	1.9	***1***	9.5
35	**SC22W145**	0.5	2.7	2.3	1.0	2.7	3.0	2.0	***2***	5.6
1	**ERNIE**	0.5	0.8	5.3	2.6	2.5	2.9	2.4	***3***	9.6
34	**SC19DH146-2**	0.3	2.3	5.7	3.0	1.4	3.0	2.6	***4***	7.6
26	**LA18003-NDH119**	0.3	1.0	3.9	3.9	3.9	6.8	3.3	***7***	9.5
6	**15VDH-FHB-MAS22-14**	0.4	5.3	4.7	2.4	3.9	2.0	3.1	***5***	9.0
3	**BESS**	0.6	2.5	5.8	2.6	4.8	3.6	3.3	***8***	8.8
2	**COKER9835**	1.5	2.1	12.3	3.2	11.5	10.6	6.9	***41***	16.2
5	**SS 8641**	1.5	4.4	29.8	4.0	17.6	10.7	11.3	***52***	14.3
.	**Nursery Mean**	**1.0**	**6.3**	**9.9**	**3.9**	**6.5**	**9.6**	**6.3**	**.**	**10.7**

The disease pressure in the spring 2025 FHB nursery was exceptionally high, with moderately resistant cultivars displaying around 30-40% severity. There was sufficient FHB pressure in both the 2024 and 2025 nurseries to eliminate susceptible Clemson lines (DHs and F5s) that were in their final year of headrow evaluation prior to entering into yield testing. Because of phenotypically selecting for FHB resistance at this headrow stage, the Clemson program wheat preliminary yield trial (WPT) is increasing the frequency of FHB resistance alleles into the program, which is known based on QTL predictions from GBS data that was generated on 816 F_6 lines (**Table 2**).

Objective 2. Genetic screening to facilitate genomic and marker-assisted selection.

Marker predictions at the $F_{5:6}$ stage has made a significant impact on tracking the frequency of resistance alleles, and this knowledge has increased the selection intensity for FHB resistance earlier in the breeding cycle. An emphasis in the Clemson breeding program has been to stack both H13 and Fhb1 to account for increased outbreaks in Hessian fly and FHB, respectively. These are the two most effective genes available in southern soft red winter wheat germplasm. As a result of both dedicated phenotyping and marker-assisted selection, there were 47 breeding lines in the 2024-2025 WPT that possessed the resistance allele at both QTLs, which is significantly higher than past years. Meanwhile, another 114 lines (14% frequency) had both H13 and the Jamestown 1B allele for FHB resistance.

Table 2. Number and frequency of predicted FHB resistance alleles present in 2024-2025 Clemson wheat preliminary trial (WPT) entries.

QTL(s)	H13 + Fhb1	H13	Fhb1	F1BJ	F1AN	F4AN
Number	47	225	173	372	319	54
Frequency	6%	28%	21%	46%	39%	7%

F1BJ, FHB 1B QTL from Jamestown; F1AN and F4AN, FHB 1A and 4A QTLs from Neuse

Objective 3. Prediction of progeny variance and transgressive segregation for FHB resistance. The significant results toward this objective were published in Ballén-Taborda et al. (2024), which includes validation of progeny prediction using historical datasets from the southern soft red winter wheat region. The Clemson program continues to utilize genomic predictions for progeny performance estimates to determine which parents to cross for new biparental populations. The significant result is that progeny prediction can aid in determining which crosses to make, but parents should be narrowed down based on multiple factors, including favorable alleles at known QTL and combinations thereof. Once parents are chosen based on field performance and marker data, progeny prediction using PopVar or similar software can be implemented to prioritize crosses among the available parents.

List key outcomes or other achievements.

The Clemson breeding program has advanced to entering wheat lines into every established cooperative yield nursery. The largest achievement of a breeding program is variety release, which the Clemson program has not yet done. However, the next observation to track a program's progress is the performance of the program's entries in these advanced nurseries that evaluate traits across environments. The 2025 Gulf Atlantic Wheat Nursery (GAWN) results to date show two promising Clemson entries, SC22W361 and SC22W281 (**Table 3**). SC22W361 has had high yield and test weight in the 2024 SunWheat and now 2025 GAWN and has H13 allele for Hessian fly resistance, and the strong combination of FHB resistance genes Fhb1, 1B Jamestown, and 1A Neuse. The 2025 SunWheat results were similar (**Table 4**), with two Clemson entries yielding at or near the top of the trial, including the top yielding line (doubled haploid SC21DH145-68) that has both H13 and Fhb1. Based on the progress in both incorporating FHB resistance and maintaining yield and test weight, the program appears ready to release a cultivar by the end of the 4-year project period (*i.e.*, spring 2026).

Table 3. Top yielding entries for the 2024-2025 GAWN are listed, along with their markers related to biotic stress resistance, which were provided by the Eastern Regional Small Grains Genotyping Lab.

ENT	DESIGNATION	MARKERS_DEFENSE	YLD	*Rank*	TW	*Rank*
57	21VTK6-21	b1,H13,F1BJ,Lr18,Yr17,Sbm1	84.2	*1*	57.2	*46*
56	VA23W-532	b1,H13,F1BJ,F1AN,Lr18,Yr17,Sbm1	81.7	*2*	56.1	*53*
46	SC22W361	b1,H13,Fhb1,F1BJ,F1AN,Lr18,Yr17,Sbm1	81.7	*2*	59.1	*7*
37	NC14757LDH-15	b1,H13,F1AN,F4AN,Sr36/Pm6,1RS:1BL,Sbm1	79.8	*4*	57.6	*34*
29	LAVT19VDH-FHB-MAS11-10	b1,H9,H13,Fhb1,F1BJ,F6AN,Lr18,Sbm1	79.7	*5*	58.2	*19*
38	NC20-21971	b1,H13,F4AN,Lr18,Sr36/Pm6,Bdv2/3,Sbm1	79.4	*6*	58.7	*12*
58	21VTK13-26	b1,H13,F1BJ,Lr18,Yr17,Sbm1	79.4	*6*	56.8	*49*
45	SC22W281	b1,H13,H7D,F1AN,Lr18,Lr23,Yr17,Sbm1	78.7	*9*	59.5	*1*

Table 4. Top yielding entries for the 2024-2025 SunWheat are listed, along with their markers.

ENT	DESIGNATION	MARKERS	YLD	*Rank*	TW
63	SC21DH145-68	**Fhb1**,**H13**,F4AN,Lr18,Yr17,Yr4BL,Pm1a,Sr24_Lr24,Sbm1	76.5	*1*	55.6
34	GA17047-24-2-12-24LE45	**H13** 1B_JT 4A_N Lr18 Yr17 Sr6D Sbm1	74.9	*2*	56.9
31	GA17310-6-4-7-24LE21	**H13** Yr17 Yr4BL Sbm1	73.4	*3*	57.0
1	HILLIARD	0	70.6	*4*	56.9
88	AR15735-1	0	74.9	*5*	56.9
52	SCLA18290LDH-008	F1AN,F4AN,H7D,Yr17,Sbm1	71.9	*6*	57.5
2	AGS 3022	0	71.7	*7*	57.6
25	GA18183ID-2-10-6-24E11	**H13** 4A_N Yr17 Lr68 Sr6D Yr4BL Sbm1	74.9	*8*	57.6

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

The Clemson Cereal Grains Breeding & Genetics program has three PhD students that are actively involved in small grains breeding, despite not being directly supported by the Scab Initiative. Active involvement includes assisting with FHB inoculum preparation and application, headrow data collection, headrow harvest, and post-harvest seed processing. The students are learning tangentially through this project very important skills in plant pathology and breeding that will be valuable to them after graduation. Research technicians continue to lead the day-to-day efforts that relate to FHB nursery screening (Objective 1), and they are picking up a number of soft skills and scientific information through this annual effort. Dr. Carolina Ballén-Taborda has published and presented on wheat FHB related research, having the opportunity to network through SunGrains meetings and various conferences.

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

Data for the Uniform Southern Scab Nursery and FHB nursery data for the GAWN, SunWheat, and SunPre get shared immediately upon running statistics through periodic emails to each breeder that is part of a given nursery. Summary and raw plot data are both shared to allow for additional statistical analyses to be run such as generating BLUEs and BLUPs to aid selection decisions. As mentioned above, DHs that were selected from the Florence FHB misted nursery were shared to all seven other VDHR-SWW breeding programs for evaluation in headrows in the subsequent field season. Information was also shared with breeders during in-person meetings at the 2024 SunGrains summer meeting (Lake Lure, NC), the 2024 FHB Forum (Austin, TX), and the 2025 SunGrains spring field tour (various TX locations).

For the third consecutive year, PI Boyles hosted a winter small grains field day at the Clemson University Pee Dee Research and Education Center (Florence, SC) for wheat industry representatives and growers to attend and learn about new wheat cultivars, ongoing research efforts, and emerging products for better management. There were roughly 40 attendees present at the event, which included visiting the FHB nursery. In addition, a wheat production guide was developed in winter 2024-2025 for South Carolina growers by Clemson University and Dr. Christina Cowger at USDA-ARS Raleigh. PI Boyles included information on FHB resistance for commercially available varieties that was based on multiple years of FHB nursery data through screening of the South Carolina Official Variety Trial entries over the past five years. Additional information was shared in this guide about FHB resistance genes and pertinent management

information for mitigation of the disease in the state. This guide will be made available to growers in summer 2025 in time to support variety selection decisions for fall 2025 planting.

Applied research findings on progeny prediction (Objective 3) were published in May 2024 by Clemson postdoc Dr. Ballén-Taborda in the journal Crop Science (see publication information below). A second publication in this research area is in final stages of submission. Two oral presentations were provided by PI Boyles at the ASPB Mid-Atlantic Meeting (College Park, MD) and a Clemson departmental seminar to highlight ongoing efforts in FHB resistance and other biotic stresses in wheat and other cereal crops.

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

The biggest hurdle for the project is final release of a wheat cultivar by Clemson. This is the main focus in the final project year, which includes final field testing but, most importantly, foundation seed purification and increase. The wheat line(s) will be submitted for release and likely go through the bidding process for exclusive or semi-exclusive licensing to a seed company for sale and distribution.

FDK analysis is ongoing and will finish in August 2025, with samples being ground and shipped to the VA Tech DON testing lab immediately after being imaged with the Vibe QM3. We will share final FDK and DON data for the USSN to Jeanette Lyerly (nursery coordinator) and share FHB data for additional trials to all invested breeding programs. The efforts in the FHB misted nursery will be maintained to generate quality data for all VDHR-SWW breeding programs. Although this has become routine within the CP, this effort remains a top priority for continual improvement in FHB resistance in southern soft red winter wheat. The South Carolina OVT entries will be screened in the 2025-2026 FHB nursery to report this data to growers in an updated production guide and through various online Clemson extension platforms.

There were 57 DHs harvested in May 2025 that will be processed and entered into first year yield trials, as well as small seed lots will be shared to the seven additional VDHR-SWW breeding programs. Genotyping will be conducted on these 57 DHs and 771 new F_5 breeding lines to predict GEBVs prior to selecting a subset that will advance into multi-state yield evaluation. Planting of all trials included the FHB nursery will begin in November 2025, with seed preparations well underway.

A second manuscript on progeny prediction is in the final stages of internal review that will be submitted for publication, with the target submission in August 2025.