USDA-ARS | U.S. Wheat and Barley Scab Initiative

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-2-097
USDA-ARS Agreement Title:	Developing Fusarium Head Blight (FHB) Resistant Wheat for the Coastal Plain
Principle Investigator (PI):	Richard Boyles
Institution:	Clemson University
Institution UEI:	H2BMNX7DSKU8
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$86,189
PI Mailing Address:	Clemson University, Pee Dee Research and Education Center 2200 Pocket Rd, Florence, SC 29506
PI E-mail:	rboyles@clemson.edu
PI Phone:	843-519-0488
Period of Performance:	May 1, 2022 – April 30, 2026
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
VDHR-SWW	A Double Haploid Initiative to Speed Development of FHB Resistant Soft Winter Wheat.	$18,263
VDHR-SWW	Expediting Development of Wheat with Improved FHB Resistance for the Coastal Plain	$67,926
	FY22 Total ARS Award Amount	$86,189

I am submitting this report as an: ☒ Annual Report

I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.

Digitally signed by Richard Boyles
Date: 2023.08.05 08:50:06 -04'00'

Principal Investigator Signature | Date Report Submitted

‡ BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
EC-HQ – Executive Committee-Headquarters
FST-R – Food Safety & Toxicology (Research)
FST-S – Food Safety & Toxicology (Service)
GDER – Gene Discovery & Engineering Resistance
HWW-CP – Hard Winter Wheat Coordinated Project
MGMT – FHB Management
MGMT-IM – FHB Management – Integrated Management Coordinated Project
PBG – Pathogen Biology & Genetics
TSCI – Transformational Science
VDHR – Variety Development & Uniform Nurseries
NWW –Northern Soft Winter Wheat Region
SPR – Spring Wheat Region
SWW – Southern Soft Red Winter Wheat Region

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

---

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

   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.)*

   a) **What were the major activities?**

Another set of three biparental F_1 crosses (SC22W128, SC22W291, and SC22W362) were sent to Heartland Plant Innovations (HPI) in July 2022, with expectations to receive 200 DH lines from each population (600 total DHs) before fall 2023 planting. The 2023 set of crosses selected for DH production were made in January, which includes the following pedigrees: LA14234CBW-31 / NC15V25-20, SCGA14298-32-6 / NC16VT30-7-47, and 15VTK-1-101 / SCLA18WF0304-13. F_1 seed quantity will be adequate of each cross to send to HPI for 150-200 DHs per population.

In June 2023, three additional crosses— SC23W213 (LA14234CBW-31 / NC15V25-20), SC23W219 (SCGA14298-32-6 / NC16VT30-7-47), and SC23W276 (15VTK-1-101 / SCLA18WF0304-13)— were sent to Allison Anthony at the USDA Plant Science Research Unit to attempt to get 100 DHs per population. Remnant seed for this same set of crosses were shipped to HPI as well.

South Carolina (Clemson University) DH lines SC21W088 (15VDH-FHB-MAS38-01 / LA12275DH-56), SC21W145 (GA15VDH-FHB-MAS23-18LE43F / 14VDH-SRW14-150), and SC21W240 (SCGA17VADH-FHB-MAS1702-179-6 / TX16DDH579) were evaluated during the 2022-2023 field season. All 337 DHs were planted in headrows within the Florence FHB inoculated nursery to evaluate FHB resistance in first year of testing. From the field evaluation, sixty-three DH lines were selected and harvested in spring 2023 for yield testing in 2023-2024. All 63 DHs will be genotyped in fall 2023 to generate genomic estimated breeding values (GEBVs) and major effect marker prediction using machine learning on genome-wide markers (Winn et al. 2022).

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. One SunGrains line, SCLANC11558-33, is being increased by the South Carolina Crop Improvement Association for certified seed for 2023 release. The release documentation and report is being drafted for approval in summer 2023.

b) What were the significant results?

Of the 63 DHs selected from the SC 2021 crosses (crosses submitted to HPI in spring 2021) in the 2022-2023 FHB nursery, all of the pedigrees are segregating for Fhb1 and H13. This combination of QTL is important as FHB and Hessian fly have been problematic in the southeastern US in recent years. Also, H13 has proven to be only one of two (other than H7D) robust resistance Hessian fly QTL in adapted soft red winter wheat backgrounds. Other older QTL such as H9 and H10 alone no longer provide full protection against the intense fly damage that has been recently observed, particularly in GA, LA, and TX. These 63 DHs exhibited, at minimum, a moderate level of FHB resistance when grown under heavy pressure in the inoculated FHB nursery in Florence. Fewer than 5% (~15 DHs) of lines failed to fully vernalize and another 10% were past the maturity window desirable for production in the Atlantic Coastal Plain, which still left nearly 300 DHs to critically assess for productivity traits and FHB symptoms. Thus, there is a high level of confidence that, with the above average FHB pressure and good growing conditions experienced in 2022-2023, the 63 DHs selected for yield testing and genotyping will be well adapted and competitive in 2023-2024 yield trials.

With the consistent funding from the USWBSI VDHR-SWW CP for doubled haploid production, the Clemson University wheat program will have an estimated 1,500 DHs to assess for cultivar development and improved FHB resistance during a 3-yr period (Table 1). The value of getting premium genetics to the release faster through this technology cannot be understated. This resource was vital to the new Clemson program by filling in the breeder pipeline with advanced breeding lines 2-3 years faster by leveraging DH technology and investment. Using the germplasm sharing arrangement of SunGrains, the Clemson program is pushing to release wheat DH line SCLANC11558-33 this summer (2023) that was originally developed by Paul Murphy in the NC State small grains breeding program. Sharing of DHs across the VDHR-SWW CP has been and continues to be a unique way to leverage resources and increase the return on investment in DH production.

Table 1. List of recent VDHR doubled haploid (DH) populations and their current status in the breeding pipeline.

Designation	Pedigree	Status
SC21W088	15VDH-FHB-MAS38-01 / LA12275DH-56	63 of 337 DHs advanced from FHB nursery headrows for 2023-2024 yield testing.
SC21W145	GA15VDH-FHB-MAS23-18LE43F / 14VDH-SRW14-150	
SC21W240	SCGA17VADH-FHB-MAS1702-179-6 / TX16DDH579	
SC22W128	15VDH-FHB-MAS33-13 / GA151313-LDH-192 -20E48	DH lines are with Heartland Plant Innovations being grown out for seed. Expected to receive for fall 2023 planting.
SC22W291	GA151313-LDH-192 -20E48 / SCLA19WF2110	
SC22W362	TX16DDH579 / 15VDH-FHB-MAS33-13	
SC23W037	LA14234CBW-31 / NC15V25-20	Crosses made in January 2023 and F_1 seed was shipped for DH production in June 2023.
SC23W150	15VTK-1-101 / SCLA18WF0304-13	
SC23W185	SCGA14298-32-6 / NC16VT30-7-47	

c) List key outcomes or other achievements.
Nothing to Report.

3. What opportunities for training and professional development has the project provided?
Nothing to Report.

4. How have the results been disseminated to communities of interest?
Information is shared annually with the VDHR-SWW CP breeders about the number of DH lines selected and harvested in a given spring. For example, breeders were notified of the 63 DH lines (and their pedigrees) that were selected in the Florence FHB Nursery, along with the amount of remnant seed available for sharing up to 5 grams per breeder (8 breeders * 5 grams = 40 grams). Unfortunately, only 12 of these 63 had more than 120 grams of harvested seed due to very low mean seed number per DH received back from Heartland Plant Innovations (Table 2).

Table 2. DH lines selected in spring 2022 with available remnant seed for sharing with VDHR-SWW CP breeders.

Designation	Pedigree	Weight
SC21DH240-1	SCGA17VADH-FHB-MAS1702-179-6 / TX16DDH579	66.3
SC21DH145-66	GA15VDH-FHB-MAS23-18LE43F / 14VDH-SRW14-150	60.0
SC21DH145-88	GA15VDH-FHB-MAS23-18LE43F / 14VDH-SRW14-150	46.3
SC21DH240-105	SCGA17VADH-FHB-MAS1702-179-6 / TX16DDH579	31.9
SC21DH240-90	SCGA17VADH-FHB-MAS1702-179-6 / TX16DDH579	29.3
SC21DH145-68	GA15VDH-FHB-MAS23-18LE43F / 14VDH-SRW14-150	26.7
SC21DH145-70	GA15VDH-FHB-MAS23-18LE43F / 14VDH-SRW14-150	22.4
SC21DH145-86	GA15VDH-FHB-MAS23-18LE43F / 14VDH-SRW14-150	15.8
SC21DH240-113	SCGA17VADH-FHB-MAS1702-179-6 / TX16DDH579	15.4
SC21DH088-124	15VDH-FHB-MAS38-01 / LA12275DH-56	13.9
SC21DH088-99	15VDH-FHB-MAS38-01 / LA12275DH-56	8.7
SC21DH240-112	SCGA17VADH-FHB-MAS1702-179-6 / TX16DDH579	5.7

Project 2: 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.)*

a) What were the major activities?

Objective 1. Similar to the prior year, the inoculated FHB nursery in Florence, SC included replicated entries from the Uniform Southern Scab Nursery (USSN), Uniform Southern Soft Red Winter Wheat Nursery (USSRWWN), Gulf Atlantic Wheat Nursery (GAWN), and SunWheat. The nursery was successfully planted on 28 November 2022, and a favorable production environment provided uniform and healthy headrows leading up to spring inoculation. Inoculum was developed from a collection of isolates provided by Dr. Christina Cowger in January 2023 using the mung tea protocol. The mung tea solution containing adequate *F. graminearum* spores was poured onto autoclaved corn kernels held in metal catering trays. Kernels imbibed with this mung tea inoculum were kept at room temperature for 10-14 days and then dried on greenhouse benches for another 3-7 days. Two batches of inoculum were prepared to account for split grain spawn applications that were completed on 15 March and 29 March (exactly two weeks apart) prior to wheat heading. The advanced nursery entries were administered a total of 40 g m^{-2} while DH rows (*n*=337) and F5 breeding lines (*n*=2,902) were administered 20 g m^{-2} of scabby corn to encourage enough pressure to display symptoms but avoid too high of pressure to prevent seed increase for next season's yield testing. FHB field ratings were collected at two timepoints in May to avoid maturity bias, and headrow plots (*n*=633) from the FHB misted nursery were manually harvested on 30 May and threshed without air to obtain seed samples of every plot. As of 25 July 2023, all seed samples from the USSN, SC OVT, and USSRWWN have been threshed, cleaned, imaged with the Vibe QM3 for FDK, ground, and shipped to the VA Tech DON Lab for testing.

Objective 2. Tissue growouts were completed in October 2022 for collection and subsequent DNA extraction (DNA extraction completed by the Eastern Regional Small Grains Genotyping Lab) enabled genome-wide sequencing using Illumina sequencing to generate ~30,000 SNP markers for genomic prediction. Predictions (GEBVs) were completed by Jeanette Lyerly at NC State in March 2023 for 680 Clemson breeding lines (23 DHs, 647 F_6/F_7s, and 10 H25/H33 introgressions). The GEBVs (for yield, test weight, DON, FDK, Hessian fly, rusts, etc.) predicted for these entries in the 2022-2023 wheat preliminary trial (WPT) were used along with yield data to support selection decisions in complement to field data.

Objective 3. To create a FHB-specific multi-parent advanced generation intercross population (MAGIC), initial crossing of FHB parents (Table 2) to combine most native QTLs for FHB resistance was successful in the 2022 spring wheat crossing nursery. The first round of intercrossing (F_1 x F_1; or 4-way) was completed (12 unique crosses based on a full diallele crossing scheme with four F_1 pedigrees) in early February 2023. Resulting four-way crosses will be grown in propagation trays, tissue will be collected from seedlings, and tissue samples will be sent to the ERSGGL for marker analysis. Four-way seedlings with best combinations of FHB resistance alleles will continue through vernalization for a third and final round of intercrossing to obtain eight-way recombinant progeny. Development of this intercross population is intended for the sole purpose of cultivar development, by stacking effective all FHB QTL into a single line that demonstrates adaptation across the southeastern USA.

Postdoctoral research fellow Dr. Carolina Ballen-Taborda submitted a manuscript to Theoretical and Applied Genetics in May 2023 that detailed results on a retroactive analysis of prediction progeny performance with genomics using the "PopVar" R package. This manuscript highlighted opportunities for making dedicated crosses using breeding lines across research programs that would provide an increased likelihood of generating segregating progeny that possessed valuable trait combinations and general adaptation. This manuscript is still under review as of 25 July 2023.

Table 2. List of parents and their FHB resistance QTL selected the development of a multi-parent advanced generation intercross (MAGIC) population.

Parent	**FHB QTL**
AR15V31-26-2285N	Fhb1, F5ANi, F1AN
15VDH-FHB-MAS33-13	Fhb1, F3BM
GA151313-LDH-192 -20E48	F1BJ, F1AN
GA161240LDH-113 -20LE6	F1BJ, F1AN
NC11546-14	Fhb1, F1BJ, F3BB, F1AN, F6AN
NC18-16913	Fhb1, F1BJ, F1AN, F3BM, F6AN
SCLA19WF2110	F1BJ, F1AN
Hilliard	F1BJ

The biggest hurdle foreseen is the ability to successfully disseminate FHB resistance data collected from the project to wheat growers and other stakeholders now that ScabSmart has been decommissioned. The plan that came out of attending the Steering Committee in Minneapolis in April 2023 was to work more closely with local extension and the USWBSI management (MGMT) team to find ways to ensure information and data reach the target audience, which is predominantly seedsmen, growers, and end-users.

b) What were the significant results?

The Florence, SC inoculated FHB nursery displayed above average FHB symptoms in spring 2023. The mean FHB rating was 3.3 while mean FDK was 15.2 using the Vibe QM3. In the Florence FHB nursery, four of the eight Clemson (SC) entries were in the top category of FHB resistance based on FDK using the Vibe QM3 (Table 2).

Table 2. Current FHB resistance data on standard checks and Clemson entries from the 2022-2023 Florence FHB nursery.

DESIGNATION	HD	FHB	FDK
ERNIE	92.5	4	20.9
COKER9835	95.5	6	19.3
BESS	98.5	1.5	17.1
JAMESTOWN	86.5	5.5	10.1
SS 8641	92	7	44.0
15VDH-FHB-MAS22-14	89.5	2.5	6.8
SC19WF3P0304-5	91.5	2	8.4
SC22W145	95	2	9.3
SC22W198	92	2.5	6.2
SC22W205	98.5	2	11.2
SC22W416	96.5	1.5	5.6
SCLA18WF0304-13	91	4	12.8
SCLA18WF0708-12-2	90.5	4.5	17.7
SCLA18WF0708-4-1	91	3.5	17.0
Mean	**92.9**	**3.5**	**14.7**
CV(%)	**1.3**	**25.7**	**23.3**
LSD(.10)	**2.1**	**1.4**	**5.9**
R-square	**0.93**	**0.79**	**0.87**

Genomic predictions on 680 Clemson WPT entries were completed in March 2023 (led by NC State) to assess yield, test weight, FHB resistance, and other traits. This information was used in conjunction with combine data and field observations to make best selection decisions to advance lines to later stage testing in 2023-2024. Predictions on FHB resistance have shown to be quite accurate to increase the mean level of resistance in the Clemson breeding program and increase the frequency of FHB QTL in isolation or in combination.

Four-way intercrosses were completed in March 2023 using a MAGIC full diallele mating design, with eight parents in total that combine to have eight unique FHB QTL (Table 1). Plants from these intercrosses will be vernalized, genotyped, and transplanted for a second and final round of intercrossing to create eight-way combinations (all eight parents represented once in every pedigree) that will collectively be segregating for all eight FHB QTL. This approach should result in segregating progeny that have unique FHB QTL stacks and recombinations that lead to either direct cultivar releases or parents for subsequent crossing and introgression of multiple QTL for FHB resistance.

c) List key outcomes or other achievements.
Nothing to Report.

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

PI Boyles continues to attend the Annual FHB Forum and get exceptional networking experience every December. Dr. Boyles attended the meeting in Tampa 2022 and is scheduled to present his recent work on FHB resistance breeding in Cincinnati in December 2023. Dr. Boyles now is chairing the VDHR-SWW CP committee and on the USWBSI Steering Committee to get additional administrative and service experience. Again, meeting associated with these responsibilities are providing excellent networking opportunities with people in the field of plant breeding and pathology.

Postdoctoral research fellow Dr. Carolina Ballen-Taborda has been working closely with Jeanette Lyerly at NC State and others (including Dr. Gina Brown Guedira) to build capabilities in genomics, bioinformatics, and computational sciences, especially as these relate to genomic prediction.

William Caughman and Ryan Holmes, research associates in the program, are now well-versed to lead pathology-related tasks including FHB inoculum preparation and application. Mr. Caughman now has five years of experience with the misted FHB irrigation system that is deployed to increase relative humidity and spread of disease throughout the nursery. These are practical skills that will be used in future experiments and certainly a reliable asset for supporting cultivar development efforts to develop new crop varieties.

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

FDK results and FHB field ratings from the 2022-2023 inoculated FHB nursery for the Uniform Southern Scab Nursery were circulated to VDHR-SWW CP breeders and cooperators on 26 June 2023. FDK results and FHB field ratings for the SC OVT and USSRWWN were disseminated to the VDHR-SWW group 26 July 2023. Genomic prediction results led by NC State are available to all SunGrains breeders through Google Drive. Final SC OVT FHB results once DON data are received back from VA Tech will be incorporated into Clemson's new online variety selection tool, Medius. The population variance work completed by Dr. Carolina Ballen-Taborda on FHB and other important wheat traits have been submitted to Theoretical and Applied Genetics for peer-review.

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your FHB work that were a result of funding from your FY22 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period** should be included.

Did you publish/submit or present anything during this award period May 1, 2022 – April 30, 2023?

☒ Yes, I've included the citation reference in listing(s) below.

☐ No, I have nothing to report.

Journal publications as a result of FY22 award

List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like.

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published [include DOI#]; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Ballén-Taborda C, Lyerly J, Smith J, Howell K, Brown-Guedira G, DeWitt N, Ward BP, Babar MA, Harrison SA, Mason RE, Mergoum M, Murphy JP, Sutton R, Griffey CA, Boyles R. Identifying superior parental combinations through simulation of progeny performance in winter wheat. Theor Appl Genet.
Status: Under review, Acknowledgment of Federal Support: Yes

Boyles R, Ballén-Taborda C, Brown-Guedira G, Costa J, Cowger C, DeWitt N, Griffey CA, Harrison SA, Ibrahim A, Johnson J, Lyerly J, Marshall DS, Mason RE, Mergoum M, Murphy JP, Santantonio N, Saripalli G, Sutton R, Tiwari V, Van Sanford D, Winn ZJ. Approaching 25 years of progress toward Fusarium Head Blight resistance in southern soft red winter wheat (*Triticum aestivum* L). Plant Breeding.
Status: Accepted, Acknowledgment of Federal Support: Yes

Mergoum M, Johnson JW, Buck JW, Buntin GD, Sutton S, Lopez B, Chen Z, Mailhot DJ, Bland D, Harrison SA, Murphy JP, Mason RE, Sutton RL, Babar MA, Brown-Guedira GL, Ibrahim AMH, Boyles R, Baik B-K, Marshall DS, Griffey CA, Cambron SE, Chen X, Cowger C (2022) A new soft red winter wheat cultivar 'GA 08535-15LE29' adapted to Georgia and the US southeast region. *J Plant Registr* 16:597–605. doi:10.1002/plr2.20235
Status: Published, Acknowledgment of Federal Support: Yes

Ballén-Taborda C, Lyerly J, Smith J, Howell K, Brown-Guedira G, Babar MA, Harrison S, Mason RE, Mergoum M, Murphy JP, Sutton R, Griffey CA, Boyles R* (2022) Utilizing genomics and historical data to optimize gene pools for new breeding programs: A case study in winter wheat. *Front Genetics* 13:964684. doi:10.3389/fgene.2022.964684
Status: Published, Acknowledgment of Federal Support: Yes

Winn Z, Lyerly J, Ward B, Brown-Guedira G, Boyles R, Mergoum M, Johnson J, Harrison S, Babar A, Mason RE, Sutton R, Murphy JP (2022) Profiling of Fusarium head blight resistance QTL haplotypes through molecular markers, genotyping-by-sequencing, and machine learning. *Theor Appl Genet* 135:3177–3194. doi:10.1007/s00122-022-04178-w
Status: Published, Acknowledgment of Federal Support: Yes

Books or other non-periodical, one-time publications as a result of FY22 award

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (book, thesis, or dissertation, other); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report.

Other publications, conference papers and presentations as a result of FY22 award

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication.

Boyles R. Building greater resiliency in southern small grains, NC and SC Seedsmen Association, Charleston, SC (24 July 2023)
Status: Oral presentation given, Acknowledgment of Federal Support: Yes

Boyles R. Spanning state lines and land-grants to enrich small grains cultivar development, NC Small Grain Growers Association, Durham, NC (11 Jan 2023)
Status: Oral presentation given, Acknowledgment of Federal Support: Yes

Ballen-Taborda C, Lyerly J, Smith J, Howell K, Brown-Guedira GL, Babar MA, Harrison S, Mason RE, Mergoum M, Murphy JP, Sutton RL, Griffey CA, Boyles R. Leveraging Genomics and Historical Data to Accelerate Wheat Cultivar Development in South Carolina, ASA, CSSA, & SSSA International Annual Meeting, Baltimore, MD (November 8, 2022).
Status: Abstract published and oral presentation archived online, Acknowledgment of Federal Support: Yes

Boyles R. Harnessing diversity and leveraging genomics for cereal crop improvement, University of Nebraska, Lincoln, NE (October 21, 2022)
Status: Oral presentation given, Acknowledgment of Federal Support: Yes