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

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

<u>**Objective 1.**</u> Phenotype screening of FHB resistance for advanced breeding lines.
<u>**Objective 2.**</u> Genetic screening to facilitate genomic and marker-assisted selection.
<u>**Objective 3.**</u> 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?

<u>***Objective 1.*** *Screening breeding lines for FHB resistance*</u>
The spring 2023 inoculated FHB nursery was a resounding success as there were above average FHB symptoms found consistently throughout the 1.5 acre nursery, which was planted at the Pee Dee Research and Education Center in Florence, SC. The FHB grain spawn inoculum was developed by research technician William Caughman in February after recieving isolates from Dr. Christina Cowger (ARS-Raleigh). This grain spawn inoculum was split-applied by hand two weeks apart to space out applications over various periods of wheat growth stages. Inoculum application followed establishment of mist-irrigation using a robust filtered sprinkler system that was purchased using USWBSI funding received in 2019.

Heading date was collected for each entry headrow to examine the correlation between maturity and FHB resistance traits. In May 2023, research technician Ryan Holmes collected FHB field ratings (two time points based on genotype maturity) while the entire program assisted with headrow harvest for subsequent FDK and DON analysis. There were 625 samples that were threshed, sieved, and analyzed for FDK using the Vibe QM3 Grain Analyzer. All but one of these samples were then submitted to the VA Tech DON Testing Laboratory in three separate batches. Samples that were analyzed represented the following advanced trials: Uniform Southern Scab Nursery (USSN), SC Official Variety Trial (OVT), Uniform Southern Soft Red Winter Wheat Nursery (USSRWWN), Gulf Atlantic Wheat Nursery (GAWN), and SunWheat. Each entry from these trials were replicated twice in the field. Final DON data was received from VA Tech in November 2023. Statistical analysis was run on these datasets, and results were shared with cooperating breeders.

For the 2023-2024 inoculated FHB nursery in Florence, all headrows were successfully planted on November 15, 2023. Over the winter, inoculum was prepared again by technician William Caughman after receiving new isolates from Dr. Christina Cowger. Grain spawn inoculum was administered in two applications in March when most plants were 2-4 weeks from heading. A dry spring hurt the spread of FHB symptoms throughout the nursery, but infected spikes were observed in susceptible check 'SS 8641' on April 16, 2024. Field ratings were collected and samples were harvested without any issues, and samples are currently in various stages of processing. Samples from the USSN and USSRWWN, which are given highest priority, were shipped to the VA Tech DON Testing Laboratory on July 15, 2024.

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

A total of 501 Clemson wheat breeding lines (63 selected DHs and 438 selected $F_{5:6}$s) were grown out in October 2023 to collect seedling leaf tissue. Tissue was collected from 2-week old wheat seedlings for each genotype by research technicians and graduate students in the program. The USDA Eastern Regional Small Grains Genotyping Lab in Raleigh received the plates of desiccated tissue for DNA extraction and preparation for Illumina sequencing. Sequencing was outsourced to the Michigan State genomics core facility, and raw reads were successfully shared in January 2024. The established genomic prediction training population was leveraged to run the new breeding lines through the prediction pipeline seed, which was led by Jeanette Lyerly at NCSU. Genomic estimated breeding values (GEBVs) for 498 of the 501 Clemson breeding lines were shared late March 2024, in time of field selections and combine harvest. Using the genome-wide SNP data, markers were imputed using machine learning to predict the presence or absence of important QTL such as Fhb1, F1B Jamestown, H13, Yr17, and Sbm1. All of this information, in addition to field observations on various agronomic and disease traits, allowed PI Boyles to confidently select the best lines (~80 of the 498) to advance for multi-location, replicated testing in 2024-2025.

Objective 3. *Prediction of progeny variance for FHB resistance and other key traits.*

The SunGrains multi-location, multi-year, and multi-trait historical genotypic and phenotypic data was compiled and used to train a robust linear model to predict superior cross-combinations for determining the best wheat crosses to make for downstream selection. This dataset consisted of 1,411 lines, 29 locations (in 10 States), 15 years (2008-22), and the following phenotypic numbers: 20,470 observations for grain yield, 17,419 for test weight, 14,534 for heading date, and 9,368 for plant height. Cross-combination parameters were also generated for FHB traits using a historical phenotypic dataset collected from VDHR-SWW CP inoculated FHB nurseries. Raw phenotype values were used to estimate genetic values for these traits by fitting the following linear model using the 'lme4' package in R. The R package PopVar (Mohammadi et al. 2015) was used to simulate progeny means and genetic variance of all possible pairwise parental combinations in a half-diallel mating design. PopVar parameters estimated and evaluated were progeny mean, genetic variance, inferior 10% of progeny mean, and superior 10% of progeny mean. Ridge-regression best linear unbiased prediction (rrBLUP) was used to cross-validate GEBVs and to estimate marker effects. For rrBLUP, simulations for each population 'nSim' was set to 25, the predicted progeny size per cross 'nInd' was set to 200, and other parameters were left as default. To understand the influence of input data (SNP number or phenotype) on predictions, four simulation experiments were completed using varying combinations of input parameters. For each trait, Pearson's correlations between predicted progeny parameters and observed values of lines advanced from those families were calculated. Relationships were further characterized through scatter plots between predicted PopVar parameters versus observed traits to study whether predictions for the 670 SunGrains lines agreed with breeders' decisions to advance lines to later stage evaluation nurseries (SunWheat, GAWN, and USW) or to drop from the breeding program following preliminary evaluation (SPE and SPL). For further details on the results, please see Materials and Methods in Ballén-Taborda et al. 2024 Crop Science (doi:10.1002/csc2.21266).

What were the significant results?

Objective 1. *Screening breeding lines for FHB resistance*

Of the 624 samples with DON data from the 2022-2023 FHB nursery, the mean DON level was 5.9 ppm (**Table 1**). However, the range was from 0.1 to 47.8 ppm. From these 624 samples, the Pearson pairwise correlation between DON and FDK estimated from the Vibe QM3 was 0.78. This strong relationship is critical because breeders are also to make timely selection decisions on FDK with confidence as DON data results frequently are received after the deadline for advancing lines to the next testing stage. As a reference, the two samples with the lowest reported DON value were ranked #3 and #7 for lowest % FDK (out of 624 samples). The two samples with the lowest FDK estimates had DON values of 0.36 and 0.4 ppm (sixth and seventh lowest value, respectively). In the Uniform Southern Scab Nursery (USSN) evaluated in Florence, 10 entries of 57 total had mean DON values lower than the standard FHB resistance check Bess value of 1.22 ppm. There were 25 experimental entries that outperformed the resistance check Ernie (DON of 2.4 ppm), including four of the eight entries submitted from the Clemson program. Seven of the eight SC entries had DON values below the trial mean of 4.2 ppm. For reference, susceptible checks Coker 9835 and SS 8641 had average DON values of 13.8 and 18.5 ppm, respectively. This clearly shows the trend of breeding programs strengthening their level of FHB resistance circulating in gene pools. Six of the eight programs had one or more entries that displayed better FHB resistance than Ernie. While overall DON levels were higher for the SC Official Variety Trial entries compared to the USSN entries, there were 46 samples of the 150 (31%) evaluated that reported a DON value below Ernie's 2.4 ppm average. This shows significant progress has been made to commercialize wheat cultivars with moderate resistance to scab in the region.

Table 1. Final statistics from 2023 are reported for Fusarium-damaged kernels (FDK) and deoxynivalenol (DON).

	Samples	FDK, %			DON, ppm		
Nursery	*n*	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**
USSN	126	2.3	15.2	52.6	0.3	4.2	25.2
OVT	150	2.3	17.6	55.5	0.1	6.1	36.0
USSRWWN	74	4.5	16.1	37.4	0.4	4.6	19.7
GAWN	118	3.7	21.1	65.4	0.1	7.7	47.8
SunWheat	160	4.1	19.8	48.9	0.2	6.4	30.3
Total	628	2.3	18.2	65.4	0.1	5.9	47.8

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

Genomic predictions on 498 Clemson breeding lines (435 F_6s and 63 DHs) were finalized in late March 2023 (led by Jeanette Lyerly) to generated GEBVs for yield, test weight, FHB resistance, and many other important traits. In addition to GEBVs, marker calls for 33 QTL (nine FHB resistance QTL) were predicted from a custom developed machine learning model to use flanking SNPs near established PCR-based marker sites to impute (*i.e.*, predict) allele presence at these loci. This added value produced from genome-wide marker data increases the selection accuracy in preliminary breeding generations and facilitates the stacking of FHB resistance QTL much more easily by tracking their presence/absence at earlier stages in the breeding pipeline. Joint GEBVs and marker calls were used in conjunction with combine data and field observations to make line selections to advance into 2024-2025 later stage, multi-state yield trials. GEBVs and QTL predictions related to FHB resistance remain accurate to make progress toward increasing the baseline level of FHB resistance in the Clemson program, as well as identify top lines that contain 3-6 favorable alleles at known FHB resistance QTL.

Objective 3. *Prediction of progeny variance for FHB resistance and other key traits*

The PopVar prediction tool was utilized to inform crossing decisions in the 2024 greenhouse crossing nursery (**Fig 1**). In this cycle, 322 crosses (115 topcrosses and 207 biparentals) were made based on priority assignments given by PopVar and QTL combinations. Seed from 88 crosses were packaged in headrow trays in early March and shipped to Fort Collins, CO to plant and advance the F_1s in a summer nursery in order to receive F_2 seed in time for fall planting in SC. The progeny prediction technology was adopted after finding, from the excellent work by Dr. Ballén-Taborda, that mean, superior mean, and genetic variance parameters that are generated from the models were moderately accurate for several traits. While the program is currently limited to low accuracy for yield and yield-related traits, agronomic traits such heading date and plant height are predicted much better, which can narrow down the populations that are more likely to possess lines that meet agronomic and production criteria for cultivar release.

Parent 1 Desig	Parent 2 Desig	Priority	Comb QTL	Index	YLD μsp	YLD Rank	Yield σ2	TW μsp	TW Rank	HD μ	PH μ
GA141045-9-3-2-21LE7	NC15305-43	1	H13a,F1AN,Yr17,Sbm1H13a,Fhb1,F1BJ,F4AN,Yr17,Lr18,Pm1a_het,Sbm1	0.44	72.2	154	3.3	58.4	161	104.8	35.3
LAAR15166W-30-1-3	NC14757LDH-44	1	Fhb1,F1BJ,F1AN,Yr17,Yr4BL,Sbm1H13a,F1BJ,F4AN,F6AN,Yr17,Pm1a_het,Sbm1	0.60	70.1	352	5.0	58.1	270	102.5	34.5
NC13217-W2111	NC14757LDH-44	1	Fhb1,Lr18,Sr24,Yr4BL,Pm1aH13a,F1BJ,F4AN,F6AN,Yr17,Pm1a_het,Sbm1	0.51	70.7	295	5.7	56.9	496	104.6	33.8
SC19DH146-2	GA141045-9-3-2-21LE7	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13a,F1AN,Yr17,Sbm1	0.35	73.6	53	2.9	58.8	48	103.7	34.6
SC19DH146-2	NC14757LDH-44	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13a,F1BJ,F4AN,F6AN,Yr17,Pm1a_het,Sbm1	0.20	73.9	38	5.7	57.8	407	102.5	33.1
SC19DH146-2	NC15305-43	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13a,Fhb1,F1BJ,F4AN,Yr17,Lr18,Pm1a_het,Sbm1	0.27	74.3	19	3.5	58.2	252	103.8	34.7
SC19DH146-2	SC19DH462-5	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1HF_7D_het,F1BJ,Yr17_het,Lr18,Sbm1	0.19	75.2	4	4.7	58.1	262	103.6	34.2
SC19DH146-2	SC22W129	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1HF_7D,F1BJ,F1AN,Yr17,Lr18,Pm54,Sbm1_het	0.24	75.2	5	3.7	58.3	208	101.2	35.6
SC19DH146-2	SC22W205	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13a,F1AN,F4AN,F6AN,F3BM,Lr18,Sbm1	0.25	73.0	78	5.8	58.3	210	102.4	33.3
SC19DH146-2	SC22W281	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13a,HF_7D_het,F1AN_het,Yr17,Lr18,Sbm1	0.48	72.2	149	2.8	58.8	39	101.9	34.2
SC19DH146-2	SC22W361	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13a,Fhb1_het,F1BJ,F1AN,Yr17,Lr18,Sbm1	0.40	73.7	47	2.4	58.7	55	103.2	34.2
SC19DH146-2	SC23W106	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13,Fhb1,F1AN,F1BJ,F4AN,Lr18,H7D,Yr17,Sbm1	0.30	74.2	25	3.1	58.3	199	102.9	33.3
SC19DH146-2	SC23W143	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13,F1AN,Lr18,Sbm1	0.32	75.8	1	2.6	58.4	151	102.8	35.4
SC19DH146-2	SC23W199	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13,F1AN,F1BJ,Lr18,Yr17,Sbm1	0.31	73.9	36	3.2	58.7	73	102.2	34.8
SC19DH146-2	SC23W557	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13,F1AN,F4AN,Lr18,Yr17,Sbm1	0.34	74.2	26	2.7	58.5	110	102.3	34.5
SC19DH146-2	SCNC14761-42_H25	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13,F3BM,F6AN,Sbm1	0.30	72.3	145	7.3	58.3	181	103.1	33.7
SC19DH146-2	SCNC19VDH-FHB-MAS02-31	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13, Fhb1	0.33	74.1	28	2.8	58.3	189	103.3	34.3
SC19DH146-2	SCNC22-10569	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13, Fhb1het, Bdv2	0.26	73.4	60	4.8	58.1	305	102.6	33.0
SC19DH146-2	SCNC22-5229	1	Fhb1,F1BJ,Yr17,Lr18,Sbm1H13	0.32	72.7	102	4.6	58.1	286	103.9	34.1
SC19DH462-5	GA17634DH-08-21E36	1	HF_7D_het,F1BJ,Yr17_het,Lr18,Sbm1Yr17,Sr_6D,Yr4BL,Sbm1	0.28	72.7	107	6.2	57.8	394	102.5	33.8
SC19DH462-5	LAAR15166W-30-1-3	1	HF_7D_het,F1BJ,Yr17_het,Lr18,Sbm1Fhb1,F1BJ,F1AN,Yr17,Yr4BL,Sbm1	0.42	71.8	192	4.7	58.3	170	103.7	35.6
SC19DH462-5	NC14757LDH-44	1	HF_7D_het,F1BJ,Yr17_het,Lr18,Sbm1H13a,F1BJ,F4AN,F6AN,Yr17,Pm1a_het,Sbm1	0.31	72.6	124	5.4	57.3	479	103.4	33.3
SC19DH462-5	NC15305-43	1	HF_7D_het,F1BJ,Yr17_het,Lr18,Sbm1H13a,Fhb1,F1BJ,F4AN,Yr17,Lr18,Pm1a_het,Sbm1	0.31	73.2	70	3.8	57.7	434	104.7	34.9
SC19DH462-5	SC22W129	1	HF_7D_het,F1BJ,Yr17_het,Lr18,Sbm1HF_7D,F1BJ,F1AN,Yr17,Lr18,Pm54,Sbm1_het	0.21	74.4	18	4.8	57.9	370	102.1	35.8
SC19DH462-5	SC22W281	1	HF_7D_het,F1BJ,Yr17_het,Lr18,Sbm1H13a,HF_7D_het,F1AN_het,Yr17,Lr18,Sbm1	0.47	71.6	209	4.0	58.2	222	102.7	34.4

Figure 1. PopVar output that generates best cross-combinations between parents available in the winter 2024 wheat greenhouse nursery. Blue highlighted marker lists in the combined QTL column denote pedigrees that would be fixed or segregate for Fhb1.

List key outcomes or other achievements.

The Clemson breeding program has greatly improved in its ability to advance competitive breeding lines into final evaluation stages. For example, all three SC entries in the 2023-2024 USSRWWN yielded above the mean (over all locations), including SCGA151058-2, which was the 2nd highest yielding entry in the trial (**Fig 2**). SCGA151058-2 was the 4th highest yielding entry in the NC statewide variety trial (OVT) in 2023-2024 while another line, SCLA19WF2110, ranked 2nd in statewide yield in this same trial. The Clemson breeding program is working to finish up analyzing 2023-2024 data to make decisions on releasing these advanced breeding lines as cultivars for commercialization.

DESIG	PED	YLD_BUPA	TW_LBBU	HD_JUL	HT_IN	PMD09	STRIPE09	LFRUST09	BYDV09	HF_PIT	BLS09	FHB09
VA21W-112		82.0	55	106	36	2	0	1	0	6	4	6
SCGA151058-2		81.2	58	103	37	2	0	1	3	65	5	4
LA15203-LDH197		81.1	56	103	39	2	0	2	1	13	3	3
Hilliard		79.2	56	106	39	2	0	1	1	45	5	4
GA141028-13-3-4 -22LE25		78.7	55	108	34	1	2	0	0	0	5	6
KWS543		78.6	56	109	36	1	0	6	2	38	3	3
Pioneer Brand 26R41		77.3	55	109	35	3	0	4	1	0	3	4
LA18003-NDH119		76.9	57	105	41	1	1	0	1	6	3	3
GA15577-3-10-2 -22LE38		76.9	57	105	38	2	3	1	1	20	3	5
TX20D5145		76.9	56	106	39	2	0	1	1	14	3	4
SCLA18WF0108-12-1		76.7	56	104	37	3	0	1	0	0	3	4
GA151450-13-6-9 -22LE29		75.8	55	108	35	2	2	0	1	26	4	7
TX20D5116		75.8	56	105	35	2	0	5	1	10	3	5
KWS542		75.4	57	108	40	2	1	4	2	0	2	3
LA17006-LDH042		75.4	58	108	37	2	0	1	1	25	2	4
TWR39925		74.6	56	102	35	4	0	2	4	9	3	5
Jamestown		74.1	57	100	35	3	0	2	1	30	4	3
18VTK5-95		73.6	55	108	36	2	1	1	1	38	3	4
TWR39919		73.4	57	106	37	2	0	3	0	48	3	4
GA16349 ID-8-1-5 -22LE31		73.4	53	108	37	2	1	1	0	0	4	6
SCLA18WF0705-4		73.2	58	105	41	3	2	0	1	14	4	4
Mean		73.1	55.6	106.8	37.5	1.9	1.8	1.6	0.9	18.7	.	4.3

Figure 2. Preliminary data report for the 2023-2024 Uniform Southern Soft Red Winter Wheat Nursery.

Experimental Summary		STATEWIDE	
Company/Brand	Variety	Yield (bu/A)	Test Weight (lb/bu)
Progeny	PGX23-15	104.2	57.9
Clemson	SCLA19WF2110	103.9	59.4
Va Tech	VA19FHB-36	100.5	58.6
Clemson	SCGA151058-2	100.0	59.4
Va Tech	VA19W-29	99.9	57.8
University of Georgia	GANC12915-167-21E3	99.5	56.3
Clemson	SCLA18WF0304-13	97.4	60.3
NCSU	SCNC16VT30-7-47	97.1	59.3
Dyna-Gro	9551	96.5	56.4
Southern Harvest	SHX3224	96.4	56.5
NCSU	SCNC13217-W2111	96.4	57.6
NCSU	NC14706-25	96.1	57.0
NCSU	NC20-21971	95.9	59.1
University of Georgia	GA15490ID-19-5-21LE2	95.2	56.8

Figure 3. Preliminary data report for the 2023-2024 North Carolina statewide variety trial.

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

Ms. Kimberly Baskins was supported by the project in FY23 as a temporary worker (*i.e.*, summer hire) to assist with 2023 wheat harvest, FHB sample processing, and data compilation and analysis. However, after the departure of a program's research technician Ryan Holmes (took a local valued position with Bayer AG) in March 2024, the program was fortunate to have Kimberly accept and fill the vacant technician position to avoid major project delays or fail to attain key research objectives. Ms. Baskins assumed this new role in May 2024 after a competitive search and screening process, and she will be funded in part by this VDHR-SWW CP. As a recent graduate of Francis Marion University, Ms. Baskins is enthusiastic about working toward these project objectives and is more than capable of doing the required tasks.

Dr. Carolina Ballén-Taborda has made exceptional progress in developing and validating a progeny prediction workflow to use genomic data on prospective wheat parent lines to predict the top cross-combinations. In other words, this technology enables the Clemson program to determine what two parents should be crossed together that give rise to superior progeny lines containing an optimal combination of genetic markers. Because of this recent progress, Ballén-Taborda gave a workshop session in July 2023 at the USDA WheatCAP Genomic Selection Workshop that was held in Raleigh, NC to share associated scripts and coding with early career wheat breeders and scientists. This provided a great opportunity for graduate students and postdocs to learn this how to properly vet and implement this technology in an applied cultivar development program. Though this, Dr. Ballén-Taborda was able to build her skills in learning how to more effectively transfer this knowledge to others and educate interested individuals on a valuable breeding tool.

PI Boyles was able to attend and present at the 2023 National FHB Forum held in Cincinnati, OH. At the forum, as chair of the VDHR-SWW steering committee, Dr. Boyles led an individual CP session as well as co-led a joint session with the management (MGMT) group. These leadership roles and ability to present research findings to the entire USWBSI FHB community certainly contributed to professional development as an early career small grains breeder and geneticist. PI Boyles, as detailed below in the dissemination section, was able to present research findings related to his VDHR-SWW project to various audiences, including key stakeholders. It is important to note that, through these events, Dr. Boyles has been expanding his collaborative research network that is fostering new proposals and additional research projects.

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

Results on progeny prediction were recently published in May 2024 by Clemson postdoc Dr. Ballén-Taborda in the journal Crop Science. Historical and recent progress on FHB resistance-related cultivar development by the southern VDHR breeders was published in the special issue "Breeding and breeding research for resistance to *Fusarium* in cereals" of the journal Plant Breeding in February 2024. Both journal articles were published with open access and thus are freely available to all. Results on FHB improvement were also presented by PI Boyles at the 2023 National FHB Forum in Cincinnati, OH. Dr. Ballén-Taborda presented research findings related to those published in Crop Science at the 2023 National Association of Plant Breeders annual meeting in Greenville, SC in July. As highlighted above, her research advances in building a

progeny prediction pipeline were shared with early career wheat breeders and scientists at a USDA WheatCAP Genomic Selection Workshop held in Raleigh, NC in July 2023. Also in July, PI Boyles presented updates on breeding winter small grains for disease and pest resistance to the NC and SC Seedsmens Associations at a joint meeting held in Charleston, SC.

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

For the first two project objectives, the same activities as the previous two years will be followed to continue effective screening of lines for FHB resistance using the inoculated nursery and genetic data, which will collectively be used to make quality breeding decisions. For Objective 3 (*Prediction of progeny variance*), we will analyze empirical field data to determine just how accurate progeny prediction can be. For this study, phenotypic data were generated on approximately 40 individual F_2 plants from 30 biparental populations (~2,400 plants per season) and their parents that were space planted in a grid design over two seasons (2021-2022 and 2022-2023) in Florence, SC at the Pee Dee Research and Education Center. This massive undertaking to generate this empirical dataset was performed to gain a better understanding of how well genomic prediction approaches can inform breeders what two parents should be crossed to develop and advance segregating populations that will ultimately give rise to superior cultivars. *Why is this so important? Why spend so much energy and effort on validating this breeding tool?* Crossing is the initial step in a breeding pipeline, and unfortunately, is a quite inefficient process as only a small fraction of families from a given crossing cycle will be represented in advanced breeding lines that reach final stages of testing. On average, about 6% of pedigrees (30 of 500) that are created from dedicated crossing and early generation advancement by the Clemson wheat program will make it to second year yield trials. Therefore, the Clemson program is vetting the predictability of population variance parameters to accurately determine what breeding lines in the crossing nursery should be crossed together. If we can find ways to improve accuracy of this tool, breeding programs can lower the number of crosses they make every breeding cycle, and if desired, increase number of plant selections within these fewer populations. By doing this, we can significantly increase breeding efficiency, return on investment, and probability of selection a superior cultivar.