FY21 FINAL Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-0-146
USDA-ARS Agreement Title:	Improving FHB Resistance in Barley and Wheat using Breeding and Genomics Methods
Principle Investigator (PI):	Nicholas Santantonio
Institution:	Virginia Tech.
Institution UEI:	QDE5UHE5XD16
Fiscal Year:	2021
FY21 USDA-ARS Award Amount:	$181,959
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USWBSI Individual Project(s)

USWBSI Research Category[1*]	Project Title	ARS Award Amount
BAR-CP	Cultivar Development, and Mapping of FHB Resistance QTL in Native Cultivars Nomini and Eve	$59,201
VDHR-SWW	Improving FHB Resistance in Winter Wheat via Traditional, GS, MAS and DH Methods	$108,527
VDHR-SWW	Double Haploids to Expedite Development of FHB Resistant Soft Winter Wheat Varieties	$14,231
	FY21 Total ARS Award Amount	$181,959

I am submitting this report as a: ☒ FINAL 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.

Principle Investigator Signature   Date Report Submitted

                                                7/26/2023

[signature: Nicholas Santantonio]

[1‡] 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: Cultivar Development, and Mapping of FHB Resistance QTL in Native Cultivars Nomini and Eve

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

The specific objectives of this project are: 1) evaluate available barley germplasm for novel sources of FHB resistance; 2) develop barley cultivars with enhanced resistance to FHB and lower DON and; 3) map and validate QTL for FHB resistance in our native winter barley sources

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?
Our program has always struggled to get good scab infection in barley due to early maturity and cool, often dry, temperatures during flowering. Harvest season 2021 was exceptionally low in disease pressure, so much so that the data was largely ignored for selection and other breeding purposes. Conversations with pathologists and other breeders suggested virulence of the pathogen may have decreased after many years under culture in the lab. Three new isolates originally collected in Virginia were obtained from the Schmale lab at Virginia Tech, and used to produce new corn spawn for the 2022 harvest season.

For the 2022 harvest season, 1,320 plots representing 707 barley breeding lines (hulled, hulless and malt) from the VT program, uniform FHB nurseries, regional trials, and other USWBSI collaborators were evaluated for FHB resistance in an inoculated, misted nursery at EVAREC in Warsaw, VA. Uniform nurseries screened in the scab nursery included the Winter Malt (32 entries) and the NABSEN (45 entries), while regional nurseries included the Eastern Malt (42 entries). Additionally, breeding populations derived from crosses made with FHB resistance sources (Island, AC Alberte, Atahulpa, Quest, MN Brite, FEG-4-98, and Fredrickson) are in advanced generations. This season (2022), pure lines were evaluated and selected from 941 hulled, malt and hulless FHB headrows alongside the standard breeding pipeline headrows (9,520 hulled/malt and 831 hulless) at the Eastern Virginia AREC in Warsaw, VA. Forty eight segregating barley populations for FHB resistance in our scab nursery were evaluated and advanced in the program.

Our program is currently working on a new effort to genotype all new Virginia Tech lines that enter into yield trials and the scab nursery using Genotyping By Sequencing (GBS). Virginia breeding lines and varieties from our first year, second year, advanced and regional barley yield trails were planted in the greenhouse in the fall of 2021 for tissue sampling, totalling 565 barley lines. This information is now starting to be used to train genomic prediction models for FHb and agronomic traits, leading into our FY22 proposal. Predictions of FHB traits will be used for parent and mate pair selection, as

well as early generation line advancement. A GBS pipeline was developed to call and filter variants to allow for annual updates of new genotypes and training models.

b) **What were the significant results?**

Fortunately, year 2022 had some of the highest ratings we have ever assigned, including many plots that had nearly 100% infection of almost all kernels, resulting in many FHB scores of 9 on the 0-9 scale. FDK scores confirmed this, with genetic FDK effects as high as 73% (observed for line VA19M-DH170735 LA in the eastern malt).

Table 1: Collaborative nursery means, broad-sense heritability, and error standard deviation for FHB scores (0-9) and FDK (%) for the 2022 scab nursery.

Year: 2022	Winter Malt		Eastern Malt		VA State Barley		NABSEN	
Trait	FHB	FDK	FHB	FDK	FHB	FDK	FHB	FDK
Mean	5.52	46.87	4.76	40	5.94	42.44	5.39	23.2
Broad-sense heritability	0.73	0.50	0.58	0.57	0.61	0.44	0.71	0.2
Error standard deviation	1.09	15.03	1.09	12.71	1.2	13.25	1.0	17.94

In spring of 2021 and spring of 2022, most of the barley crosses included at least one FHB resistant parent (including ARS15B12, VA13B-25 LA, VA16BFHB-268 NA, 12ID2, and 'Greg' (tested as VA15H-73)). A total of 150 new explicit FHB crosses were made across 2021 and 2022, however FHB traits are considered in almost all crossing decisions. Forty eight barley breeding populations were evaluated in an inoculated, mist irrigated scab nursery. Individual heads were selected from the best performing families and will be planted in fall of 2022, representing approximately 800 headrows.

Preliminary results from our GBS pipeline produced 45,402 markers for 559 lines. Missing data were imputed with Beagle, and we are beginning to work on LD pruning, and other marker filtering. Genomic prediction accuracy will be assessed this winter to determine how well the genotype phenotype information can be used for FHB trait selection.

Mapping populations of Thoroughbred / Nomini (RIL) and Nomini / Violetta (DH) were evaluated in an inoculated mist irrigation nursery in Virginia in 2018 and 2019, as well as Kinston, NC in 2020, where FHB ratings (0-10) in each population averaged 4.8 and 4.2, respectively. This location provided the only reliable data in 2020 as very cold conditions in Virginia and freezing temperatures in Kentucky severely damaged the barley crop. Preliminary linkage analysis has identified several QTL regions in the Thoroughbred / Nomini RIL population along chromosome 2H. Similarly, in the Nomini / Violetta DH population QTL regions were identified on chromosomes 2H and 7H. However, significant correlations differentiating row-types, plant height, and flowering

dates with FHB traits were seen in this population across 2018 and 2019 in the Virginia FHB site, as previously reported by others. Notably, this region on 2H is also associated with heading date, suggesting this locus results in escape from infection. Investigation into relationships between morphological and phenological traits, and FHB resistance loci is currently being conducted to ensure that identified QTL are not the result of pleiotropic effects. Lines from both populations exhibiting FHB and DON values lower than the parents and checks across 2018-2019 with acceptable agronomic traits were advanced into observation tests in 2020-2021.

Unfortunately, the research associate that was working on the mapping population, Joshua Fitzgerald, took a new position in industry in October of 2021 before that work was completed. A new Master's student, Frank Reith, was working to reanalyze that data to verify previous results, but so far, it is unclear whether those results are repeatable. A manuscript may be prepared if results can be confirmed.

c) **List key outcomes or other achievements.**

Pure lines derived from crosses between FHB resistant spring barley lines and adapted winter barley lines are being developed and evaluated for FHB resistance and agronomic performance. Several candidate regions have been identified as targets for marker assisted selection for FHB resistance, however it is currently unclear if these methods will be as effective as they have been demonstrated to be in winter wheat. Instead, we are moving forward with a whole genome-enabled approach for prediction and selection of FHB traits, with attention being paid to loci with large effects. Towards this end, all barley lines advanced to yield trials have been genotyped with high-density GBS markers to facilitate model development for genomic prediction of FHB traits.

An awnless line, VA16BFHB-268 NA, with superior performance to Nomini as a forage barley was developed as part of this project and released in 2021. The newly released awnless cultivar expresses moderate resistance to FHB and lower DON accumulation similar to cultivar 'Nomini' and better than cultivars 'Secretariat' and 'Thoroughbred'. This resistance was shown this last year to be durable even under heavy scab pressure, ranking 9th lowest for FDK in the Virginia State barley official variety trial (OVT), with an average FHB score of 4.1, and FDK of 35%. Similarly, a two-row hulless barley line tested as VA15H-73, and released in 2020 under the cultivar name 'Greg', also demonstrated durable resistance under heavy disease pressure, ranking 3rd lowest for FDK in the Virginia State barley OVT, with an average FHB score of 3.7, and FDK of 29%. These two recent lines released from our program and now under commercial production

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

A new graduate student, Sunilda Frias, who started her PhD in fall 2021 obtained new isolates, cultured them to produce enough inoculant to develop ~800 lbs of corn spawn for use in our scab nursery in 2022. She gained new experience working with fungal pathogens to culture and create inoculum.

A Master's student, Amelia Loeb, led the effort to score FDK in barley (which is significantly more challenging than wheat), and set up a set of calibration plates with increasing numbers of FDK kernels so that students/scientists in the future will have a reference for consistency and to aid in scoring for years when disease is less prevalent or obvious.

A new Master's student, Frank Reith, worked to reanalyze FHB data collected from the previous years for this project, including the Nomini by Thoroughbred mapping population, where he identified the same loci we had previously observed to affect FHB traits in barley (notably a locus on 2H that is also associated with heading date)

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

Data on FHB index, FDK, ISK, and DON obtained from the Virginia's state barley variety trial are reported at field days and online (https://resources.ext.vt.edu/contentdetail?contentid=3239&contentname=Small%20Grains%20in%202021) to promote selection and production of FHB resistant cultivars. Additionally, all results from the scab nursery have been sent to collaborators, and data will be posted to T3 this fall.

Project 2: Improving FHB Resistance in Winter Wheat via Traditional, GS, MAS and DH Methods

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

The specific objectives of this research are to: 1) incorporate and combine resistance genes from newly improved scab resistant germplasm and/or scab-tolerant native wheat lines to develop and release commercially viable cultivars and; 2) accelerate the release of scab resistant cultivars and/or germplasm using marker assisted breeding, genomic selection and doubled haploid methods.

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?**

   1) In 2020, infection rate was low, but still allowed for reasonable estimates. However, the 2021 FHB nursery had little to no infection despite using the same inoculates to produce corn spawn, inoculation at roughly the same developmental stage and daily misting at dawn and dusk before and during anthesis. It is likely that environmental conditions were poor for disease development, but the rate of infection was so poor that the entire nursery was effectively lost, with even the most susceptible known check varieties demonstrating little to no disease. FHB visual scores, FDK and DON were measured on all regional nurseries and early generation VT materials regardless. Data analysis showed that the data was questionable at best, so to keep from having these results skew estimates and pollute prediction models, the data was largely ignored from the 2021 season.

   Conversations with pathologists, and other breeders suggested that isolates may have become less virulent after being replicated in culture for many years. For the 2022 season, we decided to use three new isolates obtained from the Schmale lab at Virginia Tech to produce corn spawn. While it is difficult to tease apart environmental conditions from the use of these new isolates, infection rates were better than we have seen in many years.

   2) Marker haplotypes linked to 9 scab resistance genes located on wheat chromosomes 2D, 3B (Fhb1), and 5A of Ning 7840, 1B of Jamestown, 2B and 3B of Bess, 3B of Massey, and 1A, and 6A of Neuse were used to screen, characterize and select parents and their progeny for FHB resistance genes. Marker haplotypes linked to 44 disease resistance, phenological or morphological traits were also screened to select parents with other important agronomic loci. Ninety eight SRW parents were selected based on observed phenotypic scab resistance, FHB marker genotypes and agronomic performance. Of these, 22 lines had positive marker scores for Fhb1.

   For the 2021 crossing season, focus was centered on increasing the frequency of Fhb1 and FHB6B-Neusse in the population due to their repeated validation in reducing FHB

index, FDK and DON levels, with less focus paid to other loci. Since, a publication out of the breeding program University of Illinois (Guare et al 2022ab) showed high genome-enabled predictability for DON using field based phenotypes (notably, FHB index and FDK). With this evidence, and in accordance with the proposed FY22 project at the time, we switched toward a genomic prediction framework for designing crosses.

A genome-enabled prediction model was trained using genotypes and phenotypes from the 2019 and 2020 FHB nursery, including 1,645 plots representing 762 breeding lines for which FHB traits and genotypic information was available for 7,200 GBS markers distributed throughout the genome. Marker effects for FHB index, FDK and DON were predicted using a GBLUP framework and backsolving for marker effects. Only FDK and DON were considered for designing crosses, as FHB indices have been shown to only be loosely correlated with these more important FHB traits. Marker effects for agronomic traits were also predicted using yield plot data from 2021, consisting of 5,334 plots representing 1,254 breeding lines across multiple locations.

A genomic selection index was constructed by weighting, albeit somewhat arbitrarily, FHB traits and other agronomic and disease traits, including grain yield, test weight, heading date and plant height, as well as leaf rust and powdery mildew resistance (for which reliable data was readily available from multiple years). Estimates of family performance were predicted by averaging the parental genotype scores (i.e. obtaining the expected genotype of offspring), and multiplying by the marker effects for each trait and then the selection index to produce a single value of family merit. Currently, this method does not estimate family variance of each trait, but genetic covariance between parents is used to keep from selecting mate pairs between close relatives. Future research will involve how to properly weight each trait using an economic index to select mate pairs for profitability instead of specific traits on the family mean level, while also maximizing the probability of selecting superior lines by using estimates of family variation for each trait and incorporating information on population size (e.g. number of pure line selections possible).

Timing of available male and female flowers is a significant limitation to pre-designed crosses, especially toward the beginning and end of the crossing period, as fertile males can be difficult to come by for a given female line that is ready to cross. A pipeline was constructed to predict the best crosses for each day given a list of ready females and available males. During the middle of crossing season, females of interest were prioritized for emasculation while males were abundant enough not to present a significant problem, so all pairs were estimated for females ready that day.

3) Segregating populations (395 F3 populations) of FHB crosses were evaluated in the misted inoculated scab nursery, and are currently being measured for FDK for selection. In 2022, heads were selected from 55 F4 and F5 families of FHB crosses to derive approximately 150 pure lines per family for the following field season.

   Due to a miscommunication, FHB head selections from Blacksburg in 2021 were accidentally threshed together, resulting in only 1,497 FHB SRW headrows from the

Warsaw location available for selection in 2022. Of these, 120 were advanced to observational yield trials for the 2023 season, along with 880 lines from other efforts, including FHB DH (36 lines, project 3) and the primary SRW production pipeline. These 1,000 lines will be genotyped in the fall of 2022. Advancement to second year yield trials for 2024 will include the use of genomic predictions of FHB traits.

Materials from the Gulf Atlantic (62 lines) and Mason Dixon (82 lines) regional cooperative nurseries were evaluated in a misted and inoculated (cultured corn kernels) Fusarium (scab) nursery. All entries from the NUWWSN (49), PNUWWSN (41) and SUWWSN (66) (totaling 152 unique lines) were evaluated in the scab nursery and included in a single replicate observational yield trial for evaluation of other disease and agronomic traits. FHB scores, FDK and DON have been collected and distributed to collaborators.

b) **What were the significant results?**

Table 1: Collaborative nursery means, broad-sense heritability, and error standard deviation for FHB scores (0-9) and FDK (%) for the 2022 scab nursery.

Year: 2022	NUWWSN		PNUWWSN		SUWWSN		Gulf Atlantic		Mason Dixon	
Trait	FHB	FDK	FHB	FDK	FHB	FDK	FHB	FDK	FHB	FDK
Mean	3.3	24.1	2.2	20.7	2.8	22.3	4.7	38.9	4.3	44.3
Broad-sense heritability	0.64	0.58	0.67	0.52	0.78	0.70	0.69	0.48	0.82	0.52
Error standard deviation	1.17	8.7	0.66	8.1	0.70	7.0	1.04	14.7	0.88	14.0

Excellent infection was obtained for the 2022 misted inoculated scab nursery in Warsaw VA. It is unclear to what extent this was due to the use of new isolates or environmental conditions that benefitted the pathogen at the timing of infection. Regardless, our breeding program will work to obtain fresh isolates every other year or so to avoid loss of virulence in culture.

Several new experimental lines were identified with excellent agronomic performance and good FHB resistance, including VA20FHB-20 (developed in this project), 18VDH-FHB-MAS07-173-03 (project 3), 16VDH-FHB-MAS60-7-03 (project 3) and 17VTK4-29. All four have been confirmed to have at least one *Fhb1* allele through genotyping, highlighting the importance of that locus.

Table 2: Crosses sent for DH production in 2022 through genome-enabled selection or parents with complementary QTL. Lines in italics were developed through this double haploid project (3) while lines in bold were developed by project 3.

Pedigree	Year	No. DH	Fhb1[a]	FDK[b]	DON[c]	Yield[d]	Selection Index[e]
17VTK4-29 / **15VDH-FHB-MAS38-01**	2022	50	fixed	-2.22	-1.81	3.05	5.58
18VTK12-60 / **15VDH-FHB-MAS38-01**	2022	50	seg	-2.22	-2.00	2.73	5.32
15VDH-FHB-MAS38-01 // 17VTK4-29 / **15VDH-FHB-MAS33-13**	2022	60	fixed	-2.80	-1.99	2.30	5.21
15VDH-FHB-MAS38-01 / **19VT1FHB_DH-170**	2022	50	seg	-2.65	-1.64	2.04	4.94
15VDH-FHB-MAS38-01 / 15VTK-1-101 // 18VTK12-60	2022	60	seg	-1.70	-1.57	2.41	4.86
15VDH-FHB-MAS33-13 / **15VDH-FHB-MAS38-01**	2022	50	fixed	-3.37	-2.16	1.56	4.84
17VTK4-29 / **15VDH-FHB-MAS33-13** // 18VTK13-4	2022	60	seg	-1.84	-1.22	2.38	4.77
17VTK4-29 / **18VDH-FHB-MAS07-164-01**	2022	80	seg	-0.03	-1.21	2.91	4.50
15VDH-FHB-MAS33-13 / 17VTK4-29	2022	60	fixed	-2.09	-1.89	1.77	4.37

[a]status of *Fhb1* in the cross. "Fixed" means both parents are homozygous for the *Fhb1* allele, wheels seg indicates that only one parent contains the allele, and a dash indicates neither parent has Fhb1. Crosses were not designed with *Fhb1* explicitly, but were clearly favored due to low FDK and DON.

[b]Genomic prediction of FDK in standard units from the population mean (i.e. z-scores of GBLUP).

[c]Genomic prediction of DON in standard units from the population mean (i.e. z-scores of GBLUP).

[d]Genomic prediction of Grain Yield in standard units from the population mean (i.e. z-scores of GBLUP).

[e]Selection index included genomic predictions for FDK, DON, grain yield (shown in b-d), test weight, heading date, powdery mildew and leaf rust (not shown).

A total of 617 SRW crosses were conducted in the spring of 2022, of which 347 were segregating or fixed for *Fhb1*. Genomic predictions of family expectations were used for almost all crossing decisions on a daily basis using the pipeline developed to provide the best crosses given a subset of possible females and males. This included 224 topcrosses, and 79 double-crosses. Crosses sent for doubled haploid generation were chosen based on both the selection index and prior knowledge of the parents.

c) **List key outcomes or other achievements.**

1) Three SRW lines, VA17W-75, 15VDH-FHB-MAS38-01 and 15VDH-FHB-MAS33-13 with excellent to moderate FHB resistance were released in 2022. The later two were developed from DH efforts in project 3.
2) A genomic prediction and selection pipeline was developed to produce a selection index to make the best crosses given available females and males on a given day.
3) Lines from this project were advanced through all stages of the breeding program.

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

A new graduate student, Sunilda Frias, took over much responsibility in the program for the 2022 harvest year. She obtained three new isolates from the Schmale lab at Virginia Tech, increased them in culture to produce ~800 lbs of corn spawn. She also learned to score FDK and worked to produce data summaries for distribution to collaborators.

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

Agronomic and FHB traits (FHB score and FDK) of the Northern Scab Uniform Nursery, Northern Preliminary Uniform Scab Nursery, Southern Uniform Scab Nursery, Mason Dixon and Gulf Atlantic Nurseries were provided to collaborators. These results will be posted to T3 in fall of 2022.

Project 3: Double Haploids to Expedite Development of FHB Resistant Soft Winter Wheat Varieties

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

Objectives are to: (1) Increase the number of FHB resistant varieties available to farmers to reduce DON in the US grain supply by utilizing doubled haploid (DH) technology to decrease the breeding cycle leading to release of FHB resistant varieties at a faster rate with improved efficiency. A greater choice of FHB resistant varieties in the seed market is key to reducing DON presence within the national wheat supply chain. 2) Increase efficiency of coordinated project breeding programs to develop and release FHB resistant varieties. This will be accomplished by a unique sharing of selected DHs among all VDHR-SWW breeders, leading to a much larger number of FHB resistant DHs in regional yield trials in a decreased time frame. This will practically ensure that no line goes unnoticed and robust, multi-location data for individual lines will provide appropriate information needed to justify release and licensing to companies for marketing to growers. (3) Implement breeding technologies to enhance short term and long-term improvement of FHB resistance and to efficiently introgress effective resistance genes into breeding germplasm. Prior to selection of crosses for DH production, enrichment of FHB QTL and other important QTL using established markers and the USDA ARS Eastern Regional Small Grains Genotyping Lab.

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?

1) Seedlings from eight SRW single crosses were selected based on the number of fixed and segregating FHB QTL, as well as QTL for other disease traits to produce 550 DH lines that will be planted in headrows in the fall of 2022.

2) In 2022, a single cross (18VDH-FHB-MAS07-173-03 / 16VDH-SRW03-018) segregating for 7 FHB QTL was chosen to produce a larger number of DH lines (350) for selection of lines with many QTL combinations. These parents were chosen because they completely complementary FHB QTL (line 18VDH-FHB-MAS07-173-03 contains Fhb1 (3BS), FHB3B.Bess, FHB1A.Neusse, and FHB6A.Neusse, while line 16VDH-SRW03-018 contains FHB1B.Jtwn, FHB2B.Bess, FHB.3BL) yet have the same vernalization alleles, same photoperiod sensitivity alleles, and the same Rht alleles as determined by inhouse genotyping with SSR and KASP markers. Both parents were developed from this long running project, and have demonstrated strong agronomic performance, with 16VDH-SRW03-018 ranking first in the overall mean across the Uniform Southern Nursery in 2021, and 18VDH-FHB-MAS07-173-03 ranking first in the Mason Dixon trial at Warsaw, VA in 2022. We hope to select good lines with high scab resistance from this cross, but also intend to create a mapping population to investigate epistatic interactions between FHB QTL. In our last proposal (FY22), we demonstrated that FHB QTL do not appear to act in an additive fashion, and it is unclear if all have non-zero effects in all genetic backgrounds. We hope that by standardizing the genes for photoperiod sensitivity, vernalization and plant height, we can remove these confounding factors to dissect potential interactions at previously detected FHB QTL. This

effort is in contrast to the selection of DH crosses based on GEBVs of DON, FDK and other agronomic traits in Project 2.

3) Selection of lines produced through this project are being advanced to all of stages of yield trials, including first year observation (planted in Blacksburg, VA, Warsaw VA and Urbana IL) in a spare testing approach, second and third year advanced yield trials in Virginia, and in regional and uniform nurseries.

   b) What were the significant results?

   Selection of 28 lines were made among 154 DH lines developed through this project that were evaluated in headrows in the field in 2022. An additional selection was made of 8 lines from 71 DH lines shared by the South Carolina and Georgia breeding programs. These 36 lines are being advanced to first year yield trials in Blacksburg VA, Warsaw VA and Urbana IL.

   Multiple lines developed through this project have lead to elite breeding lines with enriched FHB QTL and moderate to good resistance under heavy scab pressure. These lines are now being used extensive as parents in the breeding program (see Table 1).

Table 1: Table of crosses sent for DH production in 2021 and 2022 through marker assisted selection for parents with complementary QTL. Lines in bold were developed through this double haploid project (3) while lines in italics were developed by project 2.

Pedigree	Year	No. DH	*Fhb1*[a]	# FHB alleles[b]	# FHB loci[c]	# other resistance alleles[d]	Selection Index[e]	Predicted family yield[f]
VA20W-171 / 17VTK19-15	2021	80	-	6	5	11	25.60	2.52
DH15SRW67-151 / VA19W-89	2021	50	seg	9	6	11.5	22.79	1.41
VA19W-89 / Liberty 5658	2021	50	seg	8	8	12	18.47	0.84
15VDH-FHB-MAS33-13 / VA17W-75	2021	90	seg	4	4	10	17.85	0.97
17VDH-SRW01-077 / **15VDH-FHB-MAS33-13**	2021	80	fixed	4	3	9	23.29	1.71
17VDH-SRW01-077 / DH16-SRW120-037	2021	50	fixed	5	4	8	21.86	1.62
17VTK4-29 / **15VDH-FHB-MAS33-13**	2021	90	fixed	4	3	7	20.09	1.52
17VTK6-17 / VA17W-75	2021	60	seg	4	4	10	18.76	1.25
18VDH-FHB-MAS07-173-03 / *16VDH-SRW03-018*†	2022	350	seg	7	7	12	-	

[a]status of *Fhb1* in the cross. "fixed" means both parents are homozygous for the *Fhb1* allele, wheels seg indicates that only one parent contains the allele, and a dash indicates neither parent has Fhb1.

[b]number of total FHB QTL alleles in the cross.

[c]number of loci with at least one FHB QTL allele.

[d]number of other disease resistance alleles, including leaf rust, stripe rust, powdery mildew, hessian fly, etc.

[e]Selection index with weights for genomic predictions for grain yield, known resistance genes and other QTL in 2021. Selection index in 2022 contained genomic predictions for FDK, DON, grain yield, test weight, heading date, powdery mildew and leaf rust.

[f]genomic prediction of family mean yield in standard normal units from the breeding program mean.

c) List key outcomes or other achievements.

1) Two lines, 15VDH-FHB-MAS38-01 and 15VDH-FHB-MAS33-13 were released from the program in spring 2021 that were developed directly from this DH cooperative project. Both of these lines carry *Fhb1* and have demonstrated excellent scab resistance under multiple years of evaluation, including this past year, as well as excellent agronomic performance.

2) A total of 28 DH lines out of 154 DH lines developed through this project were selected in single rows grown in the field in 2022. An additional eight DH lines selected by other cooperators in 2021 and evaluated in Warsaw VA in 2022 were selected for advancement to first year yield trials.

3) A total of 31 lines developed from this program are in later stage testing, including second (16 lines) and third (12 lines) year advanced yield trials in Virginia and regional (3 lines) nurseries.

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

Joshua Fitzgerald had been an integral part of this USWBSI project going back to 2017, serving as a scab breeder first as a postdoc and then as a research associate. He left the program in October of 2021 for an industry job, and I hope his experience in our program provided him with experience and skills that have made him successful in his new position.

A new graduate student, Sunilda Frias, took over much responsibility in the program for the 2022 harvest year. She designed and conducted the cross to develop a DH mapping population to investigate non-additive effects of FHB QTL independent of photoperiod sensitivity, vernalization and dwarfing gene effects.

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

The 28 DH lines selected from our project are being shipped to other breeding program cooperators on this project. Data from entries in later stages of trials produced from previous years of this project were distributed to cooperators, and will be uploaded to T3 this fall.

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your <u>FHB work</u> that were a result of funding from your FY21 grant award. Only citations for publications <u>published</u> (submitted or accepted) or presentations <u>presented</u> during the **award period** should be included.

Did you publish/submit or present anything during this award period?

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

X No, I have nothing to report.

Journal publications as a result of FY21 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).

Books or other non-periodical, one-time publications as a result of FY21 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).

Other publications, conference papers and presentations as a result of FY21 award

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