

Project FY22-BA-022: Genome-enabled Selection for FHB Resistance in Eastern Winter Malt and Feed Barley

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

Primary Objectives

- 1) Evaluate predictive ability of FHB traits, including visual scores, FDK and DON within the Virginia barley germplasm. Develop new barley varieties with improved resistance to fusarium head blight and excellent agronomic performance.
- 2) Determine genetic relationships (i.e. genetic correlations) between morphology (e.g. height), phenology (e.g. heading date, flowering time), and FHB traits (FHB index, FDK, DON), and their impact on agronomic performance and malting quality
- 3) Use genomic information to estimate breeding values for advancement and mating decisions to improve FHB resistance, agronomic performance and malting quality
- 4) Evaluate response to selection and impacts on malting quality.

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

What were the major activities?

Most progress so far has been made on objectives 1 and 3. Several high yielding lines with good FHB resistance are currently under evaluation in late stage regional trials. Genomic information is now being used to estimate genetic and breeding values at multiple breeding stages, such that selection on predicted FHB traits is now conducted multiple generations. Genomic prediction for parental selection is now being used, and implementation of predicted mate pair performance should be implemented for guiding crossing decisions in 2024.

Scab nursery 2023

In the previous two years, our scab nursery was intentionally late-planted (November 18, 2021 and December 2, 2022) to push flowering dates forward by several days to promote infection during warmer temperatures. In 2023, the scab nursery was planted on November 9th, while earlier than previous years, it was still reserved for last. Trials planted in the fall of 2023 included the NABSEN (55 entries/2 replicates), Eastern Malt (62/2) and the Winter Malt (26/2). All Year 2 (Y2) lines, comprised of 101 hulled lines, have also been evaluated in the scab nursery in two replicates, as well as in yield trials in Blacksburg VA and Warsaw VA. To facilitate genomic prediction of first year lines, 185 year 1 (Y1) lines in the scab nursery were randomly selected from 590 headrow selections from 2023 that were evaluated in 2024 Y1 yield trials. These 185 lines are being used to predict the remaining 405 lines not included in the scab nursery for FHB scores, FDK, and DON. Acceptable infection and disease progression were observed for the 2023 harvest year, however, infection rates were notably lower than in the previous two years, in part

due to hot and dry conditions during flowering and grain fill. Processing of FDK and DON is ongoing.

Parent Selection for 2023 crossing

FHB scores and FDK values from scab infection in the 2023 nursery are being used to build the prediction model for FHB, agronomic and other disease traits for potential parental pairs to be used for crossing in the spring of 2023. Our success in using similar methods for wheat suggest that these methods will be effective, although progress in the use of genomic information for the barley is notably behind our progress in wheat. Prediction accuracy of FHB traits is currently being assessed using data from 2022, given the excellent infection rates achieved that year. Addition of data from years 2019, 2020 may be included if prediction accuracy is shown to be increased. A total of 37 malt, 13 hulled, and 13 hullless inbred lines were selected as parents based on agronomic performance and FHB resistance. Of these, 34 have been genotyped with GBS by our program.

Our intention was to estimate and use marker effects to predict family mean performance for mate pair selection before crossing started in April of 2023. However, the retirement of our barley breeder, Wynse Brooks, produced a significant obstacle to completion of the pipeline. BLUPs were used to determine breeding values for genotyped lines, but predictions for mate pairs were delayed until crossing season of 2024. A postdoc in our lab, Felipe Sabadin, graciously stepped in to make crosses this season.

Genotyping

Since 2021, Virginia Tech has been genotyping all barley lines that enter into yield trials and the scab nursery using Genotyping By Sequencing (GBS). GBS sequences from these 590 lines were added to GBS sequences from 1,366 lines genotyped in previous years for site discovery and variant calling that was conducted in early summer of 2024. Our barley GBS pipeline has produced 23,093 markers for 1,956 breeding lines. Missing data were imputed with Beagle, and sites were filtered to remove redundant markers ($LD \geq 0.98$).

Crossing

Forty-five barley genotypes (36 inbred lines and 9 F1s) were used as parents for the 2023-2024 crossing season. Parents were chosen considering their genomic estimated breeding values for agronomic traits (grain yield, test weight, plant height, heading date, lodging, etc.), disease resistance (Fusarium head blight, leaf rust, powdery mildew, etc.), and quality malt traits (malt extract, diastatic power, wort viscosity, beta-glucan, alpha-amylase, etc.).

To determine the best crosses combining the selected parents, phenotypic data collected in the previous three years (2021, 2022, and 2023 seasons) was used with 16,379 GBS markers to estimate marker effects for yield, disease, and malt quality traits were used to predict the best crosses. Each trial was first fit individually by their experimental design, and included corrections for spatial variability where applicable. Fitted means from each trial and estimated the SNP effects for each trait (two-step model). All the possible F1 combinations were created *in silico* and their mean family performance was predicted using the estimated SNP effects. As mate-pair decision must consider multiple traits, a Smith-Hazel index was calculated using the

Table 1. Proportion of parents and crosses made in the spring of 2023, considering breeding purpose.

Type / Purpose	Parents*	Crosses**
Total (all crosses)	45	250
Malt	32	228
Forage / Feed	19	135
Fusarium Head Blight (FHB)		138
Leaf Rust		51
Zero Glycosidic Nitriles		31

* Parents can have more than one purpose for crosses.

** Crosses can have more than one purpose.

genetic correlation between traits with weights assigned by the breeder for their perceived importance. A total of 250 crosses were conducted combining agronomic, disease, and malt performance. Approximately 55% (138) of the crosses were made focusing on FHB resistance. Below is a summary of the number of parents and crosses by their category and purpose (Table 2).

Determination of genetic relationships between morphological/phenological traits and FHB traits is ongoing.

Scab Nursery 2025

Trials are currently being prepared for agronomic and FHB evaluation for the 2024 harvest season. This includes 142 selected lines from 954 USWBSI DH, 30 selected lines from 310 DH lines produced through Ackermagann Saatzeit, and 338 selected lines from 3,040 produced through our standard breeding pipeline for Y1 evaluation. Approximately 100 year-2 lines (Preliminary) and 50 to 80 Elite feed and malt lines will be advanced for further regional testing in multiple locations. The Uniform Barley, Eastern Malt, Winter Malt and NABSEN nurseries will all be planted in 2 replicates in our misted inoculated FHB nursery for 2024 evaluation. All the aforementioned Y2 and elite lines will be evaluated in the scab nursery, in replicated plots, while a subset of approximately 200 of the 510 Y1 lines for 2025 will be included in the scab nursery as well. Observed Y1 lines in the scab nursery will be used to help predict FHB traits in the remaining Y1 lines not entered into the scab nursery.

What were the significant results?

A total of 901 plots, representing 500 barley breeding lines and varieties were evaluated in a replicated field design in a misted inoculated nursery in 2024. Heritabilities for FHB were moderate to low (range 0.09-0.54), suggesting infection was not as high or uniform as in the previous two years.

Table 2: Collaborative nursery means, broad-sense heritability, and error standard deviation for FHB scores (0-9) 2023 scab nursery for FHB scores (0-9), FDK (%) and DON (ppm) for the 2023 scab nursery are shown.

Year: 2023	NABSEN			Eastern Malt			Winter Malt		
Trait	FHB (0-9)	FDK %	DON ppm	FHB (0-9)	FDK %	DON ppm	FHB (0-9)	FDK %	DON ppm
Mean	2.4	49.4	14.3	2.3	64.2	11.8	2.7	55.5	21.31
Broad-sense heritability	0.74	0.54	0.67	0.72	0.17*	0.63	0.78	0*	-.**
Error standard deviation	0.69	15.7	5.9	0.71	12.3	5.3	0.57	10.78	-

* Heritability of FDK for the Winter Malt nursery in 2023 was 0, and was low in the Eastern Malt. It is unclear why FDK had low heritability but FHB and DON had high heritabilities. Likely, a recording mistake was made, or needed to be rescored, but it is no longer possible to check, as the samples were subsequently ground for DON analysis.

** Grain was combined across reps for DON analysis, thus no heritability calculation could be made.

Table 3: Collaborative nursery means, broad-sense heritability, and error standard deviation for FHB scores (0-9) 2023 scab nursery.

Year: 2024	NABSEN	Eastern Malt	Winter Malt
Trait	FHB	FHB	FHB
Mean	3.8	2.7	3.2
Broad-sense heritability	0.31	0.46	0.09
Error standard deviation	1.3	1.0	1.16

List key outcomes or other achievements.

- 1) The number of lines entering first year yield trials was greatly expanded to increase selection intensity. approximately 800 new lines were entered into first year yield trials in 2023, and 590 were entered for 2024 first year yield trials, and 510 new lines will be entered into first year trials for 2025.
- 2) A unique source of FHB resistance has been identified in a hullless Virginia Tech line, 'Greg' (tested as VA15H-73), and crosses are being done with progeny of Greg to

continue to develop agronomically competitive hulled lines with resistance for malt and/or feed purposes.

- 3) A cross made in 2022, Avalon x VA21HFHB-19DH0301, was sent to Oregon State University for DH production. Line VA21HFHB-19DH0301 has Greg as a parent and has demonstrated FHB resistance in the field.

Results from the 2023 FHB nurseries will be disseminated in the following weeks as FDK data becomes available. All results from these nurseries will be uploaded to T3 in the fall of 2024

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

Postdoctoral associate, Dr. Moshood Bakare, learned to score FHB traits in the field, as well as FDK and DON sample preparation. Dr. Bakare worked with postdoctoral associate Felipe Sabadin to develop the prediction model for mate-pair decisions. He is currently working on a manuscript genetic relationships between FHB traits and other agronomic, phenologic and morphologic traits to determine trade-offs and unintended consequences of selection.

Dr. Sabadin, a postdoc in the small grains breeding program at Virginia Tech, took over crossing and headrow selection, with oversight from the PI and assistance from research technicians in the program. He recently was offered and accepted a tenure-track position at Utah State as an Assistant Professor of small grains breeding.

Graduate students Lexi Perry and Sunilda Frias assisted Moshood Bakare in scoring FHB traits in the field, and thus gained experience recognizing and quantifying FHB.

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

Summaries of regional nurseries in the FHB nursery have been sent to nursery collaborators, and will continue to be updated as grain samples are processed for FDK and DON. All 2023 data was sent to collaborators with FHB, FDK and DON scores. Data from 2023 and 2024 will be uploaded to T3 in the fall of 2024.

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

We are currently working on a manuscript assessing the genetic relationships between FHB traits and other agronomic, phenologic and morphologic traits to determine trade-offs and unintended consequences of selection. Additionally we will evaluate the efficacy of genomic prediction through validation of families in the 2026 scab nursery.

Approximately 1,000 barley plots will be evaluated in our misted inoculated FHB nursery, including Y1, Y2 and elite lines from our program and lines from other programs entered into regional nurseries.