

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?

Program Status

Progress has been made on all four objectives. Several high yielding lines with good FHB resistance are currently under evaluation in late stage regional trials. One line, VA22M-158 combines excellent agronomic performance with acceptable malting qualities for all malt brewing and distilling and good FHB resistance is currently under consideration for release by the germplasm committee at Virginia Tech, with expected release in late July. Genomic information is now being used to estimate genetic and breeding values at almost all breeding stages, such that selection on predicted FHB traits is now conducted through multiple stages of advancement. Genomic prediction is being used for parental selection predicting mate-pair performance to guide crossing decisions as of spring 2024.

Scab nursery 2024

The 2024 misted inoculated scab nursery was harvested in June of 2024. Good infection that year resulted in high means and high heritability of FHB, FDK and DON response (see results in Table 2 below). The nursery consisted of 901 barley plots representing 500 breeding lines across 9 trials. Trials included the national and regional nurseries NABSEN (55 entries/2 replicates), Eastern Malt (62/2), Uniform Winter Barley (30/2) and the Winter Malt (26/2). All entries from the state official variety test (OVT; 47 entries) were also included in the nursery in two

replicates. 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 along with all other lines phenotyped for FHB between 2021 and 2024 were 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 2024 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.

Scab nursery 2025

The 2025 misted inoculated scab nursery was planted on November 19th 2024 and comprised 908 plots representing 476 breeding lines across 7 trials. National and regional nurseries evaluated were the NABSEN (59 entries/2 replicates), Eastern Malt (58/2), Uniform Winter Barley (29/2), and the Winter Malt (30/2). The state OVT (54/2) was also included in the nursery. A total of 128 year-2 (Y2) lines (Preliminary) were evaluated in two replicates. A sample of 185 lines from 517 new Y1 lines was also included to predict performance of the remaining 332 Y1 lines. New Y1 lines for 2025 included 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.

The pump used to run the mist failed on the first day of misting on Friday April 25. A new pump was purchased and delivered to the scab nursery by midday Monday April 28, however, the new pump had standard 3" input and output diameters, while the old pump had non-standard 2.5" input/output diameters meaning the pickup and pipe to the irrigation lines would not fit the new pump. Couplers were sought and were installed making the pump operational but further delayed the start of misting by a couple days. Unfortunately, hot temperatures at that time drove rapid heading and flowering, leading the nurseries to mostly finish flowering before the pump was operational (standard deviation of heading date in the Eastern Malt nursery, for example, was 2.6). This led to poor infection and disease progression, while natural infection was also very low this year. Scores were noted for a few plots with visible symptoms, but the decision was made not to score all plots in the nursery due to low infection.

Importantly, *all* the lines in FHB nursery in 2025 were genotyped. This has allowed us to select on FHB resistance despite having poor infection and no phenotypes for the new Y1 lines.

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). Each year GBS sequences from Y1 lines are added to GBS sequences from previously genotyped lines for site discovery and variant calling in the late spring or summer. As of April 2025, our barley GBS pipeline has produced 24,202 markers for 2,604 breeding lines after filtering. Missing data were imputed with Beagle, and sites were filtered to remove redundant markers (LD \geq 0.99).

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

Type / Purpose	Parents*	Crosses**
Total (all crosses)	50	188
Malt	34	176
Forage / Feed	16	138
Fusarium Head Blight (FHB)	15	99

* Parents can have more than one purpose for crosses.

** Crosses can have more than one purpose.

Parent Selection for 2025 crossing

FHB scores, FDK values and DON quantities from 3,267 FHB nursery plots, representing 1,413 barley breeding lines across 32 trials from 2022 to 2024 were used to build a genomic prediction model for parent selection and subsequent prediction for mate-pair performance. Agronomic performance from 11,270 yield plots representing 2,533 breeding lines, as well as malt quality from 994 plots representing 584 breeding lines was used with other disease and FHB data to predict genetic merit of all 2,533 lines for 21 agronomic, disease and malt quality traits. This was used to select 50 parents with good agronomic, feed, malting or FHB related traits (Table 1).

The genetic merit of all 1,225 possible combinations of parents (i.e. mate-pairs) was then predicted using estimated marker effects multiplied by the expected marker score for each marker in each mate-pair. These values were then used to drive crossing decisions for 188 crosses made in the Spring of 2025. A selection index was calculated using the genetic correlation between traits with weights assigned by the breeder for their perceived importance. A total of 188 crosses were conducted combining agronomic, disease, and malt performance. Approximately 53% (99) 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 1).

What were the significant results?

Heritabilities and average scores were lower than typical, likely due to dry hot conditions during heading and flowering in the spring of 2024. This also pushed maturity earlier, with the Eastern Malt trial, for example, heading ten days earlier in 2024 than in 2025. Trial means and heritability is shown for FHB traits for regional nurseries in Table2.

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	3.8	13.6	8.4	2.7	8.0	9.3	3.2	-	-
Broad-sense heritability	0.31	0.42	0.87	0.46	0.44	0.44	0.09*	-	-
Error standard deviation	1.32	5.05	2.56	1.00	2.57	3.68	1.16	-	-

* Heritability of FHB for the Winter Malt 2024 was very low. As such, it was low on priority for FDK and DON processing. FDK was concluded just recently, but DON has yet to be quantified.

Genetic relationships between FHB and other traits

Genetic relationships between morphological/phenological traits and FHB traits have been conducted using correlations between genomic Estimated Breeding Values (gEBVs) from single trait models and multi-trait models (Table 3). Interestingly there is little agreement between correlations of single trait gEBVs and those from multi trait. For example, using single trait gEBVs, FHB index, FDK and DON have moderate correlations with one another, but the multi-trait fit, which takes into account error correlations between the traits, results in a high correlation between FDK and DON, but little relationship with either of these with FHB scores. Heritabilities are also markedly different between the two models, fit with the same data, and were generally higher in the multi-trait fit, with a ten-fold increase for FDK over the single trait heritability.

We are currently investigating the relationship between weather conditions around flowering to determine if some of these patterns can be explained by differential weather at the time of infection.

Table 3: Genetic correlations estimated from correlations of single trait gEBVs (post hoc; below diagonal) and fit in multi trait models (above diagonal) with traits FHB (index or score) FDK and DON. Narrow sense heritability estimates from single trait / multi-trait are listed on the diagonal.

	FHB	FDK	DON
FHB	0.23/0.20	-0.29	-0.06
FDK	0.41	0.08/0.85	0.89
DON	0.74	0.34	0.24/0.39

List key outcomes or other achievements.

- 1) Genomic prediction has been fully implemented in the barley breeding program at Virginia Tech and is being used for all parents selection, mate pair selection, population advancement and line advancement. Prediction is not used for line purification (i.e. F5 headrow selection), which is done visually due to thousands of individuals each year.
- 2) The number of lines entering first year yield trials has been greatly expanded to increase selection intensity in recent years. Approximately 500-800 new lines are now entered into first year yield trials, with genomic prediction being used with a subset of Y1 lines in the scab nursery to predict FHB traits and advance resistant lines.
- 3) A unique source of FHB resistance has been identified in a hulless 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.
- 4) A cross made in 2023, Avalon x VA21HFHB-19DH0301, was sent to Oregon State University for DH production and seed received in May 2025. Line VA21HFHB-19DH0301 has Greg as a parent and has demonstrated FHB resistance in the field. It will be planted in headrows for the 2026 harvest season.
- 5) Cross Greg / DH162310 was sent for DH production at OSU in 2024.
- 6) Cross VA23M-434 / VA21BFHB-19DH0303 was sent for DH production at OSU in 2025.
- 7) One line, VA22M-158 combines excellent agronomic performance with acceptable malting qualities for all-malt brewing and distilling and good FHB resistance is currently under consideration for release by the germplasm committee at Virginia Tech, with expected release in late July.

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

Postdoctoral associate, Dr. Moshood Bakare, learned to access weather data from online sources to be used in genomic prediction models that take environmental variables into account. Dr. Bakare has completed a draft of a manuscript that uses weather variables to separate genomic regions of resistance that are sensitive to environmental conditions at and around flowering from those that are more stable across environmental conditions.

Lexi Perry (MS, 2025), a recent Masters student in the small grains breeding program at Virginia Tech, has taken over breeding activities for barley and may apply to the barley breeding position opening at VT.

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. Several undergraduate students assisted with FHB inoculum in the winter of 2024/2025 gaining experience in sterile technique and the cultivation of fusarium on media.

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 2024 data was sent to collaborators with FHB, FDK and DON scores. We have done a poor job getting the barley data uploaded to T3 (but a good job with the wheat data). Special attention will be put towards getting data from 2023 through 2025 uploaded to T3 in the fall of 2025.

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

We have now a draft of a manuscript that uses weather variables to separate genomic regions of resistance that are sensitive to environmental conditions at and around flowering from those that are more stable across environmental conditions.

Bakare, M. et al. *A phenological model to evaluate host sensitivity to weather conditions for infection and disease progression of fusarium head blight*. Expected submission to Crop Science in July 2025.

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.