

Three Decades of Genomic Change in FHB Resistance: Haplotype Frequency Trajectories in Northern Plains Wheat (1992-2024)

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ABSTRACT

Fusarium head blight (FHB) remains the most economically devastating disease of wheat in the Northern Great Plains. Despite three decades of intensive breeding effort, the genomic architecture underlying resistance improvement has not been characterized at the haplotype level. We analyzed 33 years of multi-environment phenotypic data from two USDA-ARS nursery systems, the Uniform Regional Nursery (URN) and the Uniform Regional Scab Nursery (URSN), integrating genome-wide haplotype information to track how resistance alleles moved through breeding populations over time. The genetic architecture of FHB resistance proved highly polygenic, with most variation residing in small-effect loci that escape detection by conventional single-marker analysis. The haplotype-based framework identified multiple validated genomic regions that together explain substantial phenotypic variation yet individually contribute modestly to resistance. This study focuses on the genomic architecture and haplotype frequency trajectories underlying three decades of resistance improvement. At the genomic level, we observed directional selection at multiple resistance loci, including blocks overlapping known major resistance genes and novel regions across multiple chromosomes. Resistance haplotype frequencies increased significantly over the study period, while expected heterozygosity at these loci remained stable, demonstrating that breeders enriched resistance alleles without local diversity loss. A drift-control analysis confirmed that allele frequency shifts at validated resistance loci exceeded neutral expectations, providing statistical evidence for selection rather than drift. This

study demonstrates that haplotype-based frameworks can reveal the polygenic architecture of quantitative disease resistance and track evolutionary responses to selection in real populations.

Abbreviations: DIS, disease index; DON, deoxynivalenol; FHB, Fusarium head blight; GS, genomic selection; HD, heading date; He, expected heterozygosity; INC, FHB incidence; LD, linkage disequilibrium; local GEBV, local genomic estimated breeding value; MAS, marker-assisted selection; QTL, quantitative trait locus; RR-BLUP, ridge regression best linear unbiased prediction; SEV, FHB severity; URN, Uniform Regional Nursery; URSN, Uniform Regional Scab Nursery; VSK, visually scabby kernels.

INTRODUCTION

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, is the most economically devastating disease of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) in the Northern Great Plains of the United States and Canada (McMullen et al. 2012). The disease causes direct yield losses through floret sterility and shriveled kernels, but the most insidious impact is contamination of grain with mycotoxins, including deoxynivalenol (DON), which renders grain unsuitable for food and feed use at concentrations above regulatory thresholds (Windels 2000). Economic analyses have estimated cumulative losses exceeding \$3 billion in the Northern Plains since the 1990s (Nganje et al. 2004; Wilson et al. 2018). Epidemic years occur sporadically when warm, humid conditions coincide with anthesis, creating a persistent challenge for wheat producers, plant pathologists and breeders throughout the region (McMullen et al. 2012). Resistance to FHB is quantitative, polygenic, and heavily influenced by genotype-by-environment (G×E) interaction, making it one of the most challenging traits for wheat breeders to improve (Zhu et al. 2019; Buerstmayr et al. 2009; Buerstmayr et al. 2020). The two most widely recognized resistance types, Type I (resistance to initial infection) and Type II (resistance to spread within the

spike) were originally defined by Schroeder and Christensen (1963); Mesterházy (1995) later described additional categories including resistance to kernel infection, tolerance, and DON degradation. These resistance types are controlled by partially independent genetic regions, and most breeding programs evaluate multiple FHB-related phenotypes including disease index (DIS, the product of incidence and severity), Fusarium-damaged kernel percentage (VSK), and DON concentration (Mesterhazy et al. 1999; Bai and Shaner 2004).

The major resistance locus *Fhb1* on chromosome 3BS, originally identified in the Chinese cultivar Sumai 3, has been the most widely deployed source of Type II resistance (Anderson et al. 2001; Su et al. 2019; Infante et al. 2026), but dozens of additional quantitative trait loci (QTL) of smaller effect have been mapped across the wheat genome (Buerstmayr et al. 2009; Liu et al. 2019). The challenge of pyramiding these many small-effect loci while maintaining agronomic performance has driven interest in genomic selection approaches to FHB resistance breeding (Meuwissen et al. 2001; Bernardo and Yu 2007; Rutkoski et al. 2012; Arruda et al. 2016; Adeyemo et al. 2020; Adeyemo et al. 2023).

The Uniform Regional Nurseries coordinated by the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) provide a uniquely powerful resource for studying long-term breeding progress against FHB. The Uniform Regional Nursery (URN) is a long-running cooperative trial focused on agronomic evaluation of variety-release candidates from public and private hard red spring wheat breeding programs, with a subset of locations conducting inoculated and mist-irrigated FHB evaluation; coordinated FHB testing in the URN began in 1992. The Uniform Regional Scab Nursery (URSN), established later in the 1990s, was developed to facilitate FHB-resistance phenotyping of breeding lines with strong resistance but agronomic limitations, with all URSN locations inoculated and mist-irrigated (Brault et al. 2026). Over the

years, this system has accumulated phenotypic records for nearly a thousand breeding lines, creating a longitudinal dataset that captures the trajectory of genetic improvement for FHB resistance across the Northern Plains hard red spring wheat breeding programs. The inclusion of long-term check cultivars: susceptible (e.g., Marquis, Chris, Wheaton), moderately resistant (e.g., Alsen, Rollag) and resistant (e.g. ND2710) enables separation of genetic trend from year-to-year environmental variation in disease pressure.

Single-marker genome-wide association studies (GWAS), while well-suited to detect medium-to-large-effect loci that exceed multiple-testing thresholds, have limited power for the many small-effect QTL underlying polygenic disease resistance, particularly at moderate marker densities and moderate sample sizes (Venske et al. 2019; Buerstmayr et al. 2020). This limitation stems from the genetic architecture of quantitative resistance: individual loci may contribute only modest proportions of phenotypic variance and thus fail to reach genome-wide significance thresholds, yet collectively dozens of such regions, can explain substantial variation (Corwin and Kliebenstein, 2017). The challenge is compounded by allelic heterogeneity and the presence of multiple linked causal variants within a genomic region, which dilute single-marker associations (Yano et al. 2016; Visscher et al. 2017). Haplotype-based approaches, which group physically linked markers into blocks defined by linkage disequilibrium (LD) boundaries (Gabriel et al. 2002), address this limitation by capturing multi-marker effects within functional units and reducing the dimensionality of genomic data. Single-marker GWAS and haplotype-based local genomic estimated breeding value (local GEBV) frameworks serve complementary purposes: GWAS is primarily a confirmatory tool for individual large-effect loci with strong false-positive control via kinship and structure correction, while local GEBV partitions polygenic signal across LD-defined haplotype blocks and produces per-line, per-block breeding values that can be tracked across

breeding cohorts (Voss-Fels et al. 2019; Tong et al. 2024; Yadav et al. 2026; Roy et al. 2025). Local GEBVs, computed for individual haplotype blocks rather than the whole genome, enable both the identification of regions contributing modestly but consistently to resistance and the tracking of how selection has shaped haplotype frequencies at these regions across breeding cycles (Bernardo 2014).

Despite this convergence of long-term phenotypic resources and genomic tools, a critical gap remains: to our knowledge, no study has combined decades of coordinated FHB nursery data with haplotype-level genomic analysis to track the actual allele frequency changes that underlie breeding progress. Previous genetic gain analyses in wheat have quantified phenotypic trends over time (del Blanco et al. 2003; DePauw et al. 2011) or examined genomic architecture at a single time point (Arruda et al. 2016) but have not integrated longitudinal phenotypic and genomic data to characterize the evolutionary response to selection at the haplotype level. Major resistance genes such as *Fhb1* have been widely introgressed via marker-assisted selection and have contributed substantially to resistance gains in some breeding programs; however, their effectiveness can vary across genetic backgrounds and environments, and the contribution of accessory minor-effect loci remains poorly characterized (Anderson et al. 2001; Buerstmayr et al. 2020). Critically, two fundamental questions remain unanswered: do the genomic regions targeted by selection maintain stable associations with resistance across successive breeding cycles, and does directional selection at these loci erode genetic diversity? While broad studies of modern wheat have suggested that intensive breeding does not necessarily deplete overall genetic variation (Reif et al. 2005; Fu and Somers 2009), this has not been characterized specifically for polygenic FHB resistance loci over time.

Concurrent work by Brault et al. (2026) addressed the first question for three major FHB resistance QTL (*Fhb1*, *Fhb5*, *Fhb5AS*) in this nursery system, demonstrating via allele-specific markers that resistance allele frequencies increased over 30 years and contributed to phenotypic gains. The present study complements and expands upon this foundation by deploying a genome-wide haplotype-based local GEBV framework to capture the full polygenic architecture of FHB resistance. This approach enables discovery of temporally stable resistance regions beyond the known major QTL, formal classification of genomic block stability across breeding eras, quantification of haplotype frequency trajectories genome-wide, and assessment of diversity dynamics at resistance loci under selection.

The objectives of this study were to: (1) deploy a genome-wide haplotype-based local GEBV framework to identify resistance loci and compare detection power against single-marker GWAS; (2) identify haplotype blocks under directional selection and classify their temporal stability across eras using a per-era validation framework; and (3) track haplotype diversity trajectories at validated resistance loci to assess whether selection has eroded standing variation for FHB resistance in Northern Plains wheat.

MATERIALS AND METHODS

Plant Material and Phenotypic Data

A total of 943 unique hard red spring wheat (*T. aestivum*) lines were evaluated across 33 consecutive years (1992-2024) in the Uniform Regional Nursery (URN) and Uniform Regional Scab Nursery (URSN) coordinated by USDA-ARS. The dataset comprised 9,649 observations collected across 22 field locations in the Northern Great Plains, representing 279 unique location × year environments (mean of 8.5 locations per year, ranging from one in early years to 17 at

peak). All nursery trials were inoculated with *F. graminearum* isolate mixtures using corn-kernel spawn and mist-irrigated to promote disease development; *F. graminearum* macroconidial inoculum was additionally employed at all URSN locations (Brault et al. 2025).

Six traits were evaluated: FHB disease index (DIS; the product of incidence and severity, expressed as percentage), FHB incidence (INC; percentage of infected heads), FHB severity (SEV; percentage of infected spikelets on diseased heads), visual scab kernel rating (VSK; percentage of *Fusarium*-damaged kernels), deoxynivalenol concentration (DON; parts per million), and heading date (HD; Julian days). DIS, VSK, and DON were analyzed for haplotype validation and diversity. INC and SEV were analyzed for temporal trends. HD served as a negative control trait.

Seventeen check lines, present for seven or more nursery-years, were included (Table S2). These comprised eight checks with a range from resistant to moderately susceptible (Alsen, BacUp, Boost, Brick, Linkert, McVey, ND2710, and Rollag; mean DIS range 13.7-27.6) and nine susceptible checks (2375, Chris, Keene, Marquis, MN00269, Norm, Roblin, Verde, and Wheaton; mean DIS range 31.9-64.1).

Phenotypic observations were modeled in ASReml-R (Butler et al. 2017) with a single mixed model: line, year, and location as random effects and nursery (URN/URSN) as a fixed effect to absorb systematic between-nursery mean differences:

$$y_{ijkl} = \mu + n_j + g_i + y_k + l_l + \varepsilon_{ijkl} \text{ (Eq. 1)}$$

where n_j is the fixed nursery effect, $g_i \sim N(0, \sigma^2_g)$ the random line effect, $e_k \sim N(0, \sigma^2_y)$ the random year effect, $l_l \sim N(0, \sigma^2_l)$ the random location effect, and ε_{ijkl} the residual. Per-line adjusted means from this model, formally BLUPs of the line random effect, referred to as per-line adjusted means throughout, served as the phenotypic input to the haplotype validation, frequency-tracking, and GWAS-comparison analyses. Line was fitted as random because the

unbalanced 33-year testing pattern (mean 10.2 environments per line, strongly right-skewed) makes BLUP shrinkage statistically advantageous over a fixed-line specification (Piepho et al. 2008).

Genotypic Data and Haplotype Block Construction

A subset of 699 lines from the nursery system were genotyped using the USDA-SoyWheOatBar-3K multi-species SNP array, a custom Illumina platform containing 3,421 SNPs. Markers were filtered for call rate, minor allele frequency, and heterozygosity, retaining 2,825 biallelic markers. Genotyping data has been deposited in the Triticeae Toolbox (<https://wheat.triticeaetoolbox.org/>). Physical positions of SNP markers and haplotype block coordinates are referenced to the IWGSC RefSeq v2.1 wheat reference genome assembly. Linkage disequilibrium (LD)-based haplotype blocks were defined using pairwise r^2 between adjacent markers (Roy et al. 2025), grouping markers into blocks when adjacent r^2 exceeded 0.5. This process identified 1,631 haplotype blocks across the wheat genome, with an average of 1.8 markers per block. Haplotype blocks were assigned sequential numeric identifiers in the format b00001 through b01631, ordered by chromosomal position. Unique dosage strings across markers within a block are referred to as 'haplotype classes.' Because the advanced wheat lines in this panel are largely inbred and homozygous, these dosage classes closely approximate classical phased haplotypes, though heterozygous states are retained as distinct classes for statistical completeness. Of the 1,631 blocks, 1,212 blocks (74.3%) contained a single marker, 256 (15.7%) contained two markers, and 163 (10.0%) contained three or more markers. Single-marker blocks resolve two primary parental haplotypes, whereas multi-marker blocks can resolve up to 2^k combinations (where k is the number of markers). Within-block allelic configurations are summarized into a single dosage-weighted local GEBV per line, which integrates the joint marker-effect signal across the block while

collapsing the multi-allelic structure into a continuous scalar suitable for downstream regression and frequency analyses.

Local Genomic Breeding Value Framework

The local GEBV approach used here is a population-genetics framework for haplotype-block-level partitioning of marker-effect signal, distinct from genomic-selection prediction and from single-marker GWAS. Following Voss-Fels et al. (2019), Tong et al. (2024), Yadav et al. (2026), and Roy et al. (2025), marker effects are estimated once on the full panel via RR-BLUP, summed within LD-defined blocks to produce per-line local GEBVs, and ranked by haplotype-effect variance for downstream within-population validation. Validation is by per-era stratified regression rather than out-of-sample prediction, consistent with the population-genetics objective of characterizing temporal allele-frequency dynamics in a closed germplasm pool. Structure-driven inflation of marker effects is controlled by the genome-wide ridge penalty rather than by an external kinship matrix: the ridge spreads any joint-LD-block signal across correlated markers with each individual coefficient shrunk in proportion to the joint information, preventing over-attribution of phenotypic variance to any single marker (Yadav et al. 2026; Shaffer et al. 2025). Marker effects for each trait (DIS, VSK, DON) were estimated genome-wide using ridge regression best linear unbiased prediction (RR-BLUP; Endelman 2011) across all 699 genotyped lines. Local GEBVs were calculated for each haplotype block by summing the RR-BLUP-estimated marker effects within the block, weighted by allele dosage. Formally, the local GEBV at block b for line i is

$$\text{GEBV}_{ib} = \sum_{(m \in b)} \hat{\alpha}_m \times x_{im} \text{ (Eq. 2),}$$

where $\hat{\alpha}_m$ is the RR-BLUP-estimated effect for marker m and x_{im} is the reference-allele dosage in line i (coded 0/1/2). This summed-marker-effect quantity is the additive haplotype effect under

the framework definition (Tong et al. 2024); equivalent per-allele formulations (Tong et al. 2024) yield identical local GEBVs under consistent allelic coding.

For the directional selection analysis, lines were classified as carrying either the resistance or susceptibility haplotype at each block based on the local GEBV distribution. The median local GEBV served as the classification threshold; for traits where higher values indicate susceptibility (DIS, VSK, DON), lines with a local GEBV at or below the median were classified as resistant. Consistent with local GEBV frameworks established for wheat (Tong et al. 2024; Shaffer et al. 2025), this binary classification collapsed multi-allelic haplotype variation into two classes based on the aggregate marker effect.

Haplotype blocks were prioritized in two stages. In the discovery stage, blocks were ranked by the variance of their local GEBV distribution across lines (top 20 per trait), following Voss-Fels et al. (2019), Tong et al. (2024), and Yadav et al. (2026). In the validation stage, each candidate block was tested by regression of phenotype on local GEBV with FDR correction (Benjamini-Hochberg $q < 0.05$); the validated set reported in Table 1 comprises blocks passing the validation criterion, not the highest-variance blocks.

Cohort Assignment and Era Definition

Each breeding line was assigned to a single cohort based on its first year of entry into the nursery system (entry year). Four breeding eras were defined: Era 1 (1992-1999, $n = 95$ breeding lines), Era 2 (2000-2007, $n = 130$), Era 3 (2008-2015, $n = 175$), and Era 4 (2016-2024, $n = 284$). Long-term check cultivars ($n = 17$) were excluded from all era-cohort assignments but retained for environmental calibration. The breeding population comprised 926 lines (Table S1).

Directional Selection Response at Haplotype Blocks

For each of the 35 validated haplotype blocks, the binary resistance haplotype classification was regressed on breeding era (coded 1-4) using logistic regression. This tests whether breeders have systematically increased or decreased the frequency of resistance alleles at each locus over the 33-year study period. Blocks were classified based on the logistic regression results: positive selection (resistance frequency increasing, $p < 0.05$), purifying selection (resistance frequency decreasing, $p < 0.05$), or neutral ($p \geq 0.05$). All analyses excluded check lines.

To ensure these signals were robust to the temporal resolution of the predictor, a secondary logistic regression was fitted for each block using entry year as a continuous variable. Concordance between the era-based and year-continuous models was assessed to validate the observed selection trajectories.

Drift Control Analysis

The absolute frequency change ($|\Delta\text{freq}|$, Era 1 to Era 4) at the 35 validated FHB resistance blocks was compared against a null distribution of 200 non-associated blocks sampled genome-wide. Null blocks were selected based on a non-significance threshold ($P_{adj} > 0.3$) across all FHB traits and classified using the same median-split approach applied to the experimental blocks. The magnitude of frequency shifts was compared between the two sets using a Wilcoxon rank-sum test and a permutation test (10,000 replicates) to determine if observed selection trajectories exceeded the expectations of neutral genetic drift (Falconer and Mackay 1996; Walsh and Lynch 2018).

Per-Era Temporal Stability Validation

Haplotype block-phenotype associations were validated for temporal robustness following the approach of Tong et al. (2025). Within each breeding era, local GEBVs, derived from the global RR-BLUP model, were regressed on per-line adjusted means using only the breeding lines

assigned to that cohort. This stratified validation tested the association of the local GEBV with phenotype.

The top 20 blocks per trait (DIS, VSK, DON) were evaluated, encompassing 60 block-trait pairs and 220 validation tests. Significance was determined using Benjamini-Hochberg FDR correction ($q < 0.05$) within each trait-by-era combination. Blocks were classified into three stability categories: Stable (validated in ≥ 3 eras), Era-specific (validated in 1-2 eras), or Unvalidated (validated in 0 eras). The Stable classification (validation in ≥ 3 of 4 eras) requires significance in at least one early-era cohort ($n = 95$ in Era 1) combined with replication across multiple later cohorts, providing a temporal-replication test rather than a power test driven solely by the larger Era-4 cohort.

Haplotype Diversity Analysis

For each of the 35 validated haplotype blocks, the frequency of the resistance haplotype (p) was calculated within each breeding era. Genetic diversity was quantified as expected heterozygosity ($He = 2p(1 - p)$), which is equivalent to Nei's gene diversity (h) for biallelic loci (Nei 1978). While He and polymorphism information content (PIC) are highly correlated in biallelic systems, He was utilized to maintain consistency with established wheat diversity literature (Reif et al. 2005; Fu and Somers 2009; Gill et al. 2025). Given the per-era sample sizes ($n = 95$ -284), the small-sample bias in He is negligible (correction factor $n/(n-1) < 1.011$) and was therefore omitted from the final calculations. Mean He was averaged across blocks within each era to track the trajectory of genomic diversity across the 33-year study period.

Genome-Wide Association Analysis and Block-Centric Comparison

Genome-wide association studies (GWAS) were conducted for DIS, VSK, and DON using the rrBLUP R package (Endelman 2011), with the first three principal components and a marker-derived kinship matrix as covariates. The significance threshold was set at $-\log_{10}(p) = 4.67$ ($0.05 / n$ markers) as the Bonferroni corrected threshold and the suggestive threshold at $-\log_{10}(p) \geq 4.0$. Following the block-centric framework of Roy et al. (2025), the top 20 haplotype blocks per trait were categorized based on GWAS overlap. A block was classified as GWAS-supported if any marker within its physical boundaries reached the suggestive threshold; otherwise, it was classified as GWAS-missed. Per-block significance was determined by regressing local GEBVs on per-line adjusted means, with Benjamini-Hochberg FDR correction applied per trait ($q < 0.05$). Cumulative phenotypic variance explained ($R^2_{\text{cumulative}}$) was estimated by sequentially adding validated blocks in descending order of local GEBV variance into a multi-locus regression, rather than by stepwise selection, to avoid inflation associated with data-driven variable selection (Whittingham et al. 2006).

Statistical Software

All analyses were conducted in R (R Core Team 2024). Key packages included data.table (Dowle and Srinivasan 2023) for data manipulation, rrBLUP (Endelman 2011) for RR-BLUP marker effect estimation, ASReml-R (Butler et al. 2017) for mixed model analysis, and ggplot2 (Wickham 2016) for visualization.

RESULTS

Temporal Stability of Block-Phenotype Associations

292 The per-era validation analysis tested 220 block-trait-era combinations, of which validation rates
293 varied by trait and era. After FDR correction, DIS yielded two Stable (validated in ≥ 3 eras), 12
294 Era-specific (1-2 eras), and six Unvalidated blocks (Figure 1). DON yielded one Stable, 12 Era-
295 specific, and seven Unvalidated blocks. VSK yielded no Stable, 10 Era-specific, and 10
296 Unvalidated blocks.

297 The two Stable DIS blocks were b00559 on chromosome 3B (12.4 Mb; mean $R^2 = 0.075$, 3/4 eras
298 validated, consistent effect direction) and b01209 on chromosome 6A (240.3 Mb; mean $R^2 = 0.038$,
299 3/4 eras validated) (Table S4). The single Stable DON block was b00562 on chromosome 3B (23.5
300 Mb; mean $R^2 = 0.048$, 3/3 eras validated, consistent direction). Block b00559 directly overlaps the
301 *Fhbl*/TaHRC region (Table S3).

302 The stability classification for block-trait pairs is shown in Figure 1, with supporting metrics (eras
303 validated, direction consistency, mean R^2) in Table S4.

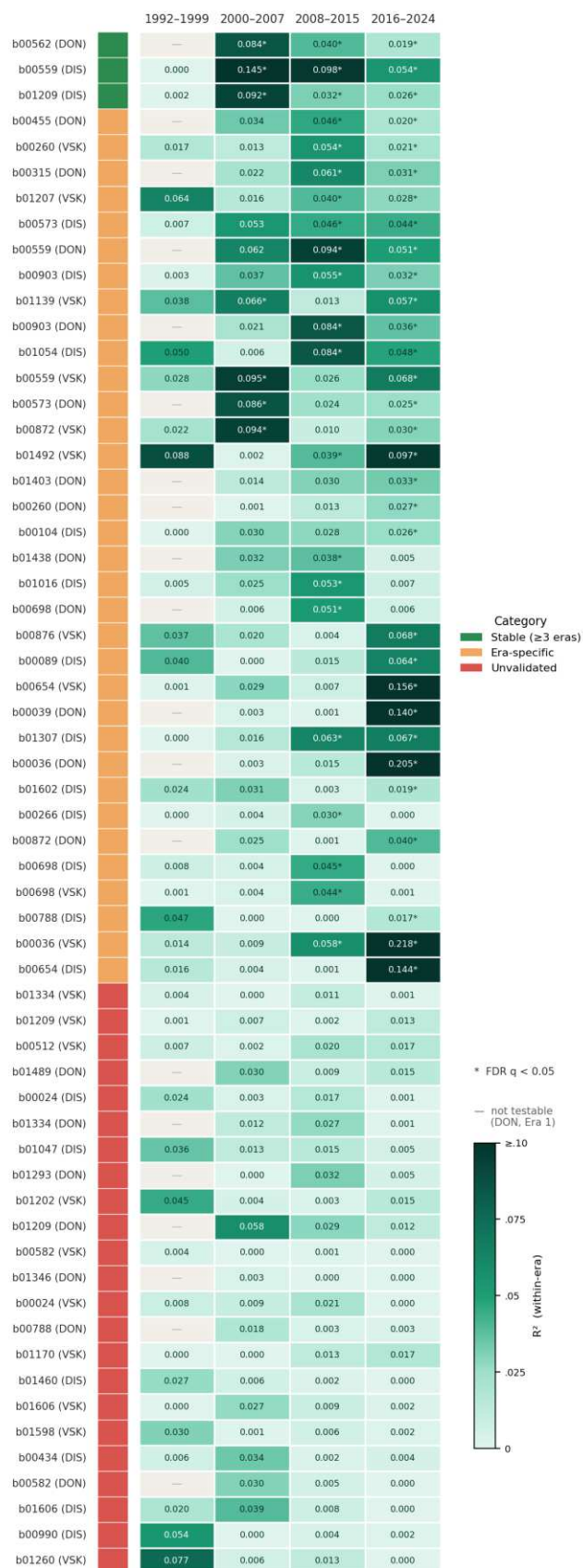


Figure 1. Stability classification for all 60 block-trait pairs, colored by category (Stable, Era-specific, Unvalidated).

Directional Selection Response at Haplotype Blocks

Thirty-five haplotype blocks were validated for significant association with FHB resistance traits through linear regression of block-level local GEBV on adjusted phenotypic values (at FDR < 0.05). These 35 validated blocks were subsequently tested for directional selection across breeding eras. Of these, 12 showed significant positive selection (increasing resistance haplotype frequency over time, $p < 0.05$), six showed significant purifying selection (decreasing frequency, $p < 0.05$), and 17 were neutral ($p \geq 0.05$) (Table 1). No blocks reached complete fixation; all 35 maintained segregating variation throughout the 33-year study period.

Table 1. Directional selection response at 35 validated FHB resistance haplotype blocks. Logistic regression of binary resistance haplotype classification on breeding era (1-4). Categories: Positive_selection (frequency increasing, $p < 0.05$), Purifying_selection (frequency decreasing, $p < 0.05$), Neutral ($p \geq 0.05$). *Full haplotype distributions are in Table S7.*

Block	Chr	Trait	Freq Era1	Freq Era4	Δ freq	P (era)	Category
b00266	Chr2A	DIS	0.537	0.887	0.350	9.41e-13	Positive_selection
b00559	Chr3B	DIS	0.074	0.475	0.402	6.66e-12	Positive_selection
b00654	Chr3D	DIS	0.621	0.926	0.305	2.54e-10	Positive_selection
b00260	Chr2A	DON	0.242	0.623	0.381	5.24e-10	Positive_selection
b00562	Chr3B	DON	0.232	0.542	0.311	2.00e-09	Positive_selection
b01209	Chr6A	DIS	0.326	0.616	0.290	3.15e-07	Positive_selection
b00024	Chr1A	DIS	0.474	0.623	0.150	0.0019	Positive_selection
b01489	Chr7A	DON	0.179	0.306	0.127	0.0020	Positive_selection
b01606	Chr7D	DIS	0.358	0.546	0.188	0.0042	Positive_selection
b00315	Chr2B	DON	0.400	0.553	0.153	0.0084	Positive_selection
b00104	Chr1B	DIS	0.463	0.563	0.100	0.0394	Positive_selection
b01492	Chr7A	VSK	0.579	0.690	0.111	0.0403	Positive_selection
b01403	Chr7A	DON	0.642	0.373	-0.269	8.79e-07	Purifying_selection
b00582	Chr3B	DON	0.453	0.271	-0.182	0.0002	Purifying_selection

b00876	Chr4D	VSK	0.768	0.634	-0.135	0.0013	Purifying_selection
b01438	Chr7A	DON	0.716	0.609	-0.107	0.0107	Purifying_selection
b00698	Chr4A	DON	0.495	0.394	-0.100	0.0222	Purifying_selection
b01334	Chr6B	DON	0.653	0.482	-0.170	0.0280	Purifying_selection
b00089	Chr1A	DIS	0.589	0.715	0.125	0.0537	Neutral
b01139	Chr5D	VSK	0.411	0.518	0.107	0.0624	Neutral
b01307	Chr6B	DIS	0.495	0.609	0.114	0.0628	Neutral
b01207	Chr6A	VSK	0.611	0.535	-0.075	0.0884	Neutral
b01016	Chr5B	DIS	0.337	0.282	-0.055	0.0955	Neutral
b01293	Chr6B	DON	0.463	0.560	0.097	0.1055	Neutral
b00573	Chr3B	DON	0.579	0.489	-0.090	0.2078	Neutral
b00872	Chr4D	VSK	0.663	0.743	0.080	0.2594	Neutral
b01202	Chr6A	VSK	0.463	0.553	0.090	0.2789	Neutral
b00455	Chr3A	DON	0.179	0.176	-0.003	0.3458	Neutral
b01054	Chr5B	DIS	0.758	0.761	0.003	0.5457	Neutral
b00512	Chr3A	VSK	0.621	0.634	0.013	0.6363	Neutral
b00039	Chr1A	DON	0.589	0.620	0.030	0.6409	Neutral
b00903	Chr5A	DON	0.179	0.208	0.029	0.7769	Neutral
b00036	Chr1A	VSK	0.663	0.697	0.034	0.9139	Neutral
b01047	Chr5B	DIS	0.463	0.496	0.033	0.9702	Neutral
b01170	Chr5D	VSK	0.495	0.507	0.012	0.9851	Neutral

319

320 The strongest positive selection signal was observed at block b00266 on chromosome 2A (DIS;
321 53.7% → 88.7%, $p = 9.41 \times 10^{-13}$; Table 1), followed by b00559 on chromosome 3B (DIS; 7.4%
322 → 47.5%, $p = 6.66 \times 10^{-12}$) and b00654 on chromosome 3D (DIS; 62.1% → 92.6%, $p = 2.54 \times$
323 10^{-10}). Block b00559, which directly overlaps the *Fhbl*/TaHRC region, showed a dramatic
324 increase from near absence (7.4%) in Era 1 to intermediate frequency (47.5%) in Era 4 (Figure 2).
325 Among the 12 positively selected blocks, seven were validated for DIS, four for DON, and one for
326 VSK, spanning chromosomes 1A, 1B, 2A, 2B, 3B, 3D, 6A, 7A, and 7D (Table 1). Six blocks
327 showed purifying selection (decreasing resistance frequency), five validated for DON and one for
328 VSK: b01403 (Chr7A), b00582 (Chr3B), b00876 (Chr4D), b01438 (Chr7A), b00698 (Chr4A),
329 and b01334 (Chr6B) (Table 1).

Year-continuous logistic regression strongly supported the era-based results. All 12 positively selected blocks and all six purifying-selected blocks remained statistically significant ($p < 0.05$) when entry year was used as a continuous predictor.

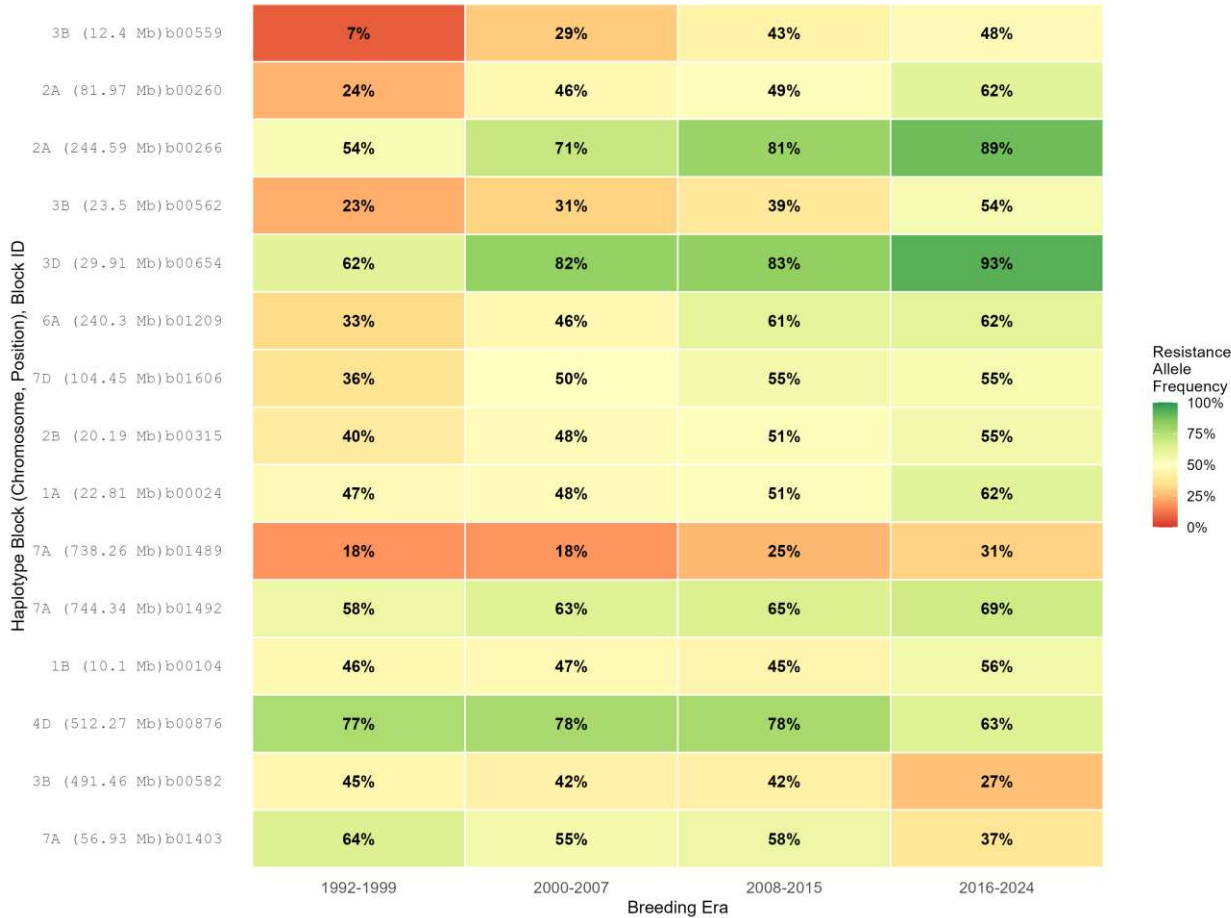


Figure 2. Resistance haplotype frequency progression across breeding eras for the 15 selected haplotype blocks. Color intensity represents the frequency of the resistance haplotype in each era. Blocks are labeled with chromosome and physical position.

Drift Control

The mean absolute frequency change ($|\Delta\text{freq}|$) at 35 FHB-validated blocks (0.134) was significantly greater than at 200 non-associated control blocks (0.061), confirmed by both a Wilcoxon rank-sum test ($p = 9.72 \times 10^{-6}$) and a permutation test ($p < 0.0001$; 10,000 replicates) (Figure S4).

GWAS versus Haplotype Block Comparison

Single-marker GWAS identified four Bonferroni-significant and one suggestive association for DIS and six Bonferroni-significant and one suggestive association for VSK, while no markers reached significance for DON (Figure 3, Table S5). The strongest GWAS peaks for DIS were on chromosomes 1D (210.3 Mb, $-\log_{10}(p) = 6.26$), 1A (422.3 Mb, 5.83), and 2A (55.9 Mb, 5.53). For VSK, the same loci on 1D and 1A showed the strongest signals ($-\log_{10}(p) = 10.9$ and 10.7, respectively).

Of the 35 FDR-corrected validated haplotype blocks ($q < 0.05$), 33 contained no internal SNP reaching even the suggestive threshold ($-\log_{10}(p) \geq 4.0$; 95% non-overlap), demonstrating that the per-block validation criterion identifies signals invisible to single-marker analysis at any standard threshold, despite the same kinship- and PC-corrected GWAS being run on the same per-line adjusted means. In contrast, direct per-block phenotypic regressions validated 14 of 20 DIS blocks, 13 of 20 VSK blocks, and 18 of 20 DON blocks at $FDR < 0.05$, even though the median strongest single-marker signal within these blocks remained well below the Bonferroni threshold (median $-\log_{10}(p) = 1.2$ -1.6 vs. threshold of 4.67). The top 20 blocks jointly explained 12.0% (DIS), 18.5% (VSK), and 14.2% (DON) of phenotypic variance when stacked sequentially (Table S5). GWAS missed 95-100% of these blocks and 96-100% of their cumulative local variance (Figure 4, Table S5 & S6).

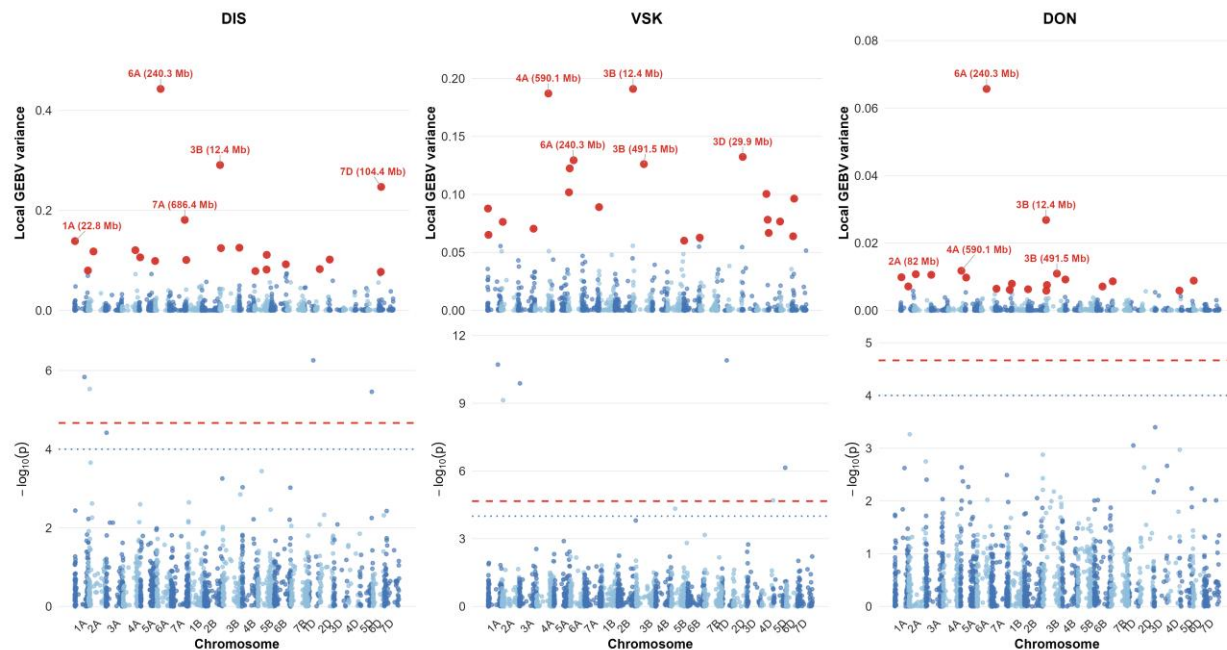


Figure 3. Genome-wide haplotype-based local GEBV variance (top row) and single-marker GWAS $-\log_{10}(p)$ (bottom row) for (A) DIS, (B) VSK, and (C) DON. Horizontal lines mark the Bonferroni-corrected threshold ($-\log_{10}(p) = 4.67$, dashed) and a suggestive threshold ($-\log_{10}(p) = 4.0$, dotted).

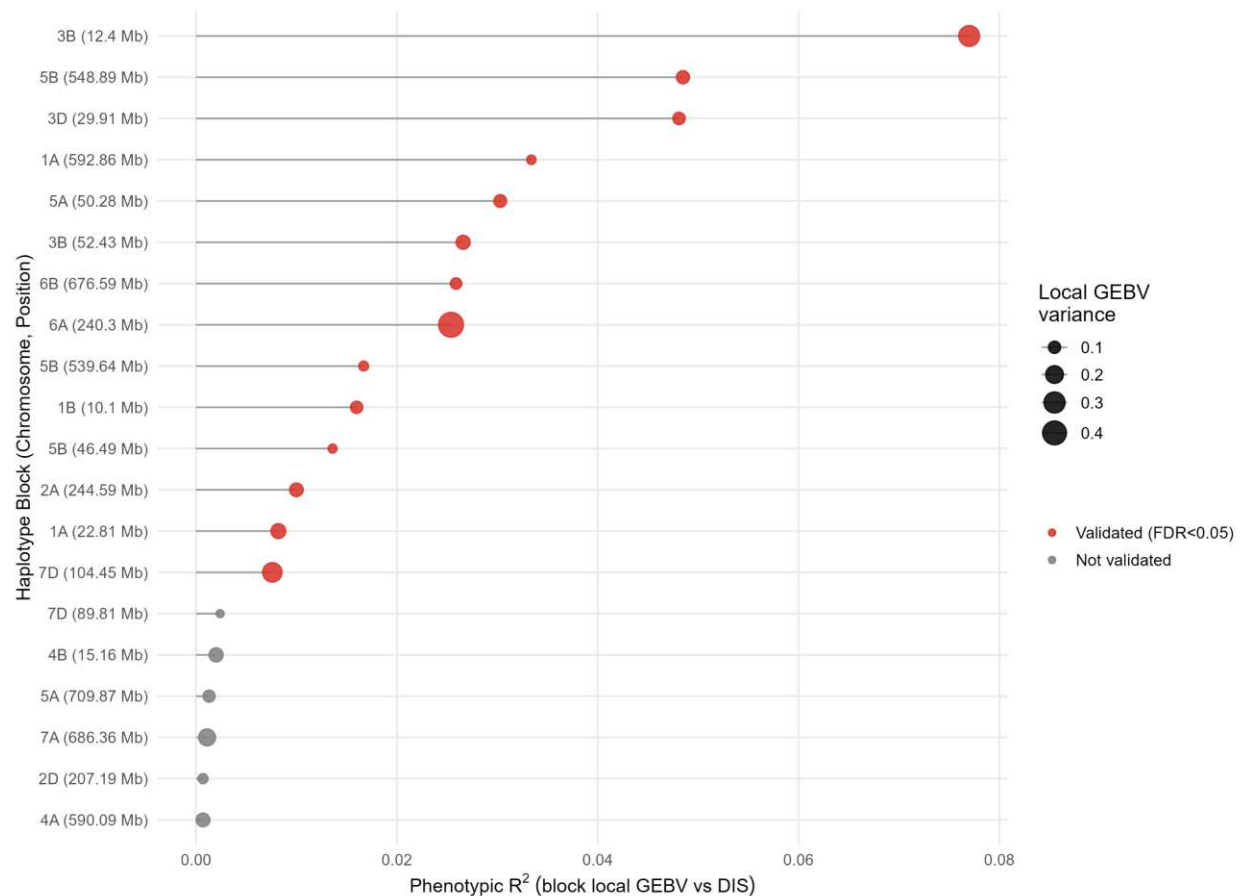


Figure 4. Per-block phenotypic R^2 (local GEBV vs. DIS) for the top 20 haplotype blocks ranked by local GEBV variance. Point size = local GEBV variance; color indicates validation status (FDR < 0.05). Block labels show chromosome and physical position.

Haplotype Diversity Across Breeding Eras

Mean expected heterozygosity (He) at the 35 validated resistance haplotype blocks was essentially stable across the four breeding eras: 0.438 (Era 1, n = 95 lines), 0.447 (Era 2, n = 130), 0.454 (Era 3, n = 175), and 0.439 (Era 4, n = 284) (Table 2). No blocks were effectively fixed (He < 0.01) in any era. Mean resistance allele frequency increased from 0.479 to 0.549 across eras (Table 2).

Table 2. Expected heterozygosity at 35 validated FHB resistance haplotype blocks across breeding eras. Per-cohort assignment: only lines entering in each era are included.

Era	N lines	Mean p	Mean He	Median He	Min He	Max He
1992-1999	95	0.4791	0.4379	0.4707	0.1365	0.4999
2000-2007	130	0.5330	0.4466	0.4830	0.2912	0.5000
2008-2015	175	0.5455	0.4538	0.4863	0.2841	0.5000

2016-2024	284	0.5491	0.4387	0.4713	0.1370	0.5007
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378

379 **DISCUSSION**

380 **Temporal Stability: Identifying Robust Markers**

381 The per-era validation analysis provides a temporal stress test for block-phenotype associations,
382 distinguishing signals that are robust across decades from those that are era-specific. Only 5% of
383 block-trait pairs achieved Stable classification (validation in ≥ 3 of 4 eras), underscoring the
384 difficulty of identifying temporally robust targets in a quantitatively inherited trait. The Stable
385 criterion is a temporal-replication test rather than a power test (Methods); cohort-size variation
386 across eras is therefore not the dominant determinant of the low Stable rate.

387 The three Stable associations: b00559 (DIS, Chr3B/*Fhb1* region), b01209 (DIS, Chr6A), and
388 b00562 (DON, Chr3B), represent the highest-confidence targets for marker deployment. It is
389 notable that two of the three Stable blocks map to chromosome 3B: b00559 directly overlaps the
390 *Fhb1*/TaHRC locus, and b00562 at 23.5 Mb may capture additional resistance variation in the
391 broader 3B region. The temporal robustness of the *Fhb1*-region signal across breeding era cohorts
392 confirms that this major QTL has maintained its predictive relationship with phenotype throughout
393 the study period. Two non-exclusive mechanisms can generate this pattern: a stable biological
394 effect of the TaHRC resistance allele across genetic backgrounds, and sustained marker-assisted
395 selection on *Fhb1* diagnostics, which maintains the association by construction. The b00559
396 frequency trajectory (7.4% $\rightarrow$ 47.5%; Table 1) is direct evidence of the latter. Brault et al. (2026),
397 analyzing the same dataset with KASP-based major-QTL genotyping, partitioned the *Fhb1* effect
398 into resistance-allele, genetic-background, and genetic-trend components, finding a significant

resistance-allele effect for DIS, VSK, and DON, supporting both mechanisms operating simultaneously. *Fhb1* Era-specific associations should not be deployed as fixed MAS targets without contemporary revalidation, but may contribute usefully to genomic prediction with appropriate down-weighting.

Selection Response: Genomic Confirmation of Breeding Progress

The identification of 12 haplotype blocks under significant positive selection provides direct genomic evidence that long-term phenotypic selection has translated into measurable allele frequency shifts (Table 1). The drift control analysis (Figure S4) confirmed that frequency changes at validated blocks significantly exceeded neutral expectations, attributing the observed shifts to selection rather than drift (Falconer and Mackay 1996; Walsh and Lynch 2018).

The strongest positive selection signal was at b00266 on chromosome 2A (Table 1), a region repeatedly involved in FHB resistance (Gervais et al. 2003; Semagn et al. 2007; Giancaspro et al. 2019). The second-strongest signal, at b00559 overlapping the *Fhb1*/TaHRC locus (Figure 2), is consistent with the progressive introgression of Sumai 3-derived resistance documented by Brault et al. (2026), who reported *Fhb1* frequency increasing from near zero in 1995 to approximately 59% in the URSN by 2024. Two analytical frameworks applied to largely overlapping data, haplotype-based local GEBV (this study) and KASP-based major-QTL genotyping (Brault et al. 2026), detect the same *Fhb1* frequency trajectory, supporting the robustness of the signal to analytical choice.

The six blocks under purifying selection were all associated with DON or VSK (Table 1), and declining resistance frequency at these loci may reflect repulsion-phase linkage with favorable agronomic alleles or hitchhiking effects, consistent with documented growth-defense tradeoffs in wheat (Brown 2002; Kolb et al. 2001; Miedaner and Korzun 2012). The strong VSK-DON

phenotypic correlation in this population is consistent with Gaire et al. (2022a), and the overall progress in reducing disease severity mirrors trends observed in other North American winter wheat nurseries (Gaire et al. 2022b).

Colocalization with Named FHB Resistance QTL

Physical comparison of the 35 validated blocks with formally designated FHB resistance genes provides context for the observed selection signals.

***Fhb1* region (chromosome 3BS, ~12 Mb).** Block b00559 (Chr3B, 12.4-14.0 Mb; Table 1) directly overlaps the *Fhb1*/TaHRC locus, where a deletion mutation in the histidine-rich calcium-binding protein confers Type II resistance (Su et al. 2019; Li et al. 2019). This block showed one of the strongest positive selection signals in the dataset (Table 1) and achieved Stable classification for DIS in the temporal validation analysis (Table S4). The near-absence of the resistance haplotype in Era 1 followed by its increase to intermediate frequency aligns closely with the KASP-based frequency trajectory of Brault et al. (2026), who reported *Fhb1* increasing from near zero in 1995 to approximately 59% in the URSN and 38% in the URN by 2024. Brault et al. (2026) further demonstrated that the phenotypic advantage of *Fhb1*⁺ over *Fhb1*[−] genotypes has narrowed over time, consistent with the accumulation of minor resistance alleles in non-*Fhb1* backgrounds, a pattern our genome-wide haplotype analysis corroborated by identifying 11 additional loci under positive selection beyond the *Fhb1* region. A second block on chromosome 3B, b00562 (23.5 Mb on Chr3B; 2 markers), showed strong positive selection for DON and achieved Stable classification (Table 1, Table S4). It is ~10 Mb distal to TaHRC and in low LD with b00559 in our panel, supporting interpretation as an independent DON-resistance QTL rather than a tag for the TaHRC haplotype. That b00559 was Stable for DIS and b00562 for DON is consistent with the

pleiotropic effects reported for the *Fhb1* region on both disease spread and mycotoxin accumulation (Buerstmayr et al. 2009; Lemmens et al. 2005).

Chromosome 2A resistance region. Two blocks mapped to chromosome 2A under strong positive selection (Table 1). Block b00266 (244.6-507.9 Mb on Chr2A; 8 markers showed the strongest selection signal in the dataset for DIS, and b00260 (82.0-86.9 Mb) showed strong positive selection for DON. Chromosome 2A harbors QFhb.mgb-2A, mapped to ~600-620 Mb (Giancaspro et al. 2019), distal to b00266 (244.6-507.9 Mb). The two are on the same chromosome but in different physical intervals, suggesting either an independent Chr2A signal or a long-range LD relationship our 3K density cannot resolve. Block b00260 (82.0-86.9 Mb on Chr2A; 5 markers) overlaps the resistance interval of Gervais et al. (2003) and partially overlaps Semagn et al. (2007), while b00266 (244.6-507.9 Mb) is more distal and not reported by either. The independent DIS (b00266) and DON (b00260) signals at different Chr2A positions suggest the chromosome carries multiple resistance determinants rather than a single pleiotropic locus.

Chromosome 3D. Block b00654 (Chr3D, 29.9-41.7 Mb; Table 1) showed the third-strongest positive selection for DIS. Although no formally designated FHB gene maps to this interval, chromosome 3D has been implicated in FHB resistance in multiple mapping populations (Buerstmayr et al. 2009). That the resistance haplotype was already at moderate frequency in Era 1 suggests this region may harbor native resistance alleles that preceded the systematic introgression of exotic sources such as Sumai 3, and the near-fixation by Era 4 indicates continued selection has acted on this locus throughout the 30-year period.

Chromosome 5A (Fhb5 region). Block b00903 (Chr5A, 50.3-106.9 Mb) was validated for DON but showed no directional selection (Table 1). The Fhb5 locus resides within this block in the pericentromeric region of chromosome 5A (Xue et al. 2011; Steiner et al. 2019) and is associated

with Type I resistance and anther extrusion. The low and stable frequency of the resistance haplotype at this block indicates that Fhb5-region resistance has not been systematically selected in this germplasm, consistent with the slower frequency increase for Fhb5 relative to *Fhb1* reported by Brault et al. (2026). Brault et al. (2026) also tracked a third QTL, Fhb5AS from PI 277012, which showed stable frequency since 2010 and significant associations with DON reduction, a pattern not directly captured by our haplotype framework but complementary to our genome-wide results.

Chromosomes 6B and 4B (Fhb2 and Fhb4 regions). No validated blocks mapped to the Fhb2 core region on chromosome 6BS (~15-45 Mb; Cuthbert et al. 2007) or to the Fhb4 region on chromosome 4BL (~640-650 Mb; Xue et al. 2010). Three blocks were validated on chromosome 6B, but all mapped to the long arm far from the 6BS location of Fhb2. This absence likely reflects limited SNP coverage of these regions on the 3K array rather than an absence of these QTL in the germplasm. Higher-density genotyping platforms would potentially detect selection signals at these loci.

The colocalization results reveal a stratified architecture of resistance deployment. The *Fhb1* region on 3BS has undergone the most dramatic frequency shift, from near absence to intermediate frequency, consistent with active introgression. Chromosome 2A carries the strongest overall selection signal across multiple resistance determinants. The 3D region shows near-fixation of a resistance haplotype not linked to any named gene. In contrast, Fhb5-linked variation on 5A remains at low frequency, and Fhb2/Fhb4 were not captured by the marker platform. This heterogeneous landscape underscores the complementary value of targeted KASP panels for named QTL (Brault et al. 2026) alongside the broader haplotype framework deployed here, which identifies additional loci under selection that would be missed by targeted approaches.

Haplotype-Based Detection of Resistance Loci Beyond GWAS Resolution

The block-centric comparison with single-marker GWAS exposes a fundamental limitation of SNP-by-SNP analysis for dissecting polygenic FHB resistance. Of the top 20 haplotype blocks ranked by local GEBV variance, GWAS failed to detect 95-100% across the three traits (Table S5, Figure 4). The haplotype framework validated 14-18 blocks per trait ($\text{FDR} < 0.05$) that were entirely invisible to single-marker analysis, while GWAS identified strongly tagged peaks on chromosomes 1A, 1D, 2A, and 5D that did not overlap with any of the top haplotype blocks for DIS. The two approaches thus recover largely non-overlapping fractions of the resistance architecture.

The multi-marker composition of our validated blocks is not a byproduct of the discovery method; it is its point. Multi-marker blocks (3+ markers) constitute only 10% of the 1,631 genome-wide blocks but 88% of the top-60-by-variance set and 80% of the 35 validated blocks (an ~8-fold enrichment; Fisher $p = 6.5 \times 10^{-17}$). These blocks dominate the top-hit set because the local GEBV sums marker effects across the block, capturing the distributed within-block signal that single-marker GWAS misses by construction. This is precisely the polygenic architecture that SNP-by-SNP analysis fails to resolve (Roy et al. 2025; Sallam et al. 2020; Bernardo 2014). For single-marker blocks, the local GEBV instead reduces to the genome-wide ridge-fitted marker effect, itself shrunk and integrated with neighboring markers across the genome (Voss-Fels et al. 2019; Tong et al. 2024). This pattern is consistent with Roy et al. (2025), who demonstrated that haplotype-based local GEBVs captured disease resistance QTL in barley that single-marker GWAS failed to detect, and with broader evidence that block-level aggregation recovers biologically relevant signals missed by single-marker analysis in wheat and other crops (Brinton et al. 2020; Venske et al. 2019). Shaffer et al. (2025) showed the same empirically: single-SNP

GWAS (FarmCPU, BLINK) detected only the largest-effect QTL (VRS1) in barley row-type, while local GEBV at the same stringency additionally identified VRS3, VRS5, and other annotated loci. Yadav et al. (2026) further showed that haplotype-based variance partitioning captured ~80% of total genetic variance for stripe rust resistance, with the SNP-only model recovering less and the combined model adding only redundant information.

Critically, several of these GWAS-invisible blocks were also under significant positive selection (Table 1), providing independent evidence that breeders have been selecting at these loci through phenotypic evaluation. For example, b00036 (Chr1A, 44-52 Mb; 5 markers) had a validation $R^2_{\text{val}} = 0.070$ for VSK (Table S5) as the joint signal across its 5 markers, while the strongest single-marker GWAS $-\log_{10}(p)$ within the block was only 0.98, consistent with single-marker GWAS at moderate density failing to resolve a joint-marker signal that the haplotype framework captures, rather than with structure-driven inflation (which our drift-control analysis empirically rules out for the validated set). Similarly, b00654 (Chr3D, 30-42 Mb), discussed above as a region harboring putative native resistance alleles approaching fixation, explained 4.8% of phenotypic variance (Table S5) while remaining invisible to single-marker analysis. The validated blocks jointly explained a substantial proportion of phenotypic variance (Table S5), yet GWAS missed nearly all of it. From a breeding perspective, relying solely on GWAS-significant markers for marker-assisted selection would target a small fraction of the available resistance architecture, whereas the haplotype framework provides a more comprehensive set of validated, breeder-actionable genomic targets (Table S5, Figure 4).

Diversity Maintenance: No Bottleneck Detected

At the 35 validated FHB-resistance haplotype blocks, expected heterozygosity remained stable across breeding eras (Table 2), indicating that directional selection at these specific loci has not

produced a local diversity bottleneck. Genome-wide diversity in this germplasm has been characterized separately by Gill et al. (2025), who reported no reduction in genome-wide diversity in North American spring wheat over a comparable period, consistent with our locus-specific observation (Reif et al. 2005; Fu and Somers 2009).

The non-monotonic H_e trajectory, increasing from Era 1 to Era 3, then declining slightly in Era 4 (Table 2), is readily explained by the mathematical relationship between allele frequency and heterozygosity. As initially low-frequency resistance alleles increased toward 0.5 under selection, H_e necessarily increased. The slight Era 4 decline reflects alleles that have been pushed past 0.5, beginning to reduce H_e as they approach higher frequencies. This is the expected population genetic signature of a system undergoing sustained directional selection with alleles at varying starting frequencies.

From a practical standpoint, the absence of fixation at any validated block means that all 35 loci retain selection potential. Breeding programs should monitor frequency trajectories and consider incorporating diversity metrics into parent selection, for example, by tracking haplotype frequencies at validated resistance blocks and prioritizing crosses that increase resistance haplotype frequencies, particularly at blocks where current frequencies remain low and the largest selection response is attainable.

The haplotype-based local GEBV framework combines two analytical choices, block-level aggregation of marker effects (vs single-marker testing) and ridge-shrinkage marker-effect estimation (vs GWAS-style mixed models). Yadav et al. (2026) provide the cleanest disentanglement of these contributions in wheat at comparable marker densities, demonstrating that haplotype-based aggregation captures variance that SNP-only ridge regression does not, with the marginal SNP contribution being negligible. Second, the stability classification can inform

559 marker deployment decisions: Stable blocks (validated across ≥ 3 breeding eras) carry the strongest
560 temporal evidence and are immediately deployable for active tracking and parent selection,
561 whereas Era-specific blocks should be revalidated in contemporary germplasm before being
562 assigned the same operational status. The per-era validation framework could be applied to any
563 long-term coordinated-trial system to assess whether genomic-selection marker effects remain
564 stable over breeding generations, distinguishing temporally-stable signals worth deploying as
565 fixed targets from era-specific signals that should be down-weighted or revalidated periodically.
566 Third, local GEBVs at validated blocks can guide complementary parent selection by identifying
567 lines with non-overlapping resistance haplotype profiles to maximize transgressive segregation
568 potential, an application formalized in the Optimal Haplotype Stacking framework of Villiers et
569 al. (2024), where selecting parent sets on haploblock-level breeding values maintains useful
570 diversity over long-term recurrent selection. For practical haplotype tracking in breeding nurseries,
571 the minimum marker set required to distinguish resistant from susceptible haplotypes depends on
572 the number of distinct haplotypes observed within a block and how many of them carry the
573 resistant classification (Table S7). In simple cases with one resistant and one susceptible haplotype,
574 flanking markers defining LD block boundaries plus at least one internal marker are generally
575 sufficient to confirm haplotype phase; blocks with three or more haplotypes or multiple resistant
576 haplotypes require genotyping all informative markers within the block. For the high-confidence
577 blocks identified here, existing 3K array markers serve this purpose, with the three Stable blocks
578 (b00559/*Fhb1*, b01209, b00562) ready for immediate tracking and Era-specific blocks
579 recommended for revalidation in contemporary germplasm before active deployment, while
580 diagnostic allele-specific PCR markers for major *Fhb1* (Su et al. 2019) enable direct allele-specific
581 tracking. Finally, extending this analysis to agronomic traits from the same nursery system could

clarify whether resistance-associated genomic regions carry yield or quality penalties. Brault et al. (2026) found no yield penalty and even a positive association with grain yield for *Fhb1* and *Fhb5AS* alleles, suggesting linkage drag from Sumai 3-derived resistance has been effectively broken. Integrating agronomic data with the haplotype framework would test for growth-defense tradeoffs at additional loci beyond these three named QTL.

CONCLUSIONS

The companion study by Brault et al. (2026) documents three decades of phenotypic improvement for FHB resistance in this nursery system; the present study characterizes the genomic architecture and haplotype frequency trajectories underlying that progress. At the genomic level, 12 of 35 validated haplotype blocks showed significant positive selection for resistance alleles, confirmed to exceed drift expectations by comparison with non-associated genomic regions. A complementary GWAS comparison demonstrated that the haplotype-based framework captured 12-18% of phenotypic variance in blocks invisible to single-marker analysis, reinforcing the value of block-centric approaches for polygenic resistance traits. The *Fhb1* region on chromosome 3B showed the most dramatic frequency shift (7.4% → 47.5%), consistent with sustained introgression of Sumai 3-derived resistance. Temporal stability analysis identified three block-trait associations robust across multiple breeding eras, including the *Fhb1* region, which represent the highest-confidence targets for marker-assisted selection. Expected heterozygosity at resistance loci remained stable over the study period, with no fixation events, indicating that standing variation has been preserved and continued selection response is achievable, with the greatest gains attainable at QTLs where resistance frequency remains low (Villiers et al. 2024). These results provide a data-driven framework for integrating genetic gain estimation, haplotype tracking,

temporal validation, and diversity monitoring into evidence-based FHB resistance breeding strategies.

Statements & Declarations

Author Contributions

MMT was responsible for formal analysis, investigation, methodology, visualization, writing the original draft, and editing the manuscript. CB contributed to investigation and methodology and provided feedback on the manuscript. EC was responsible for data curation and formal analysis, contributed to validation, and provided feedback on the manuscript. AR provided resources and feedback on the manuscript. HG contributed to investigation and provided feedback on the manuscript. AG contributed to data curation and validation and provided feedback on the manuscript. KG contributed to data curation and validation and provided feedback on the manuscript. JC contributed to data curation and validation and provided feedback on the manuscript. RD contributed to investigation and methodology and provided feedback on the manuscript. JA contributed to data curation and provided feedback on the manuscript. XL contributed to data curation and provided feedback on the manuscript. JF was responsible for conceptualization, funding acquisition, project administration, and supervision, contributed to validation, and provided feedback on the manuscript.

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Conflict of Interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Mention of trade names or commercial products in this publication is solely for the purpose of

providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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Data Availability Statement

Genotyping and phenotyping data have been deposited in the Triticeae Toolbox (<https://wheat.triticeaetoolbox.org/>). The R scripts and code used for analyses, data processing, and figure generation are also available from the corresponding author upon reasonable request.

REFERENCES

- Adeyemo E, Conley E, Bernardo R, Bajgain P, Anderson JA (2023) Combining datasets to routinely predict Fusarium head blight resistance in a wheat breeding program. *Crop Sci* 63:1197-1208
- Adeyemo E, Bajgain P, Conley EJ, Sallam AH, Anderson JA (2020) Optimizing training population size and content to improve prediction accuracy of FHB-related traits in wheat. *Agronomy* 10:543

648 Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Fetch JM,
 649 Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight
 650 resistance QTLs in two wheat populations. *Theor Appl Genet* 102:1164-1168
 651 Arruda MP, Brown P, Brown-Guedira G, Krill AM, Thurber C, Merrill KR, Forber BJ, Kolb FL
 652 (2016) Genome-wide association mapping of Fusarium head blight resistance in wheat
 653 using genotyping-by-sequencing. *Plant Genome* 9:1-14
 654 Bai G, Shaner G (2004) Management and resistance in wheat and barley to Fusarium head blight.
 655 *Annu Rev Phytopathol* 42:135-161
 656 Bernardo R, Yu J (2007) Prospects for genomewide selection for quantitative traits in maize. *Crop*
 657 *Sci* 47:1082-1090
 658 Bernardo R (2014) Genomewide selection when major genes are known. *Crop Sci* 54:68-75
 659 Brault C, Conley EJ, Green AJ, Glover KD, Cook JP, Gill HS, Read AC, Fiedler JD, Anderson JA
 660 (2025) Leveraging historical trials to predict Fusarium head blight resistance in spring
 661 wheat breeding programs. *Plant Genome* 18:e20559
 662 Brault C, Conley E, Read A, Gill H, Blecha S, Green A, Glover K, Cook J, Dill-Macky R, Fiedler
 663 J, Anderson J (2026) 30 years of breeding for Fusarium head blight resistance in wheat: a
 664 success story. Preprint. <https://doi.org/10.22541/essoar.176840925.50706754/v1>
 665 Brinton J, Ramirez-Gonzalez RH, Simmonds J, Wingen L, Orford S, Griffiths S, IWGSC, Haberer
 666 G, Spannagl M, Walkowiak S, Pozniak CJ, Uauy C (2020) A haplotype-led approach to
 667 increase the precision of wheat breeding. *Adv Agron* 162:125-156
 668 Brown JKM (2002) Yield penalties of disease resistance in crops. *Curr Opin Plant Biol* 5:339-344
 669 Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for
 670 Fusarium head blight resistance in wheat: a review. *Plant Breed* 128:1-26

671 Buerstmayr M, Steiner B, Buerstmayr H (2020) Breeding for Fusarium head blight resistance in
 672 wheat: progress and challenges. *Plant Breed* 139:429-454
 673 Butler DG, Cullis BR, Gilmour AR, Gogel BJ, Thompson R (2017) ASReml-R Reference Manual
 674 Version 4. VSN International Ltd, Hemel Hempstead, UK
 675 Corwin, J. A. & Kliebenstein, D. J. (2017). Quantitative resistance: more than just perception of
 676 a pathogen. *Annual Review of Phytopathology* 55:613-633
 677 Cuthbert, P. A., Somers, D. J., & Brulé-Babel, A. (2007). Mapping of Fhb2 on chromosome 6BS:
 678 a gene controlling Fusarium head blight field resistance in bread wheat (*Triticum aestivum*
 679 L.). *Theor Appl Genet* 114:429-437
 680 del Blanco IA, Froberg RC, Stack RW, Berzonsky WA, Kianian SF (2003) Detection of QTL
 681 linked to Fusarium head blight resistance in Sumai 3-derived North Dakota bread wheat
 682 lines. *Theor Appl Genet* 106:1027-1035
 683 DePauw RM, Knox RE, McCaig TN, Clarke FR, Clarke JM (2011) Contribution of breeding to
 684 yield and quality improvements in hard red spring wheat in western Canada. *Crop Sci*
 685 51:713-727
 686 Dowle M, Srinivasan A (2023) data.table: extension of data.frame. R package version 1.14.8
 687 Endelman JB (2011) Ridge regression and other kernels for genomic selection with R package
 688 rrBLUP. *Plant Genome* 4:250-255
 689 Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Longman,
 690 Harlow
 691 Fu YB, Somers DJ (2009) Genome-wide reduction of genetic diversity in wheat breeding. *Crop*
 692 *Sci* 49:161-168

693 Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M,
 694 Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R,
 695 Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human
 696 genome. *Science* 296:2225-2229

697 Gaire R, Brown-Guedira G, Dong Y, Ohm H, Mohammadi M (2022a) Fusarium-damaged kernel
 698 rating has higher genetic correlation with deoxynivalenol than severity or incidence:
 699 implications for breeding. *Crop Sci* 62:1459-1478

700 Gaire R, Sneller C, Brown-Guedira G, Van Sanford D, Mohammadi M, Kolb FL, Olson E,
 701 Sorrells M, Rutkoski J (2022b) Genetic trends in Fusarium head blight resistance from 20
 702 years of winter wheat breeding and cooperative testing in the Northern U.S.A. *Plant Dis*
 703 106:364-372

704 Gervais L, Dedryver F, Morlais JY, Bodusseau V, Negre S, Bilous M, Groos C, Trottet M (2003)
 705 Mapping of quantitative trait loci for field resistance to Fusarium head blight in a European
 706 winter wheat. *Theor Appl Genet* 106:961-970

707 Giancaspro A, Giove SL, Zito D, Blanco A, Gadaleta A (2019) Map-based cloning of QFhb.mgb-
 708 2A identifies a WAK2 gene responsible for Fusarium head blight resistance in wheat. *Sci*
 709 *Rep* 9:6929

710 Gill HS, Blecha S, Conley EJ, Brault C, Fiedler JD, Cook JP, Glover KD, Green AJ, Read AC,
 711 Anderson JA (2026) Genetic variation and Rht gene distribution across a century of
 712 breeding in North American spring wheat. *J Exp Bot* 202

713 Kolb FL, Bai GH, Muehlbauer GJ, Anderson JA, Smith KP, Fedak G (2001) Host plant resistance
 714 genes for Fusarium head blight: mapping and manipulation with molecular markers. *Crop*
 715 *Sci* 41:611-619

716 Lemmens M, Scholz U, Berthiller F, Dall'Asta C, Koutnik A, Schuhmacher R, Adam G,
 717 Buerstmayr H, Mesterházy A, Krska R, Ruckebauer P (2005) The ability to detoxify the
 718 mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for Fusarium
 719 head blight resistance in wheat. *Mol Plant-Microbe Interact* 18:1318-1324

720 Li G, Zhou J, Jia H, Gao Z, Fan M, Luo Y, Zhao P, Xue S, Li N, Yuan Y, Ma S, Kong Z, Jia L,
 721 An X, Jiang G, Liu W, Cao W, Zhang R, Fan J, Xu X, Liu Y, Kong Q, Zheng S, Wang Y,
 722 Qin B, Cao S, Ding Y, Shi J, Yan H, Wang X, Ran C, Ma Z (2019) Mutation of a histidine-
 723 rich calcium-binding-protein gene in wheat confers resistance to Fusarium head blight. *Nat*
 724 *Genet* 51:1106-1112

725 Liu S, Hall MD, Griffey CA, McKendry AL (2019) Meta-analysis of QTL associated with
 726 Fusarium head blight resistance in wheat. *Crop Sci* 49:1955-1968

727 McMullen M, Bergstrom G, De Wolf E, Dill-Macky R, Hershman D, Shaner G, Van Sanford D
 728 (2012) A unified effort to fight an enemy of wheat and barley: Fusarium head blight. *Plant*
 729 *Dis* 96:1712-1728

730 Mesterházy A (1995) Types and components of resistance to Fusarium head blight of wheat. *Plant*
 731 *Breed* 114:377-386

732 Mesterhazy A, Bartók T, Mirocha CG, Komoróczy R (1999) Nature of wheat resistance to
 733 Fusarium head blight and the role of deoxynivalenol for breeding. *Plant Breed* 118:97-110

734 Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-
 735 wide dense marker maps. *Genetics* 157:1819-1829

736 Miedaner T, Korzun V (2012) Marker-assisted selection for disease resistance in wheat and barley
 737 breeding. *Phytopathology* 102:560-566

738 Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of
739 individuals. *Genetics* 89:583-590

740 Nganje WE, Bangsund DA, Leistritz FL, Wilson WW, Tiapo NM (2004) Regional economic
741 impacts of Fusarium head blight in wheat and barley. *Rev Agric Econ* 26:332-347

742 R Core Team (2024) R: A language and environment for statistical computing. R Foundation for
743 Statistical Computing, Vienna

744 Reif JC, Zhang P, Dreisigacker S, Warburton ML, van Ginkel M, Hoisington D, Bohn M,
745 Melchinger AE (2005) Wheat genetic diversity trends during domestication and breeding.
746 *Theor Appl Genet* 110:859-864

747 Roy D, Dinglasan E, Fowler R, Platz G, Lance R, Synman L, Robinson H (2025) Genomic regions
748 associated with spot blotch resistance in elite barley breeding populations. *Mol Breed* 45:16

749 Rutkoski J, Benson J, Jia Y, Brown-Guedira G, Jannink JL, Sorrells ME (2012) Evaluation of
750 genomic prediction methods for Fusarium head blight resistance in wheat. *Plant Genome*
751 5:51-61

752 Sallam AH, Conley E, Prakapenka D, Da Y, Anderson JA (2020) Improving prediction accuracy
753 using multi-allelic haplotype prediction and training population optimization in wheat. *G3*
754 *Genes Genomes Genet* 10:2265-2273

755 Semagn K, Skinnes H, Bjornstad A, Maroy AG, Tarkegne Y (2007) Quantitative trait loci
756 controlling Fusarium head blight resistance and low deoxynivalenol content in hexaploid
757 wheat population from ‘Arina’ and NK93604. *Crop Sci* 47:294-303

758 Shaffer, W., Papin, V., Yadav, S., Voss-Fels, K. P., Hickey, L. T., Hayes, B. J., & Dinglasan, E.
759 G. (2025). Local genomic estimates provide a powerful framework for haplotype
760 discovery. *bioRxiv*, 2025-08

Steiner, B., Buerstmayr, M., Wagner, C., Danler, A., Eshonkulov, B., Ehn, M., & Buerstmayr, H.
 (2019). Fine-mapping of the Fusarium head blight resistance QTL Qfhs. ifa-5A identifies
 two resistance QTL associated with anther extrusion. *Theor Applied Genet* 132:2039-
 2053

Su Z, Bernardo A, Tian B, Chen H, Wang S, Ma H, Cai S, Liu D, Zhang D, Li T, Trick H,
 St. Amand P, Yu J, Zhang Z, Bai G (2019) A deletion mutation in TaHRC confers *Fhb1*
 resistance to Fusarium head blight in wheat. *Nat Genet* 51:1099-1105

Tong, J., Tarekegn, Z. T., Jambuthenne, D., Alahmad, S., Periyannan, S., Hickey, L., Dinglasan,
 E. & Hayes, B. (2024). Stacking beneficial haplotypes from the Vavilov wheat collection
 to accelerate breeding for multiple disease resistance. *Theor Appl Genet*, 137: 274

Tong H, Nikoloski Z, Altmann T (2025) Temporal validation of genomic prediction models in
 multi-environment trials. *Theor Appl Genet* 138:42

Venske E, dos Santos RS, Farias D da R, Rother V, da Maia LC, Pegoraro C, Costa de Oliveira
 A (2019) Meta-analysis of the QTLome of Fusarium head blight resistance in bread
 wheat: refining the current puzzle. *Front Plant Sci* 10:727

Villiers K, Voss-Fels KP, Dinglasan E, Jacobs B, Hickey L, Hayes BJ (2024) Evolutionary
 computing to assemble standing genetic diversity and achieve long-term genetic gain.
Plant Genome 17:e20467

Visser, P. M., Wray, N. R., Zhang, Q., Sklar, P., McCarthy, M. I., Brown, M. A., & Yang, J.
 (2017). 10 years of GWAS discovery: biology, function, and translation. *The American*
Journal of Human Genetics 101:5-22.

Voss-Fels KP, Stahl A, Wittkop B, Lichthardt C, Nagler S, Rose T, Chen TW, Zetzsche H, Seddig
 S, Baig MM, Ballvora A, Frisch M, Ross E, Hayes BJ, Hayden MJ, Ordon F, Leon J, Kage

784 H, Friedt W, Stützel H, Snowdon RJ (2019) Breeding improves wheat productivity under
 785 contrasting agrochemical input levels. *Nat Plants* 5:706-714

786 Walsh B, Lynch M (2018) *Evolution and Selection of Quantitative Traits*. Oxford University Press,
 787 Oxford

788 Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York

789 Wilson WW, Dahl BL, Njanje WE (2018) Economic costs of Fusarium head blight, scab and
 790 deoxynivalenol. *World Mycotoxin J* 11:291-302

791 Windels CE (2000) Economic and social impacts of Fusarium head blight: changing farms and
 792 rural communities in the Northern Great Plains. *Phytopathology* 90:17-21

793 Xue, S., Li, G., Jia, H., Xu, F., Lin, F., Tang, M., Wang, Y., An, X., Xu, H., Zhang, L. and Kong,
 794 Z. (2010). Fine mapping Fhb4, a major QTL conditioning resistance to Fusarium
 795 infection in bread wheat (*Triticum aestivum* L.). *Theor Appl Genet* 121:147-156

796 Xue, S., Xu, F., Tang, M., Zhou, Y., Li, G., An, X., Lin, F., Xu, H., Jia, H., Zhang, L. and Kong,
 797 Z. (2011). Precise mapping Fhb5, a major QTL conditioning resistance to Fusarium
 798 infection in bread wheat (*Triticum aestivum* L.). *Theor Appl Genet* 123:1055-1063

799 Yadav S, Dillon S, McNeil M, Dinglasan E, Jambuthenne D, Mago R, Dodds PN, Hickey LT,
 800 Hayes BJ (2026) Advancing rust resistance in elite wheat with haplotype mapping and a
 801 novel introgression strategy. *J Exp Bot* 77:2773-2792

802 Yano, K., Yamamoto, E., Aya, K., Takeuchi, H., Lo, P.C., Hu, L., Yamasaki, M., Yoshida, S.,
 803 Kitano, H., Hirano, K. and Matsuoka, M. (2016). Genome-wide association study using
 804 whole-genome sequencing rapidly identifies new genes influencing agronomic traits in
 805 rice. *Nature Genetics* 48:927-934

806 Zhu Z, Hao Y, Mergoum M, Bai G, Humphreys G, Cloutier S, Xia X, He Z (2019) Breeding wheat
 807 for resistance to Fusarium head blight in the Global North: China, USA, and Canada. Crop
 808 J 7:730-738

809 **TABLE AND FIGURE LEGENDS**

810 **Table 1.** Directional selection response at 35 validated FHB resistance haplotype blocks. Logistic
 811 regression of binary resistance haplotype classification on breeding era (1-4). Categories:
 812 Positive_selection (frequency increasing, $p < 0.05$), Purifying_selection (frequency decreasing, p
 813 < 0.05), Neutral ($p \geq 0.05$).

814 **Table 2.** Expected heterozygosity ($H_e = 2pq$) at 35 validated FHB resistance haplotype blocks
 815 across breeding eras. Per-cohort assignment: only lines entering in each era are included.

816 **Table S1.** Cohort assignments: each line mapped to a single breeding era based on first nursery
 817 entry year. Source: Manuscript_TableS1_Cohort_Assignments.csv

818 **Table S2.** Check line phenotypic summary across the 33-year testing period (17 checks, 8 resistant,
 819 9 susceptible). Source: Manuscript_TableS2_Check_Line_Summary.csv

820 **Table S3.** Per-era block validation detail: R^2 , p-value, and validation status for each block $\times$ trait
 821 $\times$ era combination. Source: per_era_validation_detail.csv

822 **Table S4.** Stability classification of haplotype blocks with supporting metrics (eras validated,
 823 effect direction consistency, mean R^2 across eras). Source: stability_classification.csv

824 **Table S5.** Block-centric comparison of haplotype framework vs. single-marker GWAS. Top 20
 825 blocks per trait ranked by local GEBV variance. GWAS-supported = at least one marker within
 826 block reached suggestive threshold ($-\log_{10}(p) \geq 4.0$).

827 **Table S6.** GWAS-significant and suggestive associations for DIS and VSK (Bonferroni and
828 suggestive thresholds).

829 **Table S7.** Directional selection response at 35 validated FHB resistance haplotype blocks.
830 Position gives the block span on the indicated chromosome; n SNPs is the number of markers
831 defining the block (see Methods). Logistic regression of binary resistance haplotype
832 classification on breeding era (1-4) yields P (era). Categories: Positive selection (frequency
833 increasing, $P < 0.05$), Purifying selection (frequency decreasing, $P < 0.05$), Neutral ($P \geq 0.05$).

834 **Figure 1.** Stability classification for all 60 block-trait pairs, colored by category (Stable, Era-
835 specific, Unvalidated).

836 **Figure 2.** Resistance haplotype frequency progression across breeding eras for the 15 selected
837 haplotype blocks. Color intensity represents the frequency of the resistance haplotype in each era.
838 Blocks are labeled with chromosome and physical position.

839 **Figure 3.** Genome-wide haplotype-based local GEBV variance (top row) and single-marker
840 GWAS $-\log_{10}(p)$ (bottom row) for (A) DIS, (B) VSK, and (C) DON. Horizontal lines mark the
841 Bonferroni-corrected threshold ($-\log_{10}(p) = 4.67$, dashed) and a suggestive threshold ($-\log_{10}(p) =$
842 4.0 , dotted).

843 **Figure 4.** Per-block phenotypic R^2 (local GEBV vs. DIS) for the top 20 haplotype blocks ranked
844 by local GEBV variance. Point size = local GEBV variance; color indicates validation status (FDR
845 < 0.05). Block labels show chromosome and physical position.

846 **Figure S1.** Check line DIS trajectories over 33 years. Absence of trend confirms environmental,
847 not genetic, variation.

848 **Figure S2.** Drift control: $|\Delta freq|$ at FHB-validated blocks vs. non-associated blocks.

Supplementary Files

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