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Unraveling the genetic of seedling Resistance to Stripe Rust in Bread Wheat through Genome-wide Association Analysis

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Abstract
Stripe rust, caused by Puccinia striiformis f.sp. tritici (Pst), is one of the most destructive diseases of wheat globally, causing yield loss of up to 100% on susceptible cultivars. In Ethiopia, the disease poses a major constraint to wheat production, especially in the highlands, where favorable environmental conditions and the emergence of virulent pathogen races considerably contribute to recurrent epidemics. The frequent emergence of novel virulent pathotypes necessitates the continuous identification and deployment of new resistant resources. This study aimed to identify genomic regions associated with seedling stage stripe rust resistance in 245 diverse bread wheat genotypes using a genome-wide association study approach. Phenotypic evaluation was conducted under controlled greenhouse conditions using a mixture of Pst isolates collected from major wheat-growing regions of Ethiopia. Infection types (IT) were scored on a 0-9 scale. Analysis of variance revealed highly significant phenotypic variation among genotypes ($P < 0.001$), with the best linear unbiased estimates ranging from 0 to 8. Approximately 72.33% of genotypes were classified as resistant, and G193 exhibited complete immunity. Population structure analysis using 10,359 quality-filtered SNP markers identified two main subpopulations with admixed backgrounds.Genome-wide linkage disequilibrium decayed to $r^2 = 0.2$ at an average distance of 5.09Mb. Using a significant threshold of -log10(P) >3, GWAS detected seven significant marker-trait associations across chromosomes 2A, 3A, 4B, 5B, 7A, and unassigned genomic regions. Five associations were consistently detected by multiple models, indicating robust loci. Functional annotation using IWGSC RefSeq v1.1 identified 40 putative candidate genes associated with cell wall reinforcement, redox homeostasis, protein turnover, signal transduction, and metabolic regulation. These genes represent biologically plausible candidates for further investigation of wheat-Pst interaction. The identified resistant wheat genotypes, associated markers, and candidate genes provide valuable resources for further validation and genetic improvement. Overall, the finding could help marker-assisted breeding for stripe rust resistance to developing stripe rust-resistant cultivars that contribute to sustainable wheat production and food security in Ethiopia and beyond.

Keywords: Bread Wheat, Candidate genes, GWAS, Marker-assisted breeding, Stripe rust, SNP markers

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a significant cereal crop that contributes to about one-fifth of daily energy and proteins requirements of more than one-third of the world's population and is important for food security (Alisaac and Mahlein, 2023; FAO, 2023). In addition to its caloric contribution, wheat is a rich source of fiber, vitamins, and minerals (Shewry & Hey, 2015; Biel et al., 2020). The unique nature of wheat gluten underpin its exceptional versatility in the food industry, making it a foundational ingredient for a wide range of food products (Gasparre and Rosell, 2022, 2023). Given the presence of rapid human population growth and climate change, sustainable wheat production is increasingly critical to meet future nutritional demands (He et al., 2026).

Wheat is widely cultivated worldwide as a staple food, with global production forecast at 841.8 million tons in 2025/26 covering 220 million hectares (Statistica, 2026). The European union, China, and India remain the top three largest wheat producers in the world, together accounting for about 48% of the world's total wheat production (USDA, 2026). In Sub-Saharan Africa, Ethiopia is the primary wheat producer owing to the suitable agro-ecological conditions and ongoing expansion in cluster and irrigated wheat cultivation (Nigus et al., 2022). In Ethiopia, wheat is produced on approximately 2.1 million hectares, with a total production estimated at about 6.7 million metric tons and average productivity of roughly 3 t/ha under rain-fed conditions, which is lower than the global average 3.6 t/ha (Tadesse et al., 2022). Although Ethiopia has made major progress in variety development and release (116 wheat varieties), wheat production remains constrained by disease and other biotic stresses, which contribute substantially yield losses and reduce the durability of improved cultivars (Savary et al., 2019; Tadesse et al., 2022).

Among the various diseases affecting wheat, stripe rust poses a significant threat to global production, causing averagely 88% wheat production loss on susceptible varieties (Beddow et al., 2015; Aslam et al., 2026). The disease results from infection by a biotrophic fungus *Puccinia striiformis* f.sp. *tritici* (*Pst*), an obligate biotrophic fungus (Chen et al., 2014). The effect of climate change on the outbreaks of the disease occurs through changes in temperature and humidity patterns, which facilitate pathogen survival, spore germination, overwintering, and disease spread. Milder conditions enable spore overwintering, thus promoting the early and severe outbreak of the disease. The *Pst* is a highly varied pathogen, where new virulent pathotype emerge yearly (Liu et al., 2017). Its capacity to sexual recombination, adapt locally, and travel over long distances

disease makes it possible for this pathogen to cause wide-spread epidemics rapidly (S. Ali et al., 2014; Farber et al., 2017). In Ethiopia, the vulnerability of some cultivars and coupled with favorable climatic conditions, led to recurrent stripe rust epidemics, notably in 1973, 1988, and 2010 with yield losses varying from 30% to 100% depending on cultivar susceptibility and epidemic severity (Daniel K & Shumi R, 2025; Jaleta et al., 2019; Meyer et al., 2021).

Increasing global food demand, coupled with the persistent threat of stripe rust disease, necessitates urgent genetic improvement of wheat to increase yield potential, adaptation and broad-spectrum and durable resistance to the disease. Effective management of stripe rust can be achieved through an integrated approach combining genetic enhancement, disease surveillance, and improved agricultural practices (Bouvet et al., 2022). Genetic improvement is recognized as the most economical, environmentally sustainable, and durable method for disease management (Ghimire et al., 2022; Mapuranga et al., 2022).

Stripe rust resistance is broadly categorized into two main types based on the plant's growth stages and genetic architecture: adult-plant resistance (APR) and all-stage resistance (ASR)(X. M. Chen, 2005). APR, also referred to as horizontal or quantitative resistance, is typically controlled by multiple genes, with additive effects and is considered more durable. In contrast, ASR is governed by one or few major genes (R genes) and often exhibits race-specific effects. ASR follows the classical gene-for-gene interaction model (Flor, 1971), in which host resistance gene recognize corresponding pathogen avirulence factors and activate defense responses.

Stripe rust resistance in wheat is governed by a large and diverse set of genes and loci. To date, 87 stripe rust resistance genes (Yr1-Yr87), and more than 350 QTL have been reported in the wheat genome (McIntosh et al., 2020; Zhu et al., 2023; Sharma et al., 2024). Out of the total named stripe rust resistance genes, 59 confer ASR, whereas 28 are associated with APR. Several permanently designated ASR genes, including Yr5, Yr15, Yr53, Yr61, Yr65 and Yr69, remains widely effective and are valuable resources for breeding, provided they are not associated with linkage drag (Youssef et al., 2026). Many ASR genes commonly encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins, which recognize pathogen effector molecules and activate effector-triggered immunity (ETI) in the host (Jan et al., 2021; Mapuranga et al., 2022). However, durability of released cultivars is often compromised by the emergence of new virulent races that overcome previously effective resistance genes, leading to resistance breakdown and cultivar replacement

(W. Liu et al., 2017). Consequently, the deployment of diverse resistance sources and the pyramiding of ASRs is important strategy for developing cultivars with more durable stripe-rust resistance. Genome-wide association mapping (GWAM) is powerful and revolutionary tool to identify and map genomic regions underlying desirable traits in crop plants. The method scan genetic variations across large set of wheat materials to link specific molecular markers with the target phenotypic trait such as yellow or stripe rust resistance. GWAS enable gene identification at higher mapping resolution because they harness historical recombination accumulated over successive generations (Kumar et al., 2021; Saini et al., 2021; Sari et al., 2020).

Therefore, the continued identification of diverse sources of stripe rust resistance gene is essential for improving wheat production in Ethiopia. Thus, this study aimed to identify genetic loci associated with ASR to stripe rust in a diverse bread wheat panel using GWAS models.

MATERIALS AND METHODS

Plant materials and evaluation of stripe rust resistance at seedling stage

A total of 300 bread wheat genotypes were evaluated in the present study, of which 293 genotypes obtained from CIMMYT (international maize and wheat improvement center), and seven were locally released varieties accessed from Kulumsa Agricultural Research Center (Supplementary Table 1). The latter group incorporated standard checks: the resistant genotype Kingbird (G25) and susceptible genotypes Paven-76 (G64) and Morocco (G302). Those genotypes obtained from CIMMYT were further grouped into 283 breeding lines and seven advanced lines (four from national variety trial and three from adaptation trials). It is important to note that, henceforth, the designation “genotype” applies equally to all breeding lines and released varieties.

The experiment was conducted at Kulumsa Agricultural Research Center (KARC), Plant pathology and protection program laboratory. Stripe rust isolates were selected from *Pst* samples (collected from Kulumsa, Bekoji, Meraro, and Sinana in 2025 main cropping seasons), accessed through KARC. The selection was based on disease severity score they created in different wheat varieties. Then, these virulent isolates were mixed and multiplied on susceptible wheat genotype (Moroco), to get enough inoculum, followed by drying of urediniospores on silica gel and storage at -80°C until use. The mix of *Pst* isolates maintained at -80°C were heat shocked in a water bath at 42°C for 5 min. The spores of the pathogen were suspended in light weight mineral oil (Soltrol

170) and adjusted to $1x10^5$ spores ml^{-1}. About 3-4 seeds of each test genotypes and susceptible genotype (Moroco) were sown in planting pot filled with soil, compost, and sand at a 2:1:1 ratio (v/v/v), replicated two times. The spore suspensions were then inoculated on 7-day-old seedlings, when two leaves were fully expanded, using atomizer inoculators (vacuum pump sprayer). Seedlings were moistened with fine droplets of distilled water 5 min after inoculation, mineral oil evaporates. The pots were placed on a sample tray with a small amount of water at the bottom and covered with plastic cage. The inoculated seedlings were incubated at temperature 8-10°C and 100% relative humidity for 24 hours of darkness. Then, seedlings were transferred to greenhouse compartments with temperature 18-22 ^{O}C, and grown for 18-20 days before recording the infection type (IT).

Phenotypic data collection and analysis

The disease response was recorded as infection types (ITs) using a 0-9 scale (McNeal et al., 1971) on the 20 days of inoculation when susceptible genotype Morocco exhibited maximum infection (Table 1). The normality distribution of IT was checked using the Shapiro-Francia test in R. ANOVA was conducted for the trait across 300 genotypes. The BLUE values were calculated using the lmer() function from the lme4 package in R. This BLUE value was used for the subsequent GWAS analysis. Genotypes with an IT score of $0 \leq IT < 3$, $3 \leq IT < 5$, $5 \leq IT < 7$and $7 \leq IT \leq 9$ were considered resistant, moderate resistant, moderate susceptible, and susceptible, respectively (Line and Qayoum, 1992).

Table 1. Description of the 0 – 9 infection type (ITs) scale used to score stripe rust response in bread wheat

scale	Types of infection	Symptoms
0	Immune	No visible sign symptoms
1	Highly resistant	Necrotic and/or chlorotic flecks; no sporulation
2	Resistant	Necrotic and/or chlorotic blotches or stripes; no sporulation
3	Resistant to Moderately Resistant	Necrotic and/or chlorotic blotches or stripes; trace sporulation
4	Moderate resistance	Necrotic and/ or chlorotic blotches or stripes; light sporulation
5	Moderate susceptible	Necrotic and/or chlorotic blotches or stripes; intermediate sporulation
6	Moderate susceptible	Necrotic and/or chlorotic blotches or stripes; moderate sporulation
7	Susceptible	Necrotic and/or chlorotic blotches or stripes; abundant sporulation
8	Highly susceptible	Chlorosis behind sporulating area; abundant sporulation

9	Very highly susceptible	No necrosis or chlorosis; abundant sporulation

Source: Washington State University's Greenhouse Protocols for Testing and Handling Stripe Rust

Genotyping and SNP quality control

A total of 35,672 GBS derived SNPs were obtained from a previous study (Mekonnen et al., 2021). Of these, 31,052 (87.05%) SNPs were successfully mapped to known chromosomal positions on the reference genome, whereas 828 (2.32%) were mapped to unknown chromosome. The rest 3,792 (10.63%) SNPs were unmapped and were excluded from further analysis. The SNPs were filtered based on callrate (≥80%), and minor allele frequencies (≥5%) using TASSEL v5.0. Moreover, genotypes with missing rate of >20% were removed from marker-trait association (MTA) analysis. Then, the resulted SNPs data were optimally imputed based on the KNN imputation method in the TASSEL v5.0. After ensuring quality checks, a total of 10,359 SNPs were used for further genetic analysis. Furthermore, 245 wheat genotypes were maintained after >80% call rate, and <5% heterozygosity.

Linkage disequilibrium

Linkage disequilibrium (LD) analysis was performed to assess the non-random association of alleles among SNP markers across the genome. LD values between pairs of SNPs were expressed in terms of the correlation of allelic frequencies (r^2) considering a window of 50 SNPs. The LD decay was estimated and visualized using RStudio, based on pairwise r^2 values estimated with TASSEL v5.0 software (Bradbury et al., 2007) and corresponding genomic distance (Mb), following the method of Hill and Weir (1988). The distance at which r^2 dropped below the chosen threshold level of 0.2 was used as an indicator of the extent of LD decay across the genome.

Population structure

A total of 5,279 informative SNP markers (call rate >80%, MAF >5%, and polymorphism information content (PIC) >0.25) were used for population structure analysis. Bayesian_model based clustering was performed using STRUCTURE via the Galaxy Europe platform (Afgan et al., 2018), with the number of subpopulation (K) tested from 1 to 10 using a burn-in period of 20,000 iterations and 100,000 Monte Carlo Markov Chain (MCMC) replicates. The optimal K was determined using StructureSelector (https://lmme.ac.cn/StructureSelector/) according to the

method of Evanno et al. (2005), and the corresponding bar plot was generated using CLUMPAK (Kopelman et al., 2015).

Principal component (PC) analysis was performed to further explore population structure. The optimal number of PCs for inclusion in the GWAS model was determined based on Silhouette method, and scree plot examination. These PC scores were subsequently fitted as covariates in the analysis to mitigate spurious associations arising from population stratification.

Marker-Trait Association Analysis

Marker-trait association (MTA) was performed using BLUEs for the phenotypic data and the filtered robust SNP markers from association panel. Six statistical models were applied within the GAPIT R package: BLINK, FarmCPU, MLMM, GLM, MLM, and SUPER. The principal components, and kinship (VanRaden, 2008) were included as covariates in the GWAS models to account for population structure and relatedness (kinship). GWAS results were visualized with the CMplot package, generating Manhattan and Q-Q plots. The model fitness for accounting for confounding factors (population structure and kinship) was tested using quantile-quantile (Q-Q) plots. Significant MTAs were declared at a significant threshold of $-\log_{10}(P) >= 3$.

Candidate gene identification and functional annotation

The significant MTAs were further examined to identify candidate genes underlying stripe rust resistance. For each significant MTA, the genomic region spanning 5.09 Mb upstream and downstream of the associated SNP was defined based on the genome-wide LD decay distance. Genes within these intervals were identified using the IWGSC RefSeq v1.1 gene annotation, based on the IWGSC RefSeq v1.0 genome assembly, obtained in GFF3 format from Ensembl Plant release 62. Only protein-coding genes were retained for downstream analysis. Functional annotation of the retained genes was conducted using EnsemblPlant BioMart (https://plants.ensembl.org) to retrieve gene ontology (GO) terms associated with candidate genes.

RESULTS

Statistical data analysis

To improve the normality of the original infection type (IT) data, a square-root transformation $IT_{tr} = \sqrt{(IT + 0.5)}$ was applied. The Shapiro-Francia test confirmed the transformed data were more normally distributed than the untransformed value (W' = 0.8569 vs 0.9141, respectively). Best

linear unbiased estimates (BLUEs) of IT ranged from 0 (immune) to 7.5 (susceptible). Based on IT categorization, 72.33% of the genotypes were classified as resistant (0≥IT< 3), 22.67% were moderately resistant (3≥IT<5), 4.33% were moderate susceptible (5≥IT<7), and 0.67% genotype as susceptible (7≥IT≤9) (Figure 1). Genotype G193 was completely resistant (immune) with IT = 0. The broad range of infection types, from immune to susceptible to stripe rust resistance supports the use of this panel for identifying resistance loci. Analysis of variance (ANOVA) on the transformed IT scores revealed significant phenotypic variation ($P < 0.001$) among the bread wheat genotypes (Table 2).

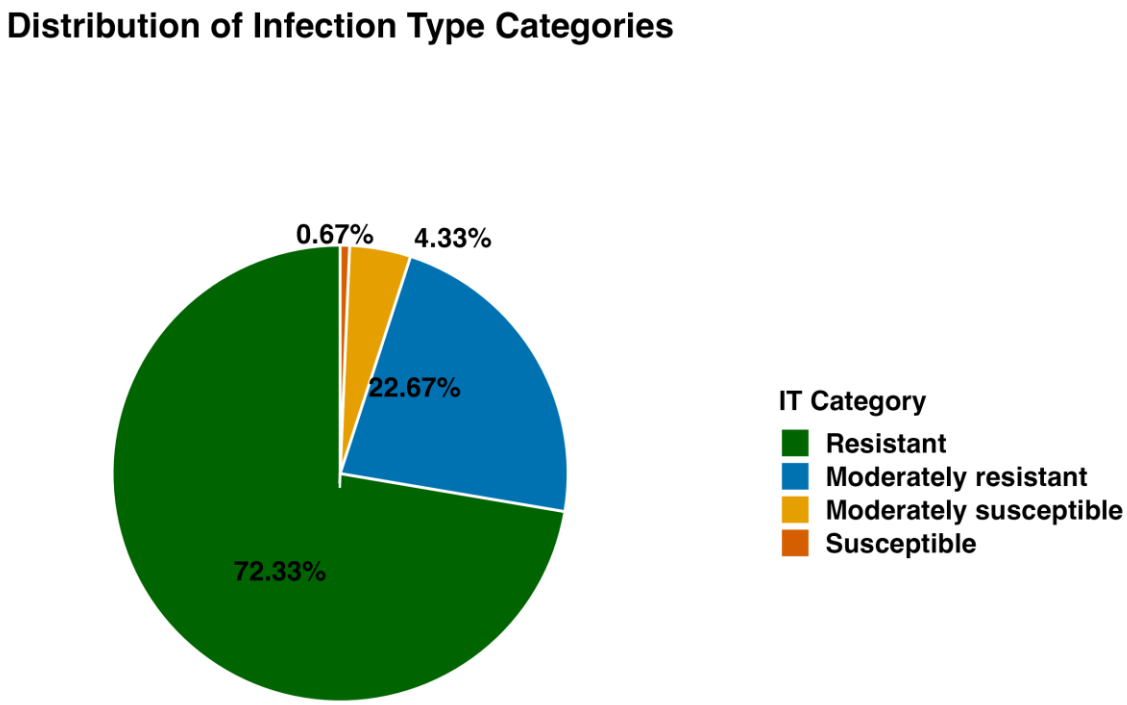

Figure 1. *Proportion of tested bread wheat genotypes classified into different stripe rust infection response categories.*

Table 2. Analysis of variance (ANOVA) for infection type (IT) scores of 245 bread wheat genotypes evaluated against mix of *Pst* isolates under controlled conditions.

Source of variance	df	MSS	*F.value*	*P value*
Genotype	299	0.2619	3.4191	2.75×10^{-25}
Residuals	300	0.0766		

Marker distribution

A high-quality set of SNP markers (10,359 SNPs) was distributed on all 21 bread wheat chromosomes, with B subgenome harboring the largest number of markers (4,401), followed by the A (3,822) and D (1,825) sub-genomes. The rest 311 SNPs were mapped to unidentified chromosome in the wheat reference genome. At the chromosome level, the number of mapped SNPs ranged from 78 on 4D to 900 on 2B. (Figure 2). This SNP dataset indicate good genome-wide coverage but with notable density variation. Most SNPs were clustered toward chromosome ends, telomeres, but the remaining sparse in conserved regions, which aligns with previous study (Wang et al., 2014).

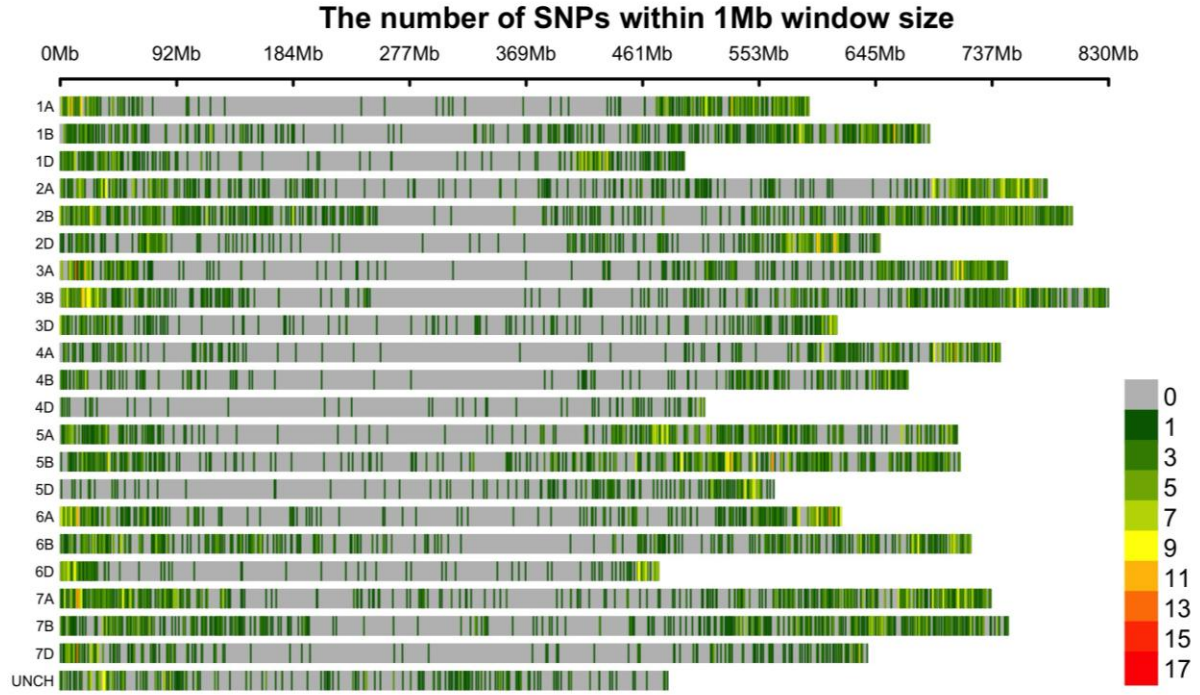

Figure 2. Distribution and density of DArTSeq SNPs across the 21 chromosomes of hexaploidy bread wheat. The color gradient indicates the number of SNPs identified within each 1Mb window

Linkage disequilibrium

Linkage disequilibrium was estimated by calculating the squared correlation coefficient (r^2) for all the 10,359 SNP markers. The LD pattern of the association panel is depicted in a scatter plot of pairwise LD (r^2) over physical distance in Mbp (Figure 3). Whole-genome LD decayed to half its maximum at 5.09 Mbp with sub-genome-specific estimates of 3.9 Mb, 6.5 Mb, and 6.2 Mb for A, B, and D, respectively. This whole genome LD decay distance (5.09 Mb) was used as a uniform window for merging MTAs to map quantitative trait loci (QTLs). While the observed decay is

consistent with previous reports in Chinese wheat landraces (5.98 Mbp; Liu et al., 2019), notable variation exists across populations.

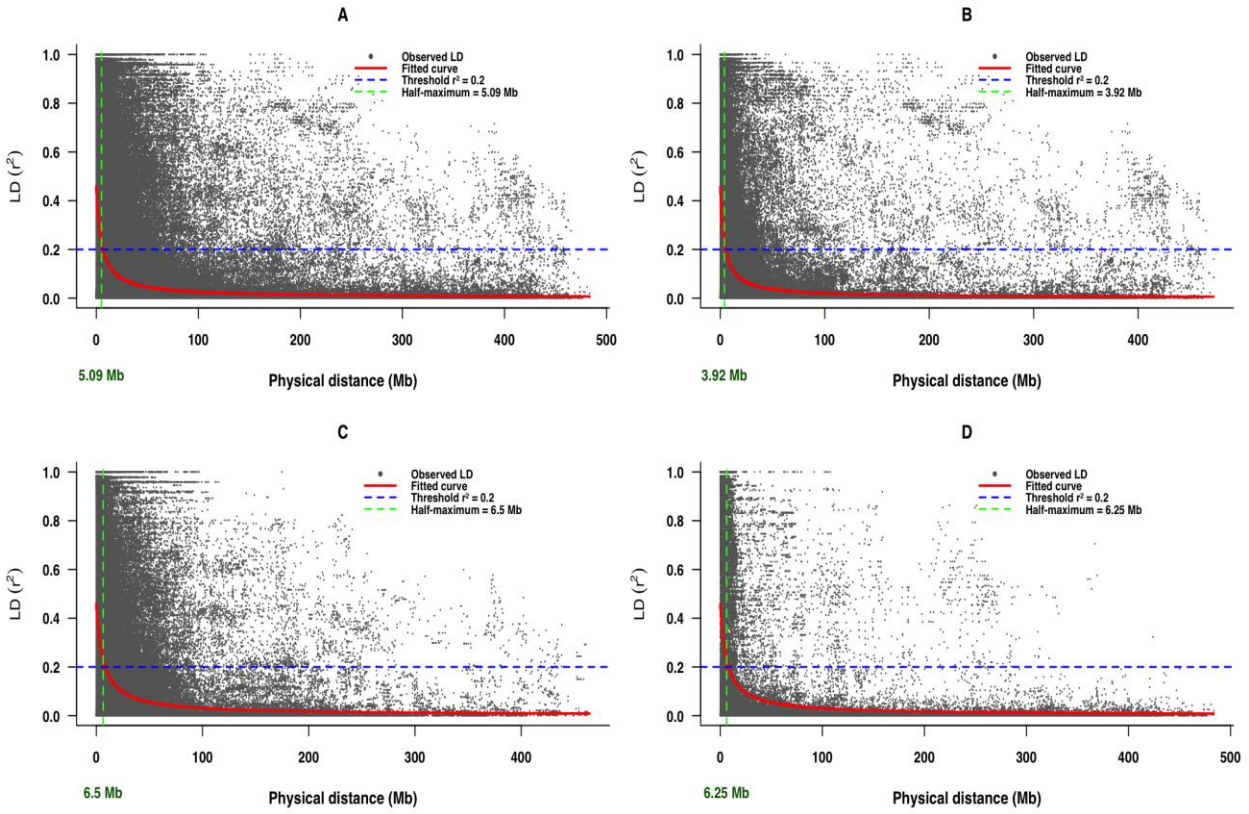

Figure 3. Scatter plot showing Linkage disequilibrium (LD) decay estimated by plotting (r^2) against genetic distance (Mb) in 245 bread wheat genotypes. The blue line indicates the threshold point ($r^2 = 0.2$) where LD dropped to 50% of its maximum value. LD decay value is at cut off point is indicated on the x-axis with the green dotted line.

Population Structure

Population structure was investigated using complementary approaches, including model-based Bayesian clustering (STRUCTURE), principal component analysis (PCA), and kinship analysis. Bayesian clustering was performed using STRUCTURE software (Pritchard et al., 2000) via the Galaxy Europe platform (Afgan et al., 2018). The optimum number of subpopulations (K) was determined using the ΔK method (Evanno et al., 2005), where the highest ΔK value was obtained at K = 2 (Figure 4B), implying that there are two genetic groups in the association panel. Individuals in each subpopulation were assigned based on a membership coefficient threshold of >0.50 (Royo et al., 2010). The admixture plot (Figure 4C) for the two subpopulation indicated that there are multiple genetic backgrounds due to gene flow and repeated crosses between germplasm from different breeding programs (Kabbaj et al., 2017; Lado et al., 2026; Vikas et al., 2022). The

two subpopulations, referred to as Cluster I and Cluster II, consists of 134 and 111 genotypes, respectively. Five out of the six commercial varieties included in this study belongs to Cluster I. Moreover, pairwise genetic relatedness of the individuals was calculated using the VanRaden method (VanRaden, 2008) as implemented in the rMVP package. The kinship matrix obtained is demonstrated as a heatmap in Figure 4E, supports the presence of two distinct groups, consistent with the STRUCTURE results.

In addition to the primary subdivision at K = 2, finer-scale substructure was detected by the Silhouette method and PCA. The Silhouette method identified K = 4 as the optimal number of clusters for the PCA-derived groupings (Figure 4A), and PCA separated the genotypes into four subclusters (Figure 4D). The low proportion of variance explained by PC1 (6.24%) and the gradual decline across PCs are consistent with the breeding history of the panel, which includes genotypes that have undergone extensive germplasm exchange and intercrossing among programs (Kabbaj et al., 2017; Lado et al., 2026). These results indicate that, although the panel is broadly structured into two major groups, additional genetic differentiation exists within these groups, possibly reflecting breeding history, geographical origin, or selection pressure. Collectively, these results confirm the suitability of the association panel for genome-wide association studies (GWAS) and underscore the importance of accounting population structure in subsequent analyses to minimize false associations.

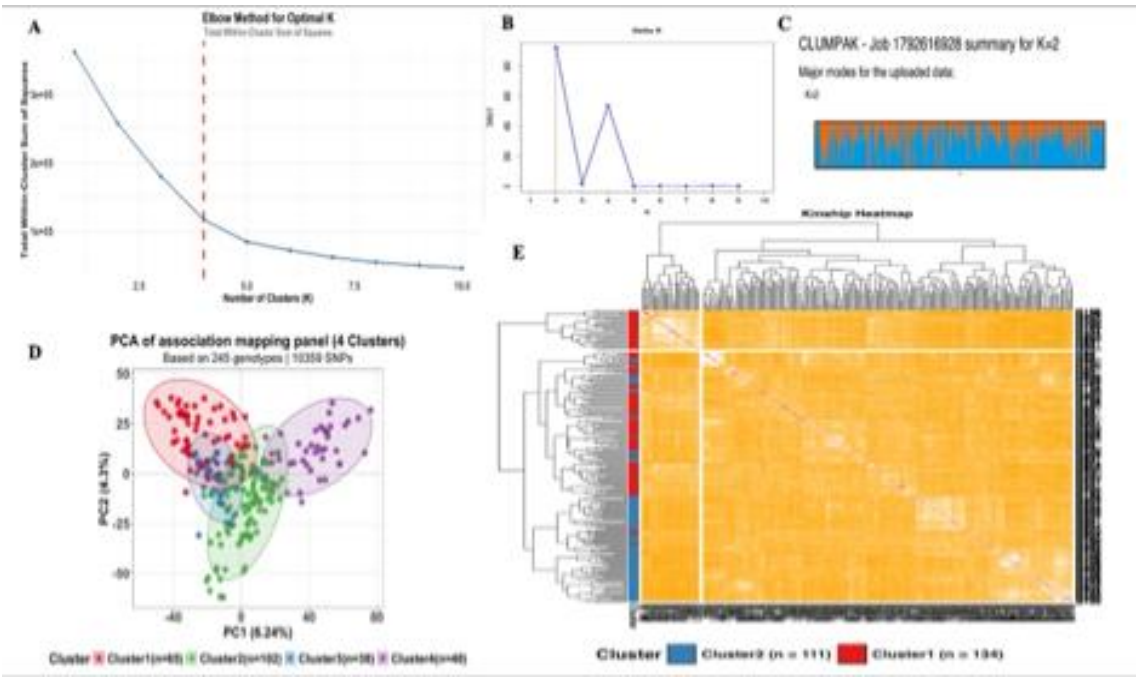

Figure 4. Population structure and kinship analysis of the wheat association panel. (A) Silhouette method determining the optimal number of cluster (K=4) in PCA plot, (B) Delta K plot from StructureSelector identifying the most probable number of subpopulations (K =2), (C) Population structure bar plot (Q-matrix) showing two clusters with admixture, the genotype of each line on the figure is represented by a colored line where each color (blue and red) reflects the membership in the K cluster (D) PCA plot indicating fine scale differentiation within the two main groups, (E) Kinship displayed through heatmap and a clustering tree outside of the matrix. Blue color, the off-diagonal region, represents a low relatedness between pairs of individuals in the panel while red shows a high correlation.

Marker-trait associations

The identification of substantial marker-trait associations (MTAs) of infection type at significance level of p-value ≤ 0.001 revealed seven markers (Table 3). These seven MTAs were distributed across chromosomes 2A, 3A, 4B, 5B, 7A, and an Unknown chromosome. Each of these chromosome's harbor one MTA, apart from 5B, which consisted of two MTAs. The identified significant MTAs were detected across the six models; BLINK, FarmCPU, MLMM, SUPER, MLM, and GLM resulting in 4, 5, 5, 4, and 5 MTAs, respectively (Figure 5, Table 3). The quantile-quantile (Q-Q) plots showed well-controlled population structure with no evidence of inflation (Figure 5), indicating that the statistical models were appropriately fitted. The significant MTAs were visualized with Manhattan plots (Figure 5) and physical map of the MTAs on chromosomes (Figure 7). Four of MTAs were located on the long arm (q) and two were on the short-arm (p) of respective chromosomes (Figure 7). Off the seven MTAs five were repeatedly detected in more than three tested models and considered to be reliable and robust quantitative trait nucleotides (QTNs) as exhibited by ggVennDiagram v1.5.7 (Gao et al., 2024) (Figure 6).

Table 3. Summary of significant marker-trait associations linked with stripe rust resistance at seedling stage, as described by GLM, MLM, MLMM, SUPER, BLINK and FarmCPU

SNP	Model	Chr.	Position	Effect	P.value	LOD
1250340|F|0-20:T>A-20:T>A	SUPER	2A	771074424	0.466	$7.02X10^{-4}$	3.15
1200629|F|0-33:C>T-33:C>T	BLINK	3A	71537069	0.7954	$3.11X10^{-4}$	3.51
1071386|F|0-17:G>T-17:G>T	GLM,	4B	18709610	0.6784	$2.26X10^{-4}$	3.65
	MLM			0.6694	$2.94X10^{-4}$	3.53
	SUPER			0.5963	$1.48X10^{-4}$	3.83
	FarmCPU			0.6785	$1.71X10^{-4}$	3.76
	MLMM			0.6694	$2.02X10^{-4}$	3.69
	BLINK			0.665	$8.87X10^{-6}$	5.05
1069330|F|0-6:T>A-6:T>A	GLM,	5B	459473976	-0.5813	$6.62X10^{-5}$	4.18
	MLM			0.5721	9.26X10-5	4.03
	SUPER			-0.4877	$9.12X10^{-5}$	4.04
	FarmCPU			-0.5813	$4.37X10^{-5}$	4.36
	MLMM			-0.5721	$5.49X10^{-5}$	4.26
	BLINK			-0.5718	$5.97X10^{-6}$	5.22
1246469|F|0-20:C>G-20:C>G	GLM	5B	455980674	-0.497	$1.05X10^{-4}$	3.98
	MLM			-0.488	$1.57X10^{-4}$	3.80
	FarmCPU			-0.429	9.88X10-5	4.01
	MLMM			-0.497	$7.29X10^{-5}$	4.14
	BLINK			-0.488	$1.00x10^{-4}$	3.99
1207653|F|0-43:C>T-43:C>T	GLM	7A	736414481	-0.649	$7.46X10^{-4}$	3.13
	MLM			-0.663	$6.33X10^{-4}$	3.19
	FarmCPU			-0.649	$6.2x10^{-4}$	3.20
	MLMM			-0.663	$4.71x10^{-4}$	3.33
	BLINK			-0.604	$6.49x10^{-4}$	3.188
995354|F|0-55:G>A-55:G>A	GLM	UNCH	323303590	0.434	$7.07X10^{-4}$	3.15
	MLM			0.426	$9.91X10^{-4}$	3.00
	FarmCPU			0.434	$5.94X10^{-4}$	3.22
	MLMM			0.426	$7.68X10^{-4}$	3.11

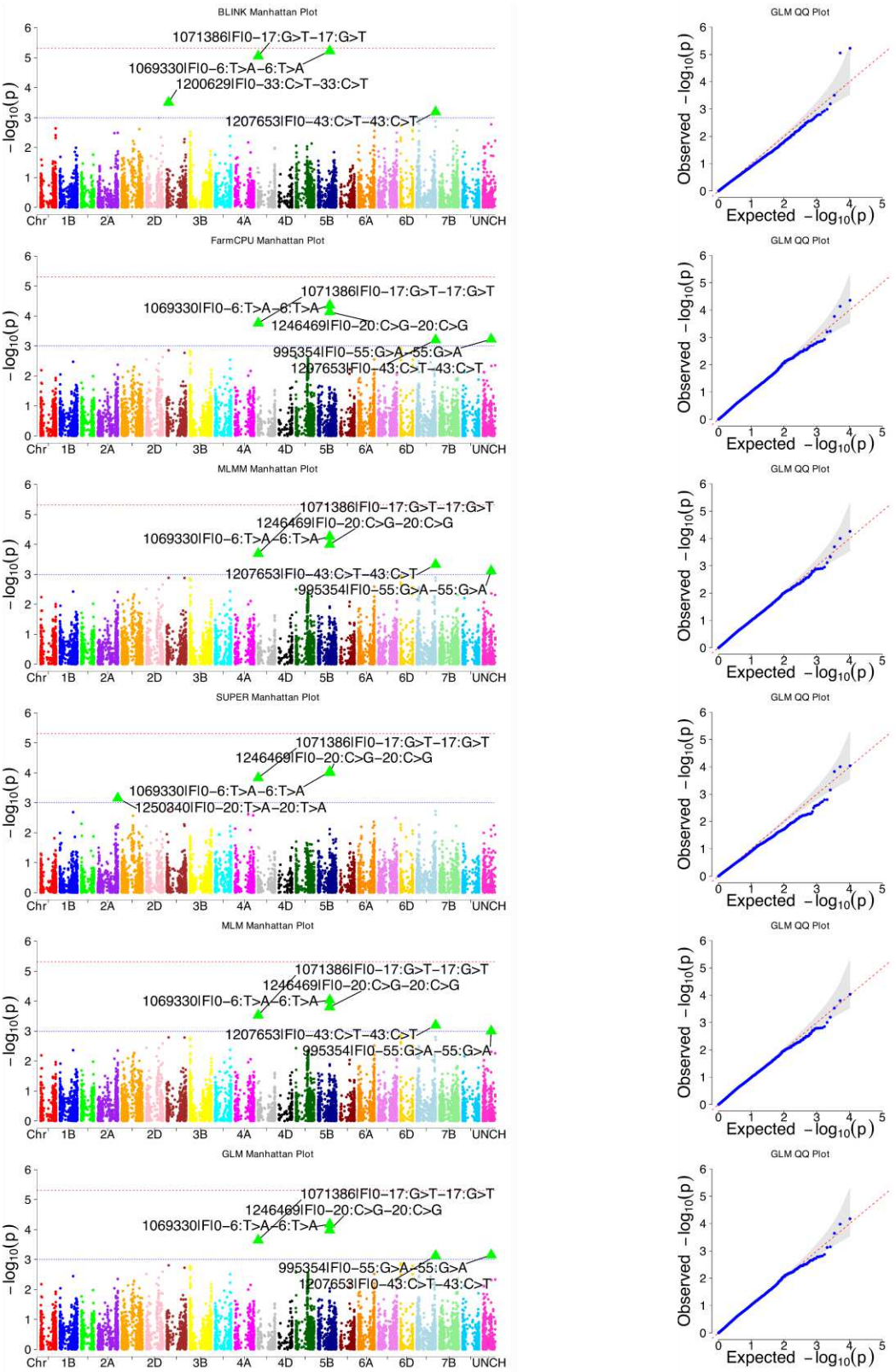

Figure 5. Manhattan and QQ plots illustrating significant marker-trait associations (LOD≥3) for seedlings infection type (IT), as detected by GLM, MLM, MLMM, SUPER, BLINK and FarmCPU models.

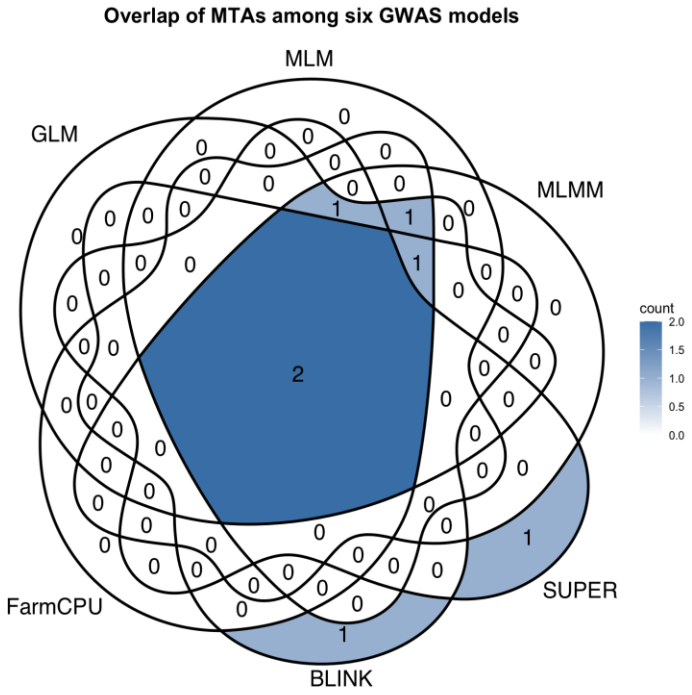

Figure 6. Venn Diagram displaying MTAs detected by the three models (GLM, MLM, MLMM, SUPER, BLINK, and FarmCPU)

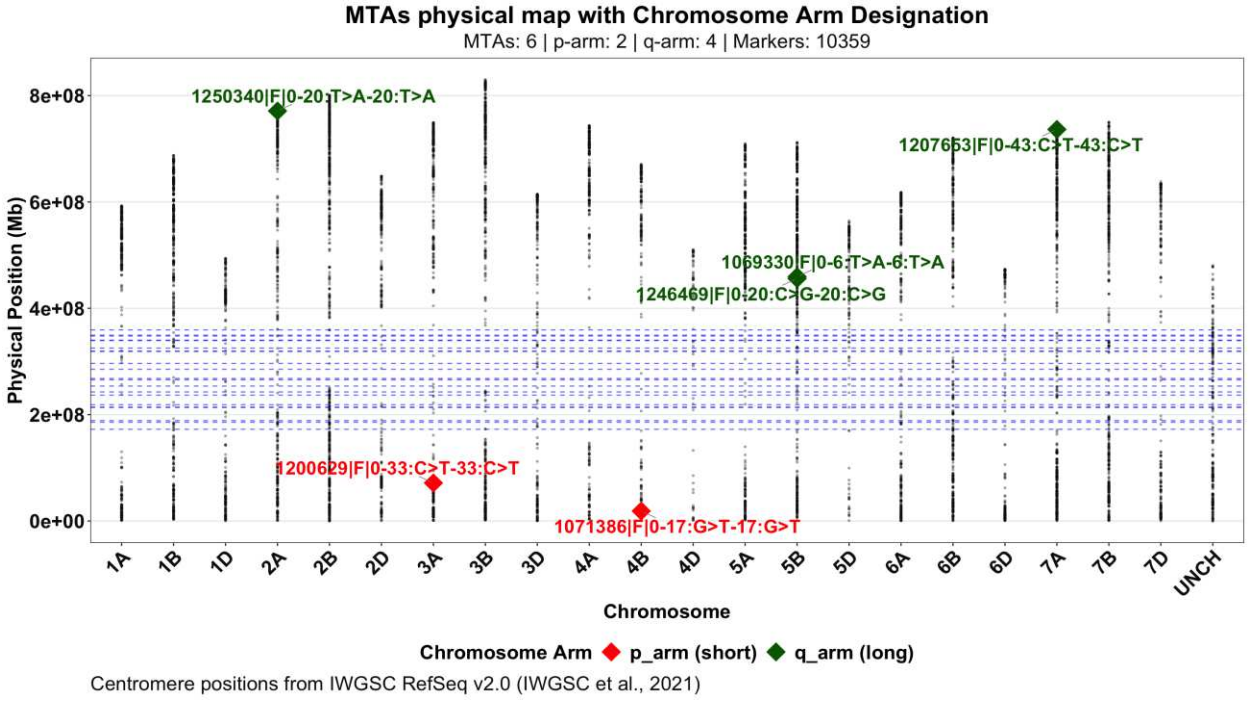

Figure 7. The physical map displaying position of each MTAs on each chromosome, the blue line indicates the centromeric region of each chromosome. The red and green marks indicate the significant markers on the short and long arms of the bread wheat chromosomes respectively.

Candidate genes screening and functional annotation

Screening genomic intervals extending 5.09 Mb on either side of significant MTAs identified 40 candidate genes associated with seedling stripe rust resistance loci (Supplementary Table 2). Functional annotation revealed that these genes encode proteins associated with several biological processes potentially relevant to fungal disease resistance, including cell wall reinforcement, signal transduction, redox regulation, vesicle trafficking, protein turnover, and metabolic adjustment.

Among the identified candidates, several genes were associated with cell wall reinforcement, and metabolic adjustment. Cell-wall-associated candidates included perodixase, dirigent, and WAT1. Fourteen candidate genes were annotated as peroxidase including TraesCS2A02G571700, TraesCS2A02G574400, TraesCS2A02G573400, and TraesCS2A02G573300. TraesCS2A02G584600, TraesCS4B02G028400, TraesCS2A02G584400, and TraesCS2A02G575700 encode WAT1-related protein, whereas TraesCS4B02G024500 encoded a dirigent protein. and TraesCS4B02G024500 encode dirigent proteins. Metabolic adjustment-associated candidate included 3-isopropylmalate dehydrogenase (TraesCS2A02G577600), pyruvate kinase (TraesCS4B02G021300), and three Pentatricopeptide repeat-containing protein encoded by TraesCS3A02G109700, TraesCS2A02G582700, and TraesCS3A02G104900.

Additional candidate genes were associated with signal transduction and vesicle trafficking. TraesCS5B02G275200 encodeD NAC domain-containing protein, whereas TraesCS2A02G580900, and TraesCS4B02G031100 encoded serine/threonine-protein kinases. TraesCS2A02G574000 was annotated as a tubby-like F-box protein. Vesicle trafficking-associated candidates included two Exocyst subunit Exo70 family proteins encoded by TraesCS2A02G583800 and TraesCS2A02G583700. Furthermore, protein turnover-associated candidates included cysteine, cysteine protease inhibitor and E3 ubiquitin-protein ligases, indicating additional potential involvement of proteolytic regulation and ubiquitin-mediated protein turnover in stripe rust resistance.

Discussion

Marker distribution, Population structure, and linkage disequilibrium

The present study employed a comprehensive genome-wide association approach to dissect the genetic architecture of seedling-stage stripe rust resistance in a diverse bread wheat panel. The high-quality set of 10,359 DArTseq SNP markers provided robust genome-wide coverage across all 21 chromosomes. The marker set was not evenly distributed among subgenome, the B subgenome carrying the highest number of SNPs (42.48%), while the D subgenome possessed the fewest (17.62%). This uneven distribution is similar to those reported in earlier studies on wheat, where the D genome is less polymorphic due to its narrower genetic diversity (Mirzaghaderi and Mason, 2019; Cavalet-Giorsa et al., 2024; Wang et al., 2024). Similarly, (Mekonnen et al., 2021) used 7,776 filtered SNPs, of which 42.5% were distributed on the B genome, and while D genome harbored the lowest number (22.3%). Clustering of markers near the chromosome ends, areas with high recombination rates, is supported by earlier research on wheat and confirms the appropriateness of the panel for high-density linkage mapping (Ali et al., 2022).

The population structure analysis revealed a primary subdivision (K = 2), and fine-scale substructure analysis using PCA, confirming that the panel was admixed rather than homogeneous. Such structure is expected in breeding materials with shared ancestry, admixture, and recurrent germplasm exchange (Van Der Nest et al., 2021; Smith et al., 2021). The clustering of five commercial varieties within cluster-I and the low variance explained by principal components (10.54%) further supports this admixture and presence of extensive germplasm exchange among breeding programs. Therefore, this structure output underscores the need to correct for relatedness and stratification to reduce spurious association in GWAS (Kang et al., 2010; Chen and Lipka, 2016).

In the present study, the whole-genome LD decayed at an average distance of 5.09 Mb ($r^2 = 0.2$) indicating moderate mapping resolution in the panel. Basically, mapping resolution depends on the study population, marker density, and criteria used for comparison (r^2-value). Besides, the study revealed existence of considerable differences among the wheat sub-genomes' LD decay distances (3.9 Mb, 6.5 Mb, and 6.2 Mb for A, B, and D, reflecting reflect uneven historical recombination and breeding selection across the wheat genomes (Liu et al., 2019; Tyrka et al., 2021; Laribi et al., 2025). Faster decay (2 Mbp) was reported in a set of CIMMYT spring bread wheat - lines (Sehgal et al., 2020). In contrast to faster LD decay, the slower decay (22 - 23 Mbp) was found in collections from Kazakhstan and Mexican (Kokhmetova et al., 2020; Vikram et al.,

2021). Such variation in the LD pattern among different association panel is likely attributable to differences in selection, mutation, admixtures, mating history, mapping population, marker type and density.

Marker-trait association

The GWAS identified 13 significant MTAs for stripe rust resistance at seedling stage across multiple chromosomes indicates a polygenic nature of resistance (Chen et al., 2012; Mahmood et al., 2022). The significant markers were detected on the chromosomes 2A, 2B, 2D, 3A, 5A, 5B, 6A, 6B, 6D, and Unknown chromosome. Similarly, Liu et al. (2017) identified 68 loci associated with seedling resistance to yellow rust in Ethiopian durum wheat distributed on chromosome 1AS, 1BS, 3AS, 4BL, and 5BL. Same authors identified stripe rust field resistance loci on durum wheat chromosomes of 1A, 1B, 2BS, 3BL, 4AL, 4B and 5AL. Likewise, Liu et al. (2018), identified 12 all-stage resistance MTAs on chromosomes 1A, 1B, 1D, 3D, 4A, 5A, 5B, 6B, and 7B in US pacific Northwest Winter Wheat Accessions by GWAS and linkage mapping methods.

In this study, the concentration of some MTAs on the q-arm may reflect the recombination frequency and better marker resolution in distal chromosome regions, which can facilitate the identification of resistance loci (Phillips et al., 2013; Darrier et al., 2017). The repeated detection of multiple MTAs across several GWAS models suggests that these loci are less likely to be false positives an may represent stable resistance-associated regions suitable for downstream validation and breeding use (Li et al., 2022; Marcano-Delgado, 2025).

Candidate gene identification and functional annotation

Gene annotation analysis identified 40 putative candidate genes within the genomic intervals surrounding significant MTAs. Given that stripe rust (*Puccinia striiformis f.sp. tritici*) is a biotrophic pathogen, candidate genes involved in cell wall reinforcement, defense signaling, redox balance, vesicle trafficking, and metabolic adjustment represent biologically plausible contributors to wheat-*Pst* interactions. These functional categories are consistent with the multilayered nature of plant immunity, which integrates pathogen recognition, intracellular signaling, transcriptional reprogramming, and downstream defense responses (Jan et al., 2026).

Several candidate genes were associated with cell-wall reinforcement, including those encoding dirigent, peroxidase, and WAT1-related proteins. These proteins are known to strengthen cell wall through lignin and related polymer deposition, thereby reducing the accessibility of host tissue to

fungal penetration. Disruption of WAT1 in Arabidopsis confers resistance to several vascular pathogens, including the fungal pathogens *Verticillium dahlia* and *V.albo-atrum*, through mechanism involving salicylic acid-associated immunity (Denancé et al., 2013; Ranocha et al., 2010). This represents a well-established defense strategy against biotrophic pathogens, including *Pst*, which rely on living host cells for successful infection and colonization.

Other candidates, such as cysteine proteinase inhibitors, E3 ubiquitin-related proteins, and serine/threonine-protein kinases, were associated with protein turnover and signal transduction. Protease inhibitors may protect host proteins against pathogen-derived proteolytic activities, whereas plant proteases and their inhibitors participate in pathogen recognition, immune signaling, and regulation of defense-associated proteolysis (Van Der Hoorn & Klemenčič, 2021). E3 ubiquitin ligases are important regulators of plant immunity because they control the stability and turnover of immune receptors, transcription factors, kinases, and other signaling components, thereby influencing both the activation and attenuation of defense responses. Similarly,serine/threonine-protein kinases may participate in phosphorylation-dependent signaling cascades that coordinate pathogen perception and downstream immune responses. In addition, exocyst proteins may support the targeted secretion of defense-related materials to infection sites (Kulich et al., 2018; Xu et al., 2025; Zhao et al., 2018).

Redox homeostasis represents another functional category among the annotated candidate genes. The identification of glutathione peroxidase and peroxidase related genes suggests potential involvement of reactive oxygen species (ROS) regulation during host-pathogen interaction. Since biotrophic fungi are often constrained by an oxidative burst that is strictly regulated, it becomes necessary to balance the production and degradation of ROS to prevent host damage while still defending themselves against the host. The presence of these anti-oxidant enzymes in the MTA regions suggests that there must be some way of regulating ROS in order to keep the HR in check (Sheng et al., 2022; do Carmo Santos et al., 2025).

In addition to that, the characterization of metabolic genes like pyruvate kinase, 3-isopropylmalate dehydrogenase, and Pentatricopeptide repeat-containing protein indicate that metabolic reprogramming could be required to cope with the energy requirements of immune responses. Though these genes do not qualify as classic resistance genes, they may act indirectly by

facilitating the physiological requirements of the host's defense system (Tauzin and Giardina, 2014; Yamada and Mine, 2024).

CONCLUSION

Stripe rust is the major bottleneck to wheat production globally. Resistance breeding is best strategy, disable and eco-friendly to control yield loss due to the disease. The frequent emergence of new pathotypes of *Pst* makes it necessary to discover, characterize, and utilize the new sources of resistance, along with molecular tools that will aid in transferring them to suitable varieties of bread wheat. In this study, the genetic bases of seedling stage to resistance of stripe rust disease had been explored in a diverse set of 245 bread wheat genotypes using genome wide association study approach. The phenotypic analysis revealed substantial genetic variability, with approximately 72.33% of the genotypes exhibiting a resistance reaction and one genotype (G193) displaying complete immunity (IT = 0), highlighting existence of promising resistance sources for further validation and use in wheat resistance breeding program. Population structure analysis identified two major sub-populations (K = 2). STRUCTURE and kinship analyses confirmed the presence of genetic groupings in the association panel, suggesting the need of accounting for population structure and kinship in subsequent MTA analysis to minimize spurious MTA.

The GWAS identified seven significant marker-trait associations on five chromosomes, with five robust QTNs consistently detected across multiple models. Functional annotation of 40 potential candidate genes in the genomic regions based on LD decay distance uncovered genes potentially involved in fungal disease defense, including cell wall reinforcement, signal transduction, redox regulation, signal transduction, vesicle trafficking, protein turnover, and metabolic adjustment. Overall, the present study has identified promising sources of seedling resistance materials to wheat stripe rust, detected statistically supported MTAs and candidate genes involved in yellow rust resistance. These findings help to initiate genome-assisted wheat resistance breeding against stripe rust. Future research should address the functional validation of the candidate genes by using either mutagenesis, gene silencing, gene editing or transformation approaches to understand their functions in stripe rust defense. The genetic resources and markers identified herein will accelerate wheat breeding programs aimed at developing durable stripe rust-resistant wheat cultivars in Ethiopia and beyond.

Author Contributions

Wegayehu Felek was involved in data collection, methodology, statistical analysis and writing - the original draft; Tilahun Mekonnen was involved in conceptualization, methodology, genotyping and reviewing; Sisay Kidane was involved in data curation, visualization, and reviewing; Kassahun Tesfaye was involved in methodology, project administration and supervision.

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Conflict of Interest Disclosure

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.

Author Agreement/ declarations

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Ethical approval:

The research does not involve human participants or animals.

REFERENCE

Afgan, E., Baker, D., Batut, B., van den Beek, M., Bouvier, D., Cech, M., Chilton, J., Clements, D., Coraor, N., Grüning, B. A., Guerler, A., Hillman-Jackson, J., Hiltemann, S., Jalili, V., Rasche, H., Soranzo, N., Goecks, J., Taylor, J., Nekrutenko, A., & Blankenberg, D. (2018). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Research*, *46*(W1), W537–W544. https://doi.org/10.1093/nar/gky379

Ali, M., Danting, S., Wang, J., Sadiq, H., Rasheed, A., He, Z., & Li, H. (2022). Genetic Diversity and Selection Signatures in Synthetic-Derived Wheats and Modern Spring Wheat. *Frontiers in Plant Science*, *13*. https://agris.fao.org/search/fr/records/675accd00ce2cede71d17ab1

Ali, S., Gladieux, P., Leconte, M., Gautier, A., Justesen, A. F., Hovmøller, M. S., Enjalbert, J., & Pope De Vallavieille, C. (2014). Origin, Migration Routes and Worldwide Population Genetic Structure of the Wheat Yellow Rust Pathogen Puccinia striiformis f.sp. Tritici. *Plos Pathogens 1 (10), 003903. (2014)*. https://agris.fao.org/search/en/providers/122652/records/64776142bc45d9ecdbc340f2

Alisaac, E., & Mahlein, A.-K. (2023). Fusarium Head Blight on Wheat: Biology, Modern Detection and Diagnosis and Integrated Disease Management. *Toxins*, *15*(3), 192. https://doi.org/10.3390/toxins15030192

Aslam A. H., Ali Z., Saleem K., Maqbool R., Dababat A. A., Ozdemir F., Lahlali R., Nurbekov A., Jarroudi M. E., Bhavani S., & Ali M. A. (2026). *Escalating threat of wheat stripe rust under climate change: Pathogen evolution, resistance durability, and future management*. https://agris.fao.org/search/zh/records/6a32c4ebb2791246588aa54d

Beddow, J. M., Pardey, P. G., Chai, Y., Hurley, T. M., Kriticos, D. J., Braun, H.-J., Park, R. F., Cuddy, W. S., & Yonow, T. (2015). Research investment implications of shifts in the global geography of wheat stripe rust. *Nature Plants*, *1*, 15132. https://doi.org/10.1038/nplants.2015.132

Biel, W., Kazimierska, K., & Bashutska, U. (2020). Nutritional value of wheat, triticale, barley and oat grains. *Acta Scientiarum Polonorum Zootechnica*, *19*(2), 19–28. https://asp.zut.edu.pl/2020/19_2/asp-2020-19-2-03.pdf

Bouvet, L., Percival-Alwyn, L., Berry, S., Fenwick, P., Mantello, C. C., Sharma, R., Holdgate, S., Mackay, I. J., & Cockram, J. (2022). Wheat genetic loci conferring resistance to stripe rust in the face of genetically diverse races of the fungus Puccinia striiformis f. Sp. Tritici. *Theoretical and Applied Genetics*, *135*(1), 301–319. https://doi.org/10.1007/s00122-021-03967-z

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics*, *23*(19), 2633–2635. https://doi.org/10.1093/bioinformatics/btm308

Cavalet-Giorsa, E., González-Muñoz, A., Athiyannan, N., Holden, S., Salhi, A., Gardener, C., Quiroz-Chávez, J., Rustamova, S. M., Elkot, A. F., Patpour, M., Rasheed, A., Mao, L.,

Lagudah, E. S., Periyannan, S. K., Sharon, A., Himmelbach, A., Reif, J. C., Knauft, M., Mascher, M., … Krattinger, S. G. (2024). Origin and evolution of the bread wheat D genome. *Nature*, *633*(8031), 848–855. https://doi.org/10.1038/s41586-024-07808-z

Chen, A. H., & Lipka, A. E. (2016). The Use of Targeted Marker Subsets to Account for Population Structure and Relatedness in Genome-Wide Association Studies of Maize (Zea mays L.). *G3: Genes|Genomes|Genetics*, *6*(8), 2365–2374. https://doi.org/10.1534/g3.116.029090

Chen, W., Wellings, C., Chen, X., Kang, Z., & Liu, T. (2014). Wheat stripe (yellow) rust caused by Puccinia striiformis f. Sp. Tritici. *Molecular Plant Pathology*, *15*(5), 433–446. https://doi.org/10.1111/mpp.12116

Chen, X. M. (2005). Epidemiology and control of stripe rust [Puccinia striiformis f. Sp. Tritici] on wheat. *Canadian Journal of Plant Pathology*, *27*(3), 314–337. https://doi.org/10.1080/07060660509507230

Chen, X., Min, D., Yasir, T. A., & Hu, Y.-G. (2012). Genetic Diversity, Population Structure and Linkage Disequilibrium in Elite Chinese Winter Wheat Investigated with SSR Markers. *PLoS ONE*, *7*(9), e44510. https://doi.org/10.1371/journal.pone.0044510

Daniel K, & Shumi R. (2025). *Evaluation of Ethiopian wheat rust trap nurseries against yellow rust (Puccinia striiformis f.sp. Tritici) in Ethiopia*. https://doi.org/10.5281/ZENODO.15119847

Darrier, B., Rimbert, H., Balfourier, F., Pingault, L., Josselin, A.-A., Servin, B., Navarro, J., Choulet, F., Paux, E., & Sourdille, P. (2017). High-Resolution Mapping of Crossover Events in the Hexaploid Wheat Genome Suggests a Universal Recombination Mechanism. *Genetics*, *206*(3), 1373–1388. https://doi.org/10.1534/genetics.116.196014

Denancé, N., Ranocha, P., Oria, N., Barlet, X., Rivière, M.-P., Yadeta, K. A., Hoffmann, L., Perreau, F., Clément, G., Maia-Grondard, A., van den Berg, G. C. M., Savelli, B., Fournier, S., Aubert, Y., Pelletier, S., Thomma, B. P. H. J., Molina, A., Jouanin, L., Marco, Y., & Goffner, D. (2013). Arabidopsis wat1 (walls are thin1)-mediated resistance to the bacterial vascular pathogen, Ralstonia solanacearum, is accompanied by cross-regulation of salicylic acid and tryptophan metabolism. *The Plant Journal*, *73*(2), 225–239. https://doi.org/10.1111/tpj.12027

do Carmo Santos, M. L., Silva Santos, A., Pereira Silva de Novais, D., Dos Santos Lopes, N., Pirovani, C. P., & Micheli, F. (2025). The family of glutathione peroxidase proteins and their role against biotic stress in plants: A systematic review. *Frontiers in Plant Science*, *16*, 1425880. https://doi.org/10.3389/fpls.2025.1425880

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

FAO. (2023). *2.1 Food security indicators – latest updates and progress towards ending hunger and ensuring food security*. https://doi.org/10.4060/cc3017en

Farber, D. H., Medlock, J., & Mundt, C. C. (2017). Local dispersal of Puccinia striiformis f. Sp. Tritici from isolated source lesions. *Plant Pathology*, *66*(1), 28–37. https://doi.org/10.1111/ppa.12554

Flor, H. H. (1971). Current Status of the Gene-For-Gene Concept. *Annual Review of Phytopathology*, *9*, 275–296. https://doi.org/10.1146/annurev.py.09.090171.001423

Gao, C.-H., Chen, C., Akyol, T., Dusa, A., Yu, G., Cao, B., & Cai, P. (2024). ggVennDiagram: Intuitive Venn diagram software extended. *iMeta*, *3*(1), e177. https://doi.org/10.1002/imt2.177

Gasparre, N., & Rosell, C. M. (2022). *Wheat gluten: A functional protein still challenging to replace in gluten free cereal-based foods*. https://agris.fao.org/search/en/records/6942c97f33b548daaa381d82

Gasparre, N., & Rosell, C. M. (2023). Wheat gluten: A functional protein still challenging to replace in gluten-free cereal-based foods. *Cereal Chemistry*, *100*(2), 243–255. https://doi.org/10.1002/cche.10624

Ghimire, B., Mergoum, M., Martinez-Espinoza, A. D., Sapkota, S., Pradhan, S., Babar, M. A., Bai, G., Dong, Y., & Buck, J. W. (2022). *Genetics of Fusarium head blight resistance in soft red winter wheat using a genome-wide association study*. *15*. https://doi.org/10.1002/tpg2.20222

He, L., Ren, C., & Rosa, L. (2026). Reclaiming abandoned croplands to adapt wheat production to a warmer world. *Communications Earth & Environment*, *7*(1), 392. https://doi.org/10.1038/s43247-026-03386-2

Hill, W. G., & Weir, B. S. (1988). Variances and covariances of squared linkage disequilibria in finite populations. *Theoretical Population Biology*, *33*(1), 54–78. https://doi.org/10.1016/0040-5809(88)90004-4

Jaleta, M., Hodson, D., Abeyo, B., Yirga, C., & Erenstein, O. (2019). Smallholders' coping mechanisms with wheat rust epidemics: Lessons from Ethiopia. *PLOS ONE*, *14*(7), e0219327. https://doi.org/10.1371/journal.pone.0219327

Jan, F., Rathore, M., Kumar Saini, D., Singh, A., Qureshi, N., Aggarwal, N., Bashir, M., Shakeel, M., Gupta, V., Kumar, S., Varshney, R. K., & Mir, R. R. (2026). Wheat's war against stripe rust: Integrating host immunity, genomics and breeding for durable resistance. *The Plant Genome*, *19*(2), e70174. https://doi.org/10.1002/tpg2.70174

Jan, I., Saripalli, G., Kumar, K., Kumar, A., Singh, R., Batra, R., Sharma, P. K., Balyan, H. S., & Gupta, P. K. (2021). Meta-QTLs and candidate genes for stripe rust resistance in wheat. *Scientific Reports*, *11*(1), 22923. https://doi.org/10.1038/s41598-021-02049-w

Kabbaj, H., Sall, A. T., Al-Abdallat, A., Geleta, M., Amri, A., Filali-Maltouf, A., Belkadi, B., Ortiz, R., & Bassi, F. (2017). *Genetic Diversity within a Global Panel of Durum Wheat (Triticum durum) landraces and Modern Germplasm reveals the History of Alleles Exchange*. https://doi.org/10.3389/fpls.2017.01277

Kang, H. M., Sul, J. H., Service, S. K., Zaitlen, N. A., Kong, S.-Y., Freimer, N. B., Sabatti, C., & Eskin, E. (2010). Variance component model to account for sample structure in genome-wide association studies. *Nature Genetics*, *42*(4), 348–354. https://doi.org/10.1038/ng.548

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*(5), 1179–1191. https://doi.org/10.1111/1755-0998.12387

Kulich, I., Vojtíková, Z., Sabol, P., Ortmannová, J., Neděla, V., Tihlaříková, E., & Žárský, V. (2018). Exocyst Subunit EXO70H4 Has a Specific Role in Callose Synthase Secretion and Silica Accumulation. *Plant Physiology*, *176*(3), 2040–2051. https://doi.org/10.1104/pp.17.01693

Kumar, D., Sharma, S., Sharma, R., Pundir, S., Singh, V. K., Chaturvedi, D., Singh, B., Kumar, S., & Sharma, S. (2021). Genome-wide association study in hexaploid wheat identifies novel genomic regions associated with resistance to root lesion nematode (Pratylenchus thornei). *Scientific Reports*, *11*(1), 3572. https://doi.org/10.1038/s41598-021-80996-0

Lado, B., Kavanová, M., Castro, A., Baraibar, S., Poland, J., Condón, F., Quincke, M., & Silva, P. (2026). The impact of breeding on barley and wheat genetic diversity and the identification of genomic regions that have undergone selection. *Euphytica*, *222*(1), 15. https://doi.org/10.1007/s10681-025-03665-7

Laribi, M., Ben M'Barek, S., Velasco-Cuervo, S. M., Hwang, S.-F., Yahyaoui, A. H., & Strelkov, S. E. (2025). Genetic diversity and population structure of a core collection of Mediterranean durum wheat accessions using DArTseq markers. *BMC Genomics*, *26*(1), 1130. https://doi.org/10.1186/s12864-025-12232-2

Li, M., Zhang, Y.-W., Zhang, Z.-C., Xiang, Y., Liu, M.-H., Zhou, Y.-H., Zuo, J.-F., Zhang, H.-Q., Chen, Y., & Zhang, Y.-M. (2022). A compressed variance component mixed model for detecting QTNs and QTN-by-environment and QTN-by-QTN interactions in genome-wide association studies. *Molecular Plant*, *15*(4), 630–650. https://doi.org/10.1016/j.molp.2022.02.012

Liu, J., Xu, Z., Fan, X., Zhou, Q., Cao, J., Wang, F., Ji, G., Yang, L., Feng, B., & Wang, T. (2018). A Genome-Wide Association Study of Wheat Spike Related Traits in China. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01584

Liu, W., Frick, M., Huel, R., Nykiforuk, C. L., Wang, X., Gaudet, D. A., Eudes, F., Conner, R. L., Kuzyk, A., Chen, Q., Kang, Z., & Laroche, A. (2014). The Stripe Rust Resistance Gene *Yr10* Encodes an Evolutionary-Conserved and Unique CC–NBS–LRR Sequence in Wheat. *Molecular Plant*, *7*(12), 1740–1755. https://doi.org/10.1093/mp/ssu112

Liu, W., Kolmer, J., Rynearson, S., Chen, X., Gao, L., Anderson, J. A., Turner, M. K., & Pumphrey, M. (2019). Identifying Loci Conferring Resistance to Leaf and Stripe Rusts in a Spring Wheat Population (Triticum aestivum) via Genome-Wide Association Mapping. *Phytopathology*, *109*(11), 1932–1940. https://doi.org/10.1094/PHYTO-04-19-0143-R

Liu, W., Maccaferri, M., Rynearson, S., Letta, T., Zegeye, H., Tuberosa, R., Chen, X., & Pumphrey, M. (2017). Novel Sources of Stripe Rust Resistance Identified by Genome-Wide Association Mapping in Ethiopian Durum Wheat (Triticum turgidum ssp. Durum). *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.00774

Mahmood, Z., Ali, M., Mirza, J. I., Fayyaz, M., Majeed, K., Naeem, M. K., Aziz, A., Trethowan, R., Ogbonnaya, F. C., Poland, J., Quraishi, U. M., Hickey, L. T., Rasheed, A., & He, Z. (2022). Genome-Wide Association and Genomic Prediction for Stripe Rust Resistance in Synthetic-Derived Wheats. *Frontiers in Plant Science*, *13*, 788593. https://doi.org/10.3389/fpls.2022.788593

Mapuranga, J., Zhang, N., Zhang, L., Liu, W., Chang, J., & Yang, W. (2022). Harnessing genetic resistance to rusts in wheat and integrated rust management methods to develop more durable resistant cultivars. *Frontiers in Plant Science*, *13*, 951095. https://doi.org/10.3389/fpls.2022.951095

Marcano-Delgado, M. (2025). *Window Size or Model Choice? Quantifying the Impact of GWAS Methods on QTN Detection in Maize*. Biology and Life Sciences. https://doi.org/10.20944/preprints202504.0254.v1

McIntosh, R. A., Dubcovsky, J., Rogers, W. J., Xia, X. C., & Raupp, W. J. (2020). *V. CATALOGUE OF GENE SYMBOLS FOR WHEAT: 2020 SUPPLEMENT*.

McNeal, F. H., Konzak, C. F., Smith, E. P., Tate, W. S., & Russel, T. S. (1971). *A uniform system for recording and processing cereal research data*. Agricultural Research Service, United States Department of Agriculture.

Mekonnen, T., Sneller, C. H., Haileselassie, T., Ziyomo, C., Abeyo, B. G., Goodwin, S. B., Lule, D., & Tesfaye, K. (2021). Genome-Wide Association Study Reveals Novel Genetic Loci for Quantitative Resistance to Septoria Tritici Blotch in Wheat (Triticum aestivum L.). *Frontiers in Plant Science*, *12*, 671323. https://doi.org/10.3389/fpls.2021.671323

Meyer, M., Bacha, N., Tesfaye, T., Alemayehu, Y., Abera, E., Hundie, B., Woldeab, G., Girma, B., Gemechu, A., Negash, T., Mideksa, T., Smith, J., Jaleta, M., Hodson, D., & Gilligan, C. A. (2021). Wheat rust epidemics damage Ethiopian wheat production: A decade of field disease surveillance reveals national-scale trends in past outbreaks. *PLOS ONE*, *16*(2), e0245697. https://doi.org/10.1371/journal.pone.0245697

Mirzaghaderi, G., & Mason, A. S. (2019). Broadening the bread wheat D genome. *Theoretical and Applied Genetics*, *132*(5), 1295–1307. https://doi.org/10.1007/s00122-019-03299-z

Nigus, M., Shimelis, H., Mathew, I., & Abady, S. (2022). Wheat production in the highlands of Eastern Ethiopia: Opportunities, challenges and coping strategies of rust diseases. *Acta Agriculturae Scandinavica. Section B, Soil and Plant Science*, *72*(1), 563–575. https://doi.org/10.1080/09064710.2021.2022186

Phillips, D., Wnetrzak, J., Nibau, C., Barakate, A., Ramsay, L., Wright, F., Higgins, J. D., Perry, R. M., & Jenkins, G. (2013). Quantitative high resolution mapping of HvMLH3 foci in barley pachytene nuclei reveals a strong distal bias and weak interference. *Journal of Experimental Botany*, *64*(8), 2139–2154. https://doi.org/10.1093/jxb/ert079

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

Ranocha, P., Denancé, N., Vanholme, R., Freydier, A., Martinez, Y., Hoffmann, L., Köhler, L., Pouzet, C., Renou, J.-P., Sundberg, B., Boerjan, W., & Goffner, D. (2010). Walls are thin 1 (WAT1), an Arabidopsis homolog of Medicago truncatula NODULIN21, is a tonoplast-localized protein required for secondary wall formation in fibers. *The Plant Journal*, *63*(3), 469–483. https://doi.org/10.1111/j.1365-313X.2010.04256.x

Royo, C., Maccaferri, M., Alvaro, F., Moragues, M., Sanguineti, M. C., Tuberosa, R., Maalouf, F., Moral, L., Demontis, A., Sayar, R., Nachit, M., Nsarellah, N., & Villegas, D. (2010). Understanding the relationships between genetic and phenotypic structures of a collection of elite durum wheat accessions. *Field Crops Research*, *119*, 91–105. https://doi.org/10.1016/j.fcr.2010.06.020

Saini, D. K., Chopra, Y., Singh, J., Sandhu, K. S., Kumar, A., Bazzer, S., & Srivastava, P. (2021). Comprehensive evaluation of mapping complex traits in wheat using genome-wide association studies. *Molecular Breeding : New Strategies in Plant Improvement*, *42*(1), 1. https://doi.org/10.1007/s11032-021-01272-7

Sari, E., Knox, R. E., Ruan, Y., Henriquez, M. A., Kumar, S., Burt, A. J., Cuthbert, R. D., Konkin, D. J., Walkowiak, S., Campbell, H. L., Singh, A. K., Ross, J., Lokuruge, P., Hsueh, E., Boyle, K., Sidebottom, C., Condie, J., Yates, S., Pozniak, C. J., & Fobert, P. R. (2020). Historic recombination in a durum wheat breeding panel enables high-resolution mapping of Fusarium head blight resistance quantitative trait loci. *Scientific Reports*, *10*(1), 7567. https://doi.org/10.1038/s41598-020-64399-1

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, *3*(3), 430–439. https://doi.org/10.1038/s41559-018-0793-y

Sharma, D., Avni, R., Gutierrez-Gonzalez, J., Kumar, R., Sela, H., Prusty, M. R., Shatil-Cohen, A., Molnár, I., Holušová, K., Said, M., Doležel, J., Millet, E., Khazan-Kost, S., Landau, U., Bethke, G., Sharon, O., Ezrati, S., Ronen, M., Maatuk, O., … Sharon, A. (2024). A single NLR gene confers resistance to leaf and stripe rust in wheat. *Nature Communications*, *15*(1), 9925. https://doi.org/10.1038/s41467-024-54068-6

Sheng, C., Yu, D., Li, X., Yu, H., Zhang, Y., Saqib Bilal, M., Ma, H., Zhang, X., Baig, A., Nie, P., & Zhao, H. (2022). OsAPX1 Positively Contributes to Rice Blast Resistance. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.843271

Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and Energy Security*, *4*(3), 178–202. https://doi.org/10.1002/fes3.64

Smith, S., Nickson, T. E., & Challender, M. (2021). Germplasm exchange is critical to conservation of biodiversity and global food security. *Agronomy Journal*, *113*(4), 2969–2979. https://doi.org/10.1002/agj2.20761

Statistica. (2026). *Production of wheat worldwide 2025/26*. Statista. https://www.statista.com/statistics/267268/production-of-wheat-worldwide-since-1990/

Tadesse, W., Zegeye, H., Debele, T., Kassa, D., Shiferaw, W., Solomon, T., Negash, T., Geleta, N., Bishaw, Z., & Assefa, S. (2022). Wheat Production and Breeding in Ethiopia: Retrospect and Prospects. *Crop Breeding, Genetics and Genomics*. https://doi.org/10.20900/cbgg20220003

Tauzin, A. S., & Giardina, T. (2014). Sucrose and invertases, a part of the plant defense response to the biotic stresses. *Frontiers in Plant Science*, *5*, 293. https://doi.org/10.3389/fpls.2014.00293

Tyrka, M., Mokrzycka, M., Bakera, B., Tyrka, D., Szeliga, M., Stojałowski, S., Matysik, P., Rokicki, M., Rakoczy-Trojanowska, M., & Krajewski, P. (2021). Evaluation of genetic structure in European wheat cultivars and advanced breeding lines using high-density genotyping-by-sequencing approach. *BMC Genomics*, *22*(1), 81. https://doi.org/10.1186/s12864-020-07351-x

USDA. (2026). *Wheat | USDA Foreign Agricultural Service*. https://www.fas.usda.gov/data/production/0410000

Van Der Hoorn, R. A. L., & Klemenčič, M. (2021). Plant proteases: From molecular mechanisms to functions in development and immunity. *Journal of Experimental Botany*, *72*(9), 3337–3339. https://doi.org/10.1093/jxb/erab129

Van Der Nest, M. A., Hlongwane, N., Hadebe, K., Chan, W.-Y., Van Der Merwe, N. A., De Vos, L., Greyling, B., Kooverjee, B. B., Soma, P., Dzomba, E. F., Bradfield, M., & Muchadeyi, F. C. (2021). Breed Ancestry, Divergence, Admixture, and Selection Patterns of the Simbra Crossbreed. *Frontiers in Genetics*, *11*, 608650. https://doi.org/10.3389/fgene.2020.608650

VanRaden, P. M. (2008). Efficient methods to compute genomic predictions. *Journal of Dairy Science*, *91*(11), 4414–4423. https://doi.org/10.3168/jds.2007-0980

Vikas, V. K., Pradhan, A. K., Budhlakoti, N., Mishra, D. C., Chandra, T., Bhardwaj, S. C., Kumar, S., Sivasamy, M., Jayaprakash, P., Nisha, R., Shajitha, P., Peter, J., Geetha, M., Mir, R. R., Singh, K., & Kumar, S. (2022). Multi-locus genome-wide association studies (ML-GWAS) reveal novel genomic regions associated with seedling and adult plant stage leaf rust resistance in bread wheat (Triticum aestivum L.). *Heredity*, *128*(6), 434–449. https://doi.org/10.1038/s41437-022-00525-1

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., International Wheat Genome Sequencing Consortium, Lillemo, M., Mather, D., … Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, *12*(6), 787–796. https://doi.org/10.1111/pbi.12183

Wang, Z., Wang, W., He, Y., Xie, X., Yang, Z., Zhang, X., Niu, J., Peng, H., Yao, Y., Xie, C., Xin, M., Hu, Z., Sun, Q., Ni, Z., & Guo, W. (2024). On the evolution and genetic diversity

of the bread wheat D genome. *Molecular Plant*, *17*(11), 1672–1686. https://doi.org/10.1016/j.molp.2024.09.007

Xu, C., Zhang, J., Li, W., & Guo, J. (2025). The role of Exo70s in plant defense against pathogens and insect pests and their application for crop breeding. *Molecular Breeding: New Strategies in Plant Improvement*, *45*(2), 17. https://doi.org/10.1007/s11032-025-01539-3

Yamada, K., & Mine, A. (2024). Sugar coordinates plant defense signaling. *Science Advances*, *10*(4), eadk4131. https://doi.org/10.1126/sciadv.adk4131

Youssef, A., Tadesse, W., Osman, N. H., Hamwieh, A., Tawkaz, S., Fouad, N., Radwan, K. H., & El-soda, M. (2026). Durable resistance to stripe rust in wheat: Integration of breeding tools and genomic technologies. *Cereal Research Communications*, *54*(1), 39–59. https://doi.org/10.1007/s42976-025-00750-4

Zhao, J., Zhang, X., Wan, W., Zhang, H., Liu, J., Li, M., Wang, H., Xiao, J., & Wang, X. (2018). Identification and Characterization of the EXO70 Gene Family in Polyploid Wheat and Related Species. *International Journal of Molecular Sciences*, *20*(1), 60. https://doi.org/10.3390/ijms20010060

Zhu, Z., Cao, Q., Han, D., Wu, J., Wu, L., Tong, J., Xu, X., Yan, J., Zhang, Y., Xu, K., Wang, F., Dong, Y., Gao, C., He, Z., Xia, X., & Hao, Y. (2023). Molecular characterization and validation of adult-plant stripe rust resistance gene Yr86 in Chinese wheat cultivar Zhongmai 895. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *136*(6), 142. https://doi.org/10.1007/s00122-023-04374-2