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RESEARCH

Genome-wide association analysis of type II resistance to Fusarium head blight in Pakistani spring wheat germplasm

Rafi Ullah · Fahim Ullah Khan · Inam Ullah · Valentina Spanic · Katarina Sunic Budimir · Attiq ur Rehman

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Abstract Fusarium head blight (FHB) is a devastating fungal disease of wheat, causing significant losses in grain yield and quality. Understanding the genetic basis of FHB resistance is crucial for developing resistant varieties. This study aimed to characterize the genetic architecture of FHB resistance in a diverse panel of Pakistani spring wheat germplasm, consisting of 150 recognized varieties, 45 landraces/lines, and two check varieties. Resistance to fungal spread along the spike (Type II resistance) was evaluated under controlled conditions, and the genotypes were categorized based on FHB scores ranging from 0 (highly resistant) to 9 (highly susceptible). We found statistically significant variation ($p \leq 0.01$) for resistance to type II FHB in the tested panel including some promising genotypes with high levels of resistance to the infection. To dissect the genetic basis of FHB resistance, multi-model GWAS were performed using 14,800 SNP markers from the 50 K SNP array. Population structure and kinship were accounted for to control false positives, using principal components and a kinship matrix. Our study identified eight quantitative trait loci (QTL) regions associated with Type II FHB resistance, distributed across six chromosomes (1A, 1B, 2A, 2B, 7B, and 7D). Among these, q_Fhb_1B on chromosome 1B was consistently detected across multiple models, underscoring its potential as a key resistance locus based on the top SNP 1B_667978743. Haplotype analysis further revealed favorable allele combinations linked to resistance, providing additional insights for marker-assisted selection. These findings offer valuable insights for genome-based breeding strategies aimed at enhancing FHB resistance while maintaining agronomic performance, thereby contributing to the development of more resilient wheat varieties suitable for FHB-prone regions.

Keywords Fusarium head blight · Spring wheat · QTL · GWAS · Type II resistance · MAS

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R. Ullah · I. Ullah
Department of Biotechnology and Genetic Engineering, Hazara University Mansehra, Mansehra 21300, Pakistan

F. U. Khan (✉)
Department of Agriculture, Hazara University Mansehra, Mansehra 21300, Pakistan
e-mail: fahimbiotech@hu.edu.pk

V. Spanic (✉) · K. S. Budimir
Agricultural Institute Osijek, Juzno Predgradje 17, 31000 Osijek, Croatia
e-mail: valentina.spanic@poljinos.hr

A. u. Rehman (✉)
Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland
e-mail: attiq.rehman@helsinki.fi

A. u. Rehman
Natural Resources Institute (Luke), Helsinki, Finland

Introduction

Wheat, rice, and maize are the major cereal grain crops. Among cereals, bread wheat (*Triticum aestivum* L.) accounts for 15% of the world's total arable land. Bread wheat, also called common wheat, is the second most productive crop in the world in terms of grain yield, whereas Central Asia is the most suitable region for bread wheat production (Soto-Gómez and Pérez-Rodríguez 2022). Pakistan is one of the world's largest wheat producers, and the crop plays a central role in the country's agriculture, economy, and food security (Abbas and Alnafrah 2024). Current wheat production is sufficient to meet present food demands but may not be adequate for future generations. However, global wheat-producing regions, including Pakistan, are threatened by several biotic (pathogens, weeds, and insects) and abiotic (temperature, salinity, and drought) factors. Rust diseases, powdery mildew, and Fusarium head blight are among the most important diseases (Prasad et al. 2021).

Fusarium head blight (FHB), a major fungal disease of wheat, barley, and maize, is primarily caused by two predominant species *Fusarium graminearum* and *F. culmorum* but other species can also cause the disease depending on environmental conditions (Becher et al. 2013). The FHB species complex produces mycotoxins that reduce grain quality and pose significant health risks to both humans and animals. (Haile et al. 2023). The weather conditions directly affect the concentration of *Fusarium* mycotoxins that accumulate in grains. It has been reported that prolonged periods of high humidity, starting from the flowering stage and continuing until harvest, significantly increase the risk of infection with *Fusarium* spp. and, consequently, the concentration of mycotoxins in grain (Brennan et al. 2005; Van der Burgt et al. 2011). At flowering FHB causes wheat spikes to become blighted, leading to significant yield losses due to flower abortion, reduced grain number, and poorly developed grains. In susceptible varieties the whole spike can become bleached within 7–10 days of infection (Sunic et al. 2023).

FHB poses a significant threat to the future wheat production in Pakistan, particularly in the northern belt of Khyber Pakhtunkhwa, including the Hazara division, where unexpected climatic events, favorable temperatures, and high humidity during the wheat growing season create ideal conditions for the disease (Gul et al. 2022). Fungicide use can reduce the severity of FHB, but the actual reductions are highly variable. Moreover, when weather conditions are unfavorable for FHB occurrence, fungicide application can be both costly and environmentally harmful (Matengu et al. 2024). In some cases, FHB losses can be reduced by proper agronomic practices or biological control. Given the high risk of future FHB epidemics, implementing preemptive measures, such as cultivation of FHB-resistant genotypes, represents the most effective and sustainable strategy for combating the disease (Spanic and Sarcevic 2023). However, FHB resistance is a complex trait comprising multiple genetic components, each controlled by various quantitative trait loci (QTL) with small to medium effects. The Type I component reflects resistance to initial infection, the Type II component indicates resistance to the spread of symptoms, and the Type III component represents resistance to toxin accumulation in the grains (Mesterházy et al. 1999). Additionally, two more types of resistance have been identified: Type IV, which pertains to resistance against kernel infection, and Type V, which focuses on grain yield tolerance. FHB-resistant genotypes are critial for disease management, as they are expected to accumulate significantly lower levels of mycotoxins compared to susceptible ones (Spanic et al. 2023).

Phenotyping for FHB resistance is often challenging due to strong genotype-by-environment (G×E) interactions, making marker-assisted selection (MAS) a valuable tool in many breeding programs. QTL for FHB resistance has been reported on all wheat chromosomes and molecular markers have been developed for most of the mapped QTL. This includes seven cataloged FHB genes, *Fhb1–Fhb7* (Zhu et al. 2020). Dai et al. (2022) reported that Sumai 3 harbors three different genes *Fhb1*, *Fhb2*, and *Fhb5* for resistance to FHB on chromosome 3BS, 4BL and 6BL, respectively. *Fhb1* is the most important and widely studied one (Buerstmayr et al. 2009) as it was fine mapped to a 1.1 Mb genomic region containing 28 candidate genes. Many spring wheat varieties contain the FHB resistance derived from Sumai 3. In a spring wheat mapping population [Surpresa × Wheaton], four QTL for type II resistance on chromosomes 3A, 5A, 6A, and 7A, explained 10.4–14.4% of the total phenotypic variation (Poudel et al. 2022). Zhu et al. (2020) evaluated Chinese wheat germplasm for FHB resistance using mixed linear model and found

five QTL on chromosomes 1, 2, 5, and 7. Notably, QTL are often specific to the populations, meaning not transferable to other populations and are often not stable across different environments (Würschum 2012). On the other hand, landraces with high or moderate FHB resistance could not be used directly as the breeding parents in modern breeding programs due to their inferior agronomic performances (Zhang et al. 2021). Therefore, incorporating their resistance into elite varieties requires a more gradual breeding approach, such as backcrossing or introgression. This process can be accelerated by identifying loci associated with FHB resistance, which can then be used in MAS to streamline breeding efforts.

Genome-wide association studies (GWAS) are promising approach for identifying QTL associated with various simple and complex traits of interest. This method leverages recombination events in the genome to achieve a higher mapping resolution, allowing for more precise identification of QTL related to the trait under investigation. Zhu et al. (2020) observed that very few GWAS on wheat have addressed FHB type II resistance. Recent studies have demonstrated the utility of GWAS in identifying genomic regions associated with FHB resistance, such as Shi et al. (2023), who detected novel loci on chromosomes 1B and 5A using high-density SNP arrays, and Gaire et al. (2021), who identified QTL from diverse sources contributing to FHB resistance traits in soft red winter wheat. Further, more powerful methods like FarmCPU and BLINK can improve the ability of GWAS to detect loci with smaller effects (Huang et al. 2019). Markers identified through GWAS can be used for MAS directly or after conversion to utility markers (Radecka-Janusik et al. 2022). The current research was conducted to study phenotypic variance among Pakistani wheat germplasm for type II resistance and to determine the genomic regions controlling FHB resistance in the diversity panel.

Methods and material

Plant material

A set of 197 spring wheat genotypes including 150 released varieties, 45 landraces/lines and two checks including Sumai 3 and Gamenya was tested in the greenhouse at Agriculture Research Institute, Croatia (Supplementary Dataset S1).

Inoculum preparation

The inoculum was prepared at a concentration of 50,000 spores mL^{-1} by mixing spores equally from two pathogenic species of *F. graminearum* (PIO 31) collected from East Croatia and *F. culmorum* (IFA 104) collected from Austria. For a mass production of the conidia of each species in the proportion 1:1., two discs (5 mm diam.) from a well-grown colony at synthetic low-nutrient (SNA) medium were transferred to the mixture of wheat and oat (3:1), previously soaked in water overnight, and autoclaved (Spanic et al. 2021).

Growth conditions, inoculation, and evaluation of reaction to Fusarium head blight

In greenhouse environments at Agricultural Institute Osijek (Croatia), the spring wheat material was grown in 2.5 L pots filled with soil (pH: 5.5–7.0, organic matter: 70.0–85.0%, N (1/2 vol.): 100–200 mg L^{-1}, P_2O_5 (1/2 vol.): 100–150 mg L^{-1}, K_2O (1/2 vol.): 200–400 mg L^{-1}) in randomized complete block design. In each pot four plants were planted in two replications. The greenhouse (Gis Impro d.o.o., Vrbovec, Croatia) was supplemented with artificial light for a 14 h photoperiod, with temperature maintained between 22 and 25 °C, and irrigation applied twice per week. Nitrogen was applied at the two-leaf development stage (GS12) using calcium ammonium nitrate (CAN) (27% N). Plants were protected against pests with the insecticide Vantex (gamma-cyhalothrin 60 g L^{-1}) (GS31). At the flowering stage (GS61), plants were inoculated with a mixture of *F. graminearum* and *F. culmorum* using the single-spikelet inoculation method (Sunic et al. 2023). 20 µL of inoculum was injected into the central spikelet of a spike using an automatic pipette (Eppendorf, Wien, Austria). To facilitate disease development, the misting system was turned on for the next 36 h keeping the spraying with foggers every hour for a period of 2 minutes. Disease ratings were conducted at 21 days after inoculation in one plant per pot in two replications. Type II resistance was assessed by counting the blighted spikelets (Sunic et al. 2023).

Genomic DNA extraction and genotyping

Seeds were surface sterilized using 3% NaOCl and were sown in plastic trays containing peat moss. Seedlings were harvested 15 days after sowing and genomic DNA was extracted from fresh leaves using phenol–chloroform method (Ahmed et al. 2009). A 50 µl aliquot of DNA (50–100 ng/µl) for each sample was used for 50 K (Triticum TraitBreed array) SNP array genotyping (Rasheed and Xia 2019). The samples were sent to Chinese Academy of Agricultural Sciences (CAAS), Beijing, China for genotyping using 50 K SNP array. For quality control, SNP markers with heterozygosity > 0.2 and minor allele frequency (MAF) < 5% were filtered using TASSEL 5.2. software and the finally retained 14,800 SNP markers for 146 genotypes were used for population structure and association analysis (Supplementary Dataset S1).

Statistical analysis for FHB disease severity

Statistical analysis of FHB disease severity was performed using Statistica 12.0 software (StatSoft Inc., Tulsa, OK, USA). Variation of disease severity was computed using one-way analysis of variance (ANOVA), followed by Fisher LSD post hoc test ($p < 0.05$). Broad-sense heritability (H^2) for FHB severity was estimated as $H^2 = Vg/(Vg + Ve)$, following the method described by Buerstmayr et al. (2000) (Supplementary Dataset S1).

Population structure analysis

The principal component analysis (PCA) and Kinship matrix (VanRaden 2008) were performed using high quality 14,800 SNP markers in Genomic Association and Prediction Integrated Tool (GAPIT) version 3 (Wang and Zhang 2021).

Genome-wide association analyses

We implemented a comprehensive analytical strategy by integrating five GWAS methods: Generalized Linear Model (GLM), Mixed Linear Model (MLM), Compressed Mixed Linear Model (CMLM), Fixed and random model Circulating Probability Unification (FarmCPU), and Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) within the GAPIT version 3 (Wang and Zhang 2021). Our association analyses were conducted with careful consideration of population structure and individual relationships, utilizing both the Q + K matrices. Q was the principal component from PCA while the K matrix was computed using the Van Raden method (VanRaden, 2008). Both GLM and MLM are single locus models and can be described as following formula: $Y = X\beta + Zu + e$, where Y is the vector of observed phenotypes; β is an unknown vector containing fixed effects, including the genetic marker, population structure (PCA in both GLM and MLM), and the intercept; u is an unknown vector of random additive genetic effects from multiple markers for individuals; X and Z are the known design matrices; and e is the unobserved vector of residuals. The u and e vectors are assumed to be normally distributed with a null mean and a variance of: $Var\begin{pmatrix} u \\ e \end{pmatrix} = \begin{pmatrix} G & 0 \\ 0 & R \end{pmatrix}$, where $G = \sigma_a^2 K$ while σ_a^2 as additive genetic variance and K as kinship matrix (included only in MLM). Whereas CMLM is an improved form of am MLM model in which individuals are grouped using clustering algorithms, including the un-weighted pair group method with arithmetic mean (UPGMA) and the statistical power is increased (Li et al. 2014). The first ten principal components were included in the analysis to account for population stratification, with the number chosen based on Bayesian information criterion (BIC)-based model selection procedure, indicating that these components capture the majority of population structure present in the genetic data (Supplementary Dataset S1). Among the multi-locus models, the FarmCPU model iteratively uses both random and fixed models to estimate pseudo-quantitative trait nucleotides (QTNs) and testing markers by using pseudo-QTNs as covariates, respectively. BLINK model replaces the binning method of FarmCPU with linkage disequilibrium to increase statistical power and decrease the computation time. Both FarmCPU and BLINK are considered particularly powerful methods to conduct GWAS by controlling false-negative rate (Liu et al. 2016). To identify significant marker-trait associations (MTAs), initially, a stringent Bonferroni corrected p-value threshold was considered for significance; however, due to the highly conservative nature of this correction in the context of linked markers and the polygenic nature of FHB resistance, no significant MTAs were detected at this level. Consequently, as a method

of correction for multiple testing issues and to identify candidate loci, a more relaxed threshold value of $-\log(p) > 3.0$ was applied (Saripalli et al. 2023). QTLs were given names based on the relative position on the chromosome where significant MTAs are identified. A QTL was considered stable if the associated SNP was detected by at least two of the five tested GWAS models. To estimate the effect of each top SNP within identified QTL, we calculated the proportion of phenotypic variance explained (R^2) using the formula: $R^2 = 2 \times MAF \times (1-MAF) \times \beta^2$, where β beta the additive effect of the SNP, MAF is the minor allele frequency (You et al. 2021).

Results

Variation in FHB resistance

The analysis of variance showed significant differences in Fusarium Head Blight (FHB) severity among the 197 wheat genotypes (Supplementary Dataset S1). This suggests that the observed variability in FHB severity is primarily driven by genetic differences among the genotypes, underscoring the presence of substantial genetic diversity for FHB resistance within the tested wheat germplasm. Similarly, broad-sense heritability (H^2) for FHB score was estimated at 0.51, based on a genetic variance (Vg) of 1.68 and an environmental variance (Ve) of 1.60. FHB scores for 197 wheat genotypes were categorized (Fig. 1) into four groups: resistant (0–2), moderately resistant (2.1–3.0), moderately susceptible (3.1–4.9), and susceptible (5 and above). Notable genotypes include Johar-16, Nowshera-96, NIA-Zakheera, Ghazi-19, MH-97, Gomal-08, and SKD-1, Punjab-1, NIFA-Insaf, Punjab-2011, Shafaq-2006, 10,810, 18,678, and 24,002 fall into resistant category with scores of 2 or below. Moderately resistant genotypes, with scores between 2.1 and 3.0, included varieties like Shalimar-88, Barani-17, Khyber-87, NIFA-Awaz, and Faisalabad-2008 and landraces viz. 11,154, 11,395, 18,671, 18,675, 10,865, 11,062, 11,154, 11,180, 11,255, 11,529, and 18,677. Genotypes classified as moderately susceptible, with scores between 3.1 and 4.9, included varieties like NIFA-Aman, Pirsabak-2004, NN-Gandum1, Auqab-2000, and

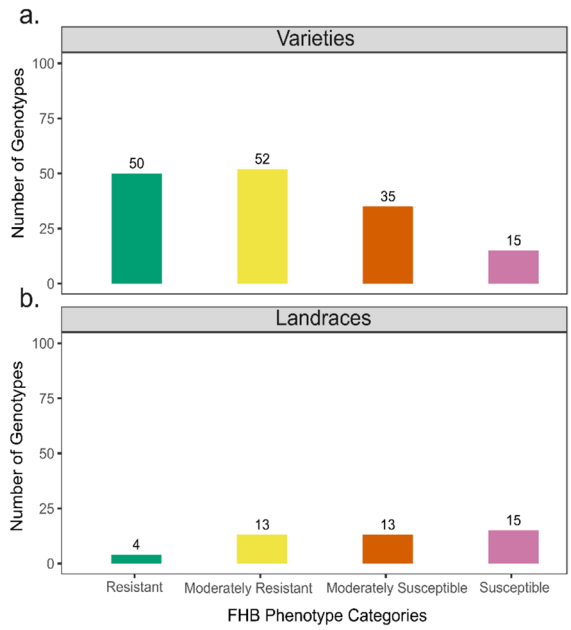

Fig. 1 Frequency distribution of the tested spring wheat panel based on FHB phenotype categories, separately displayed for varieties **(a)** and landraces **(b)**

NARC-09. Finally, the FHB susceptible genotypes, with scores of 5 and above, exhibited severe FHB symptoms and included varieties like Rohtas-90, Chakwal-86, Shalakot-13, and Inqilab-91. The most susceptible genotype in the study was 11,706, with a maximum score of 9.

Population structure revealed clear grouping of landraces and varieties

Although the inclusion of diverse varieties and landraces in this study was apparent, both PCA and Kinship matrix using 14,800 SNP markers also showed clear separation between varieties and the set of landraces used in this study, with some overlap indicating the presence of population structure in the data (Fig. 2a, b). As most of the variance was explained by the first three components (Fig. 2c), with PC1 explaining approximately 9.8%, PC2 explaining 8.5%, and PC3 explaining 5.8%, however, we included all ten principal components during the subsequent GWAS analysis when the inflection began to become clearer.

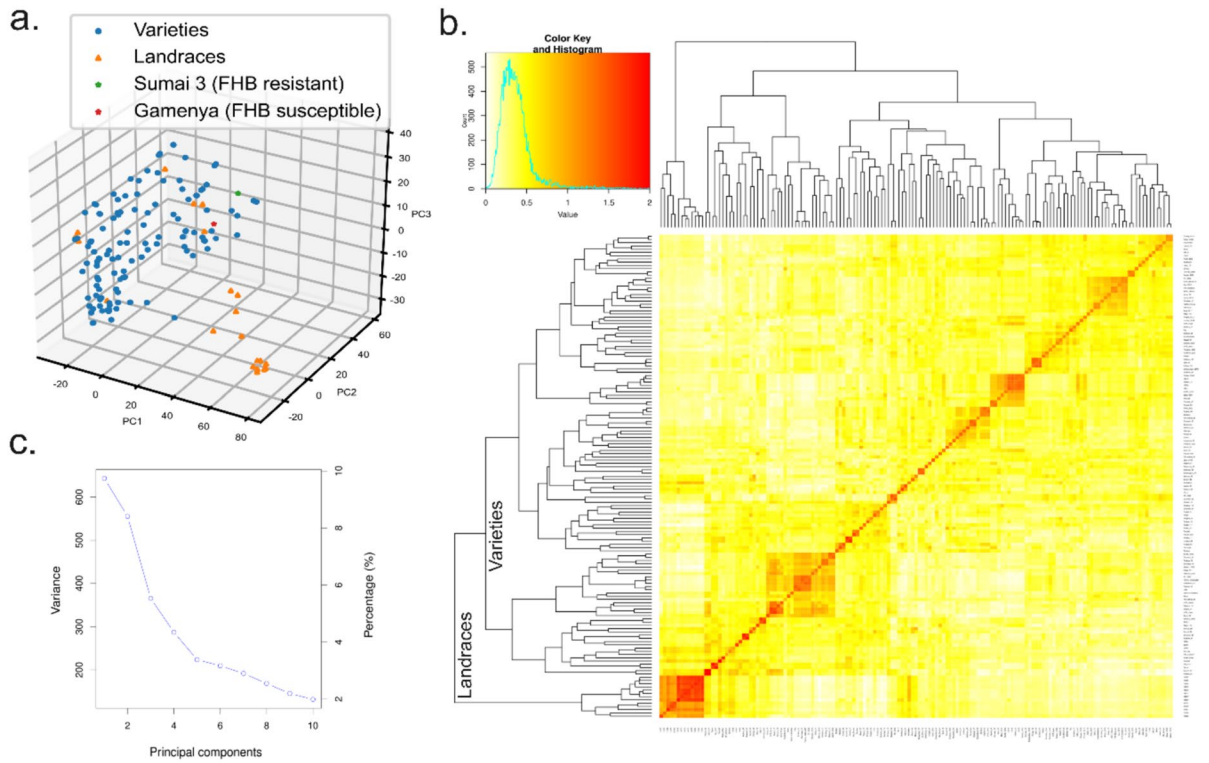

Fig. 2 **a** Population structure of wheat germplasm based on first three principal components, **b** A heatmap of the kinship matrix of the tested wheat panel, **c** Proportions of explained variance for 10 principal components indicated on x-axis **(c)**

GWAS identified multiple QTL for FHB type II resistance

We performed GWAS with five different models, including three single-locus (GLM, MLM, CMLM) and two multi-locus models (FarmCPU, and BLINK) that resulted in eight unique marker-trait associations (MTAs) on six different wheat chromosomes. These MTAs were clustered to eight QTL regions as detailed in Table 1. Notably, multi-locus models proved particularly effective in dissecting FHB resistance, not only identifying the majority of unique marker-trait associations but also demonstrating superior control of population stratification based on QQ plots (Fig. 3). Specifically, while single-locus models (GLM, MLM, CMLM) exhibited *p*-value deflation, indicative of over-correction, the multi-locus models closely adhered to the expected null distribution until approximately $-\log10(p) = 3.0$, beyond which a clear upward deviation indicated the presence of significant associations.

All the single-locus models (GLM, MLM, and CMLM) detected only one SNP on chromosome 1B, whereas both the multi-locus models (FarmCPU and BLINK) identified seven additional significant SNPs each, exceeding the threshold value of $-\log(p) > 3.0$ (Fig. 4a). The multi-locus models identified seven unique regions in addition to the one on chromosome 1B, indicating superior detecting power than the tested single-locus models. These eight MTAs correspond to eight different QTL localized on six different chromosomes, each confirmed by at least two of the tested GWAS models (Fig. 4b, c). Only one single MTA (1B_667978743) on chromosome 1B was detected by all the tested GWAS models, thereby making the q_Fhb_1B a promising QTL for FHB resistance in the evaluated wheat panel.

We also searched the literature for previously reported QTL on the same chromosomes and found that nearly all the discovered QTL were close to the previously reported markers, suggesting a

Table 1 QTL names, SNP IDs, R^2, chromosome positions, −log(p) values, minor allele frequencies for respective GWAS model

QTL	SNP	R^2	Chr	Pos	−log10 (p)	MAF	Model
q_Fhb_1A	1A_173966594	0.10	1A	173,966,594	3.11	0.12	FarmCPU
			1A	173,966,594	3.11	0.12	BLINK
q_Fhb_1B	1B_667978743	0.06	1B	667,978,743	3.21	0.09	CMLM
			1B	667,978,743	3.21	0.09	MLM
			1B	667,978,743	3.21	0.09	GLM
			1B	667,978,743	3.21	0.09	FarmCPU
			1B	667,978,743	3.21	0.09	BLINK
			1B	667,933,358	3.05	0.12	FarmCPU
			1B	667,933,358	3.05	0.12	BLINK
q_Fhb_2A.1	2A_115142667	0.12	2A	115,142,667	3.35	0.24	FarmCPU
			2A	115,142,667	3.35	0.24	BLINK
q_Fhb_2A.2	2A_707010276	0.08	2A	707,010,276	3.17	0.27	FarmCPU
			2A	707,010,276	3.18	0.27	BLINK
q_Fhb_2B	2B_694612619	0.07	2B	694,612,619	3.56	0.49	FarmCPU
			2B	694,612,619	3.57	0.49	BLINK
q_Fhb_7B	7B_690048233	0.09	7B	690,048,233	3.54	0.13	FarmCPU
			7B	690,048,233	3.54	0.13	BLINK
q_Fhb_7D.1	7D_397567807	0.16	7D	397,567,807	3.05	0.34	FarmCPU
			7D	397,567,807	3.06	0.34	BLINK
q_Fhb_7D.2	7D_581139373	0.11	7D	581,139,373	3.03	0.19	FarmCPU
			7D	581,139,373	3.03	0.19	BLINK

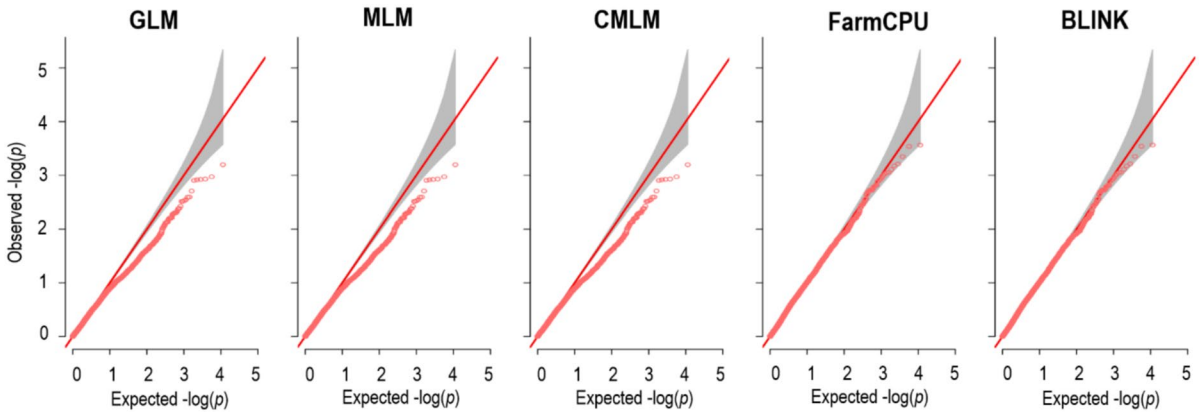

Fig. 3 Quantile–Quantile (QQ) plots of observed (y-axis) versus expected (x-axis) p-values for each of the tested GWAS models. The red diagonal line indicates the expected null distribution of p-values, with each SNP represented as red dot. The gray shaded area indicates the 95% confidence interval for each plot

possibility of similar resistance sources present in different plant materials (Table 2). Notably, the QTL on chromosomes 1B and 2B were in close vicinity of the previously published studies on FHB resistance.

Allele effects of significant SNPs

The distribution of different allelic classes of the significant SNPs for FHB resistance showed variability across the tested panel (Fig. 5). The SNP located on chromosome 7B (7B_690048233)

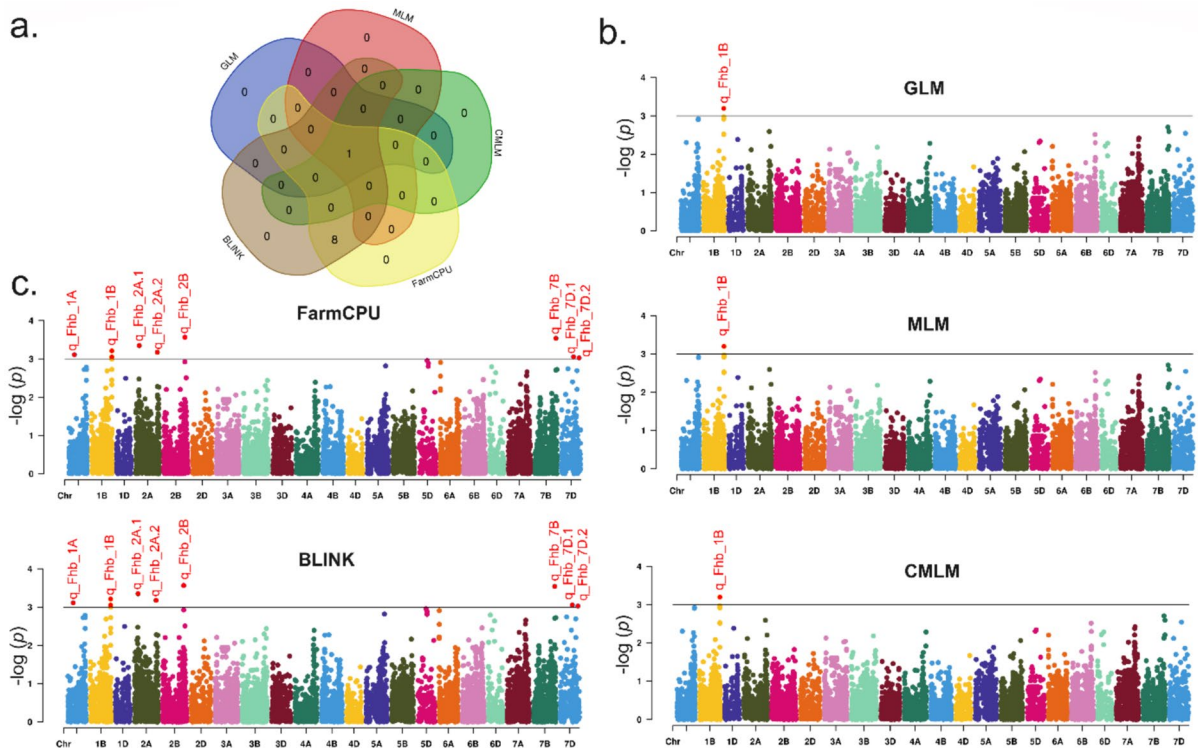

Fig. 4 **a** Venn diagram showing single shared marker-trait association between five GWAS models, **b** Stacked Manhattan plots for GWAS using single-locus GLM, MLM, and CMLM models, **c** and multi-locus FarmCPU and BLINK models for FHB resistance in wheat. The x-axis represents different chromosomes, and y-axis represents $-\log(p)$ values of the SNPs. Horizontal black line shows cutoff at $-\log(p) = 3$. Significant SNPs and respective FHB QTL names are represented in red

demonstrated the clearest allelic trend, where genotypes carrying the 'TT' allele were associated with lower FHB scores, indicative of moderate resistance, while the 'AA' allele was more frequently observed in moderately susceptible genotypes. Conversely, the 'TT' allele at SNPs on chromosomes 2A was associated with moderate susceptibility. However, visual differences in FHB scores between alleles were not statistically significant based on two-sample t-tests ($p > 0.05$ for all top SNPs). The R^2 values for these SNPs ranged from 0.06 to 0.16 (Table 1), indicating minor contributions to phenotypic variance. Despite the lack of strong statistical support, the consistent directional trends observed across genotypes suggest additive effects, supporting the classification of these loci as minor-effect QTLs.

Haplotype analysis

Haplotype analysis, which involves combining SNPs located on the same chromosome that can possibly co-segregate, proved to be more effective at identifying favorable allele combinations than individual SNP analysis. In this study, several haplotypes were tested, with some showing clear associations with either susceptibility or resistance to FHB (Fig. 6). For example, on chromosome 1B, the haplotype 'CCTT' was linked to susceptible phenotypes, as were the haplotypes 'TTGG' and 'TTTT' on chromosome 2A. Similarly, the haplotype 'AGGC' and 'AGGG' on chromosome 7D demonstrated a strong connection to FHB susceptibility as well. In contrast, the haplotype 'GGCC' on chromosome 1B was consistently associated with FHB resistance across the tested GWAS

Table 2 QTL co-segregating with previously reported FHB resistance QTL. The approximate positions of the QTL with reference to the previously reported ones are also mentioned in parenthesis in the SNP column

QTL	SNP	Chr	Pos	MAF	GWAS Model	References
q.Fhb-1A	Xgwm153 (≈675 Mb)	1A	528,309,856	0.15	BLINK	Bourdoncle and Ohm (2003)
	Xgwm153 (≈675 Mb)	1A	528,309,876	0.12	BLINK	Aviles et al. (2020)
q.Fhb-1B	Xgwm153 (≈39.15 Mb)	1B	628,826,245	0.14	FarmCPU	Buerstmayr et al. (2009)
	XEtcg.Magc-7 (≈39.15 Mb)	1B	628,826,264	0.16	FarmCPU	Zhang et al. (2004); Buerstmayr et al. (2009)
q.Fhb-2A.1	RAC875_rep_c78744_228 (≈83.18 Mb)	2A	31,957,675	0.12	BLINK	Wang et al. (2019)
q.Fhb-2A.2	–	2A	31,957,775	0.13	BLINK	Wang et al. (2023)
q.Fhb-2B	Xwmc149 (≈84.5 Mb)	2B	779,109,515	0.15	BLINK	Liu et al. (2007); Gilsinger et al. (2005)
	Xcn16-2B (≈46.19 Mb)	2B	740,802,332	0.14	BLINK	Sari et al. (2018)
q.Fhb-7B	–	7B	–	–	MIXED	Wang et al. (2019); Bourdoncle and Ohm (2003)
q.Fhb-7D.1	GBS979/GBS20328 (≈124–290 Mb)	7D	107.1–521.7 Mb	0.1	BLINK	Cativelli et al. (2013)
q.Fhb-7D.2	Xwmc405 (≈381.14 Mb)	7D	200,000,000	0.12	BLINK	Ren et al. (2019)

panel. These haplotypes can be further investigated for potential use in haplotype-based selection for FHB resistance in spring wheat.

Discussion

Fusarium head blight (FHB) continues to be a major challenge in wheat production, with disease severity varying based on environmental conditions, crop management practices, and varietal susceptibility. The severity of FHB can range from subtle symptoms that may go unnoticed to devastating epidemics resulting in severe grain yield losses and quality deterioration (Shaner 2003). *Fusarium* spp., the primary causal agents of FHB, can survive as saprophytes on plant residues or exist on plant surfaces without initially causing disease. They transition into opportunistic pathogens during anthesis, characterized by a narrow infection window and a short parasitic period (Shaner 2003). Key environmental factors contributing to FHB outbreaks include moisture, humidity, and moderate temperatures around anthesis (Cowger et al. 2020).

Breeding for FHB-resistant varieties is crucial, with a focus on developing stable resistance across different environments. Effective control strategies integrate genetic resistance with agronomic practices such as crop rotation and residue management (Bai and Shaner 2004). In this study, we assessed 197 spring wheat genotypes, including released varieties, landraces, and checks like Sumai 3 (resistant) and Gamenya (susceptible). To date, over 50 QTL related to FHB resistance have been mapped across all 21 wheat chromosomes, with particularly strong and stable QTL identified on chromosomes 3BS, 5AS, 6BS, 3A, and 2D (Buerstmayr et al. 2009; Liu et al. 2009; Bai et al. 2018). Meta-analyses identified novel loci, enriching our understanding of the genetic architecture of FHB resistance (Liu et al. 2009). QTL are often specific to the populations in which they are identified, which limits the transferability of markers into unrelated populations. Additionally, these markers may exhibit instability across environments, underscoring the need to conduct association studies to confirm existing sources of resistance and potentially discover new ones.

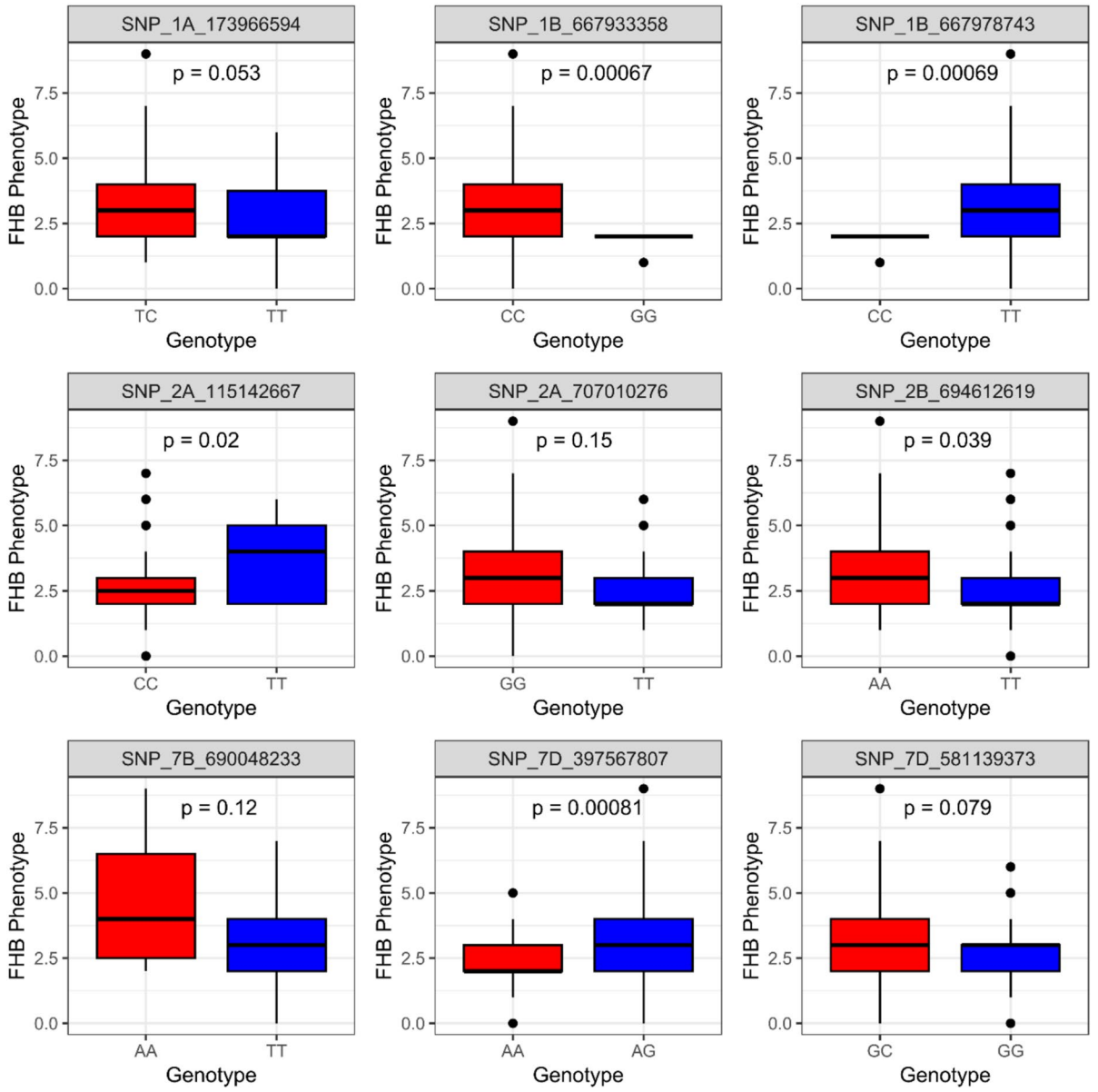

Fig. 5 Box plots of MTAs associated with FHB resistance in wheat. Chromosome and SNP information is given on the top of each box-and-whisker. Boxes represent the first quartile, the median, and the third quartile, respectively. The thick horizontal lines correspond to the median. Whiskers represent variability outside the upper and lower quartiles. *p*-values from t-tests comparing genotype groups are shown above each plot

Despite the identification of well-characterized QTL, FHB resistance is quantitatively inherited, involving multiple minor-effect genes, which complicates breeding efforts. Genotype-environment interactions further influence resistance expression, emphasizing the importance of multi-environment trials (Zhang et al. 2020). The challenge of balancing disease resistance with other agronomic traits, such as grain yield and quality, adds to the complexity of developing FHB-resistant varieties (Spanic et al. 2021). Recent studies have increasingly focused on locally adapted germplasm to avoid potential drawbacks associated with exotic resistance sources, such as linkage drag (Bohra et al. 2022). Thus, our study

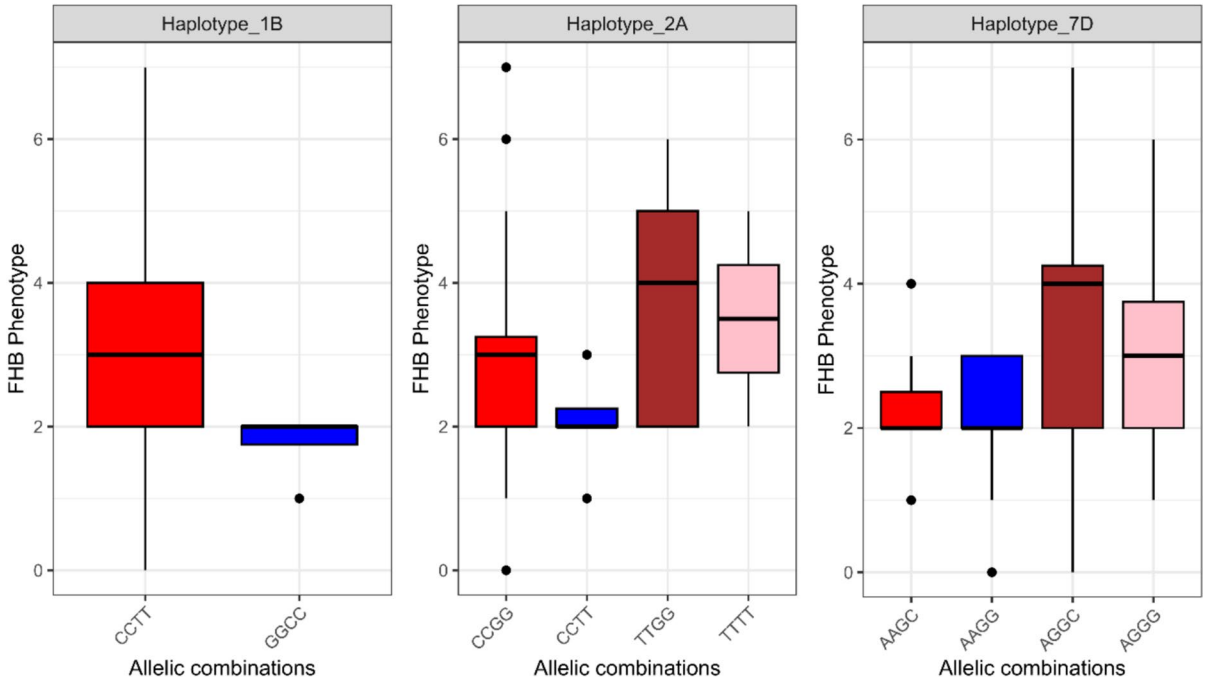

Fig. 6 Box plots of haplotypes on three different chromosomes associated with FHB resistance in wheat. Boxes represent the first quartile, the median, and the third quartile, respectively. The thick horizontal lines correspond to the mean. Whiskers represent variability outside the upper and lower quartiles

utilized locally developed varieties and landraces, well-adapted to diverse agro-ecological zones of Pakistan. This approach ensured genetic variation for successful GWAS and identified potential resistance sources for local breeding use.

Artificial inoculation methods used in this study created uniform and moderate-to-high disease pressure, ensuring accurate assessment of FHB resistance. The use of mixed *Fusarium* isolates with varying levels of aggressiveness ensured sufficient disease pressure while minimizing reliance on environmental conditions (Mesterházy et al. 2012). FHB resistance is not necessarily specific to *Fusarium* species or isolates, highlighting the need for standardized testing environments and inoculation protocols (Steiner et al. 2009). Parameters for evaluating FHB resistance include visual scoring, grain yield loss, grain quality traits, and quantification of *Fusarium* biomass or deoxynivalenol (DON) content. Our results showed substantial variation in Type II resistance among genotypes. The genotypes were categorized into four groups: 54 were highly resistant (scores of 0–2), 65 showed moderate resistance (scores of 2.1–3.0), 48 were moderately susceptible (scores of 3.1–4.9), and 30 were susceptible (scores of 5 and above). These results underscore the substantial genetic diversity for FHB resistance in the tested wheat germplasm, which is valuable for breeding programs targeting improved resistance.

The selection of appropriate models and statistical methods in GWAS is paramount for obtaining reliable results, especially considering the nature of the trait under investigation. FHB resistance, being a polygenic and multifactorial complex trait, is regulated by numerous small-effect loci. Utilizing multi-locus methods proves to be more effective and efficient in capturing these small-effect loci, as demonstrated by previous studies not only in wheat but other crops as well (Segura et al. 2012; Zhang et al. 2019; Rehman et al. 2024, 2025). However, for increased detection power and robustness, it is recommended to complement multi-locus methods with single-locus models (Li et al. 2017a, b). Furthermore, the integration of multiple GWAS methods is advantageous, serving as a cross-check mechanism to enhance the confidence of identified QTL (Sorrells et al. 2010). We

found multi-locus models advantageous in particular as most of the unique QTL were detected with this approach, in addition to detecting the same QTL identified by traditional single-locus models. Furthermore, our analysis indicated that while single-locus models exhibited *p*-value deflation, suggestive of potential over-correction and a loss of statistical power, the multi-locus models demonstrated more robust statistical performance, with their SNP distribution closely adhering to the expected null distribution before showing clear deviations indicative of potential genetic associations. It is important to note that these associations were identified using a significance threshold of $-\log10(p) = 3.0$. This approach was necessitated as a more stringent Bonferroni correction, which, as widely discussed in the context of GWAS, is considered a conservative method for selecting a threshold due to its assumption that every genetic variant tested is independent of the rest (Kaler and Purcell 2019), did not yield any significant associations. This highlights the challenge of detecting complex trait loci under such conservative multiple testing adjustments.

Our study identified QTL for FHB resistance on chromosomes 1A, 1B, 2A, 2B, 7B, and 7D. These findings contribute to the broader understanding of the genetic architecture of FHB resistance in wheat (McMullen et al. 2012). While the well-known FHB resistance loci *Fhb1* on chromosome 3B (Cuthbert et al. 2006) and *Fhb2* on chromosome 6BS (Cuthbert et al. 2007) are critical, the QTLs identified in our study represent minor-effect loci, with relatively lower R^2 values. These loci did not show strong statistical support in allele-wise comparisons but exhibited additive trends that may be relevant under polygenic selection. Importantly, the newly detected loci, particularly on chromosomes 1A, 2A, and 7B, may play supporting roles in Type II resistance (resistance to spread within the spike), and warrant further validation in larger or multi-environment populations.. When combined with known loci such as *Fhb1* and *Fhb2*, the loci identified in this study may also have the potential to contribute to overall FHB resistance in wheat. However, given that these loci were detected at a LOD threshold of 3, further validation and fine mapping are necessary to confirm their significance and utility in breeding programs (Bai and Shaner 2004; McMullen et al. 2012; Kage et al. 2017) QTL.

Our study estimated a broad-sense heritability (H^2) of 0.51 for FHB severity, reflecting a moderate genetic contribution. This aligns with previous reports, though values vary widely with conditions. For example, Buerstmayr et al. (2000) found high heritability ($H^2 > 0.75$) under controlled environments, while Ghimire et al. (2022) reported a range of 0.36–0.85 across environments. Similarly, Urrea et al. (2002) observed H^2 of 0.65 for severity and 0.46 for DON in barley, confirming moderate heritability in cereals. In the current study, four out of 43 landraces showed resistance with FHB scores between 0 and 2, while 11 landraces exhibited moderate resistance to with FHB score less than 3.0. Significant progress in identifying FHB resistance loci has been made, but translating these findings into commercial varieties remains challenging. Resistance derived from exotic sources often comes with undesirable agronomic traits, necessitating careful selection during backcrossing (Steiner et al. 2009). Marker-assisted selection has been essential in accelerating the introgression of key QTL into elite lines. However, the polygenic nature of FHB resistance implies that MAS alone may be insufficient. Integrating genomic selection approaches, which account for multiple small-effect loci, can improve prediction accuracy and breeding efficiency (Otto et al. 2002; Wang et al. 2025).

Chromosome-specific findings

Chromosome 1A

Chromosome 1A has been identified as a significant contributor to FHB resistance in several studies. Buerstmayr et al. (2009) identified multiple QTL on 1A, emphasizing its role in providing resistance against FHB. Similarly, Buerstmayr et al. (2020); Yuan et al. (2013) confirmed the importance of this chromosome in controlling resistance, particularly in relation to deoxynivalenol accumulation. Fine mapping efforts by He et al. (2019) have further refined these QTL, by pinpointing their precise locations and contributing to a better understanding of their functional roles. The characterization of these QTL offers valuable insights for selecting resistant varieties and improving FHB resistance through breeding.

Chromosome 1B

Chromosome 1B also plays a crucial role in FHB resistance. Zhang et al. (2004) first mapped significant QTL on this chromosome, which was further confirmed by more recent studies by Hu et al. (2020). These studies demonstrated the presence of multiple QTL, suggesting a complex genetic architecture for resistance on 1B. Guo et al. (2015) utilized high-density SNP markers to enhance the mapping resolution, which improved our understanding of the QTL' contributions to resistance. These findings underscore the potential of chromosome 1B for breeding programs aimed at enhancing FHB resistance in wheat.

Chromosome 2A

Chromosome 2A has been recognized for its role in FHB resistance through various studies. Wang et al. (2019); Li et al. (2016) identified and characterized QTL on this chromosome, highlighting its contribution to resistance mechanisms. The fine mapping performed by McCartney et al. (2005); He et al. (2018) has been instrumental in pinpointing QTL locations with greater precision. Studies such as those by Wang et al. (2023); Cao et al. (2022) have provided further insights into the genetic control of resistance on chromosome 2A. The identification and validation of QTL on this chromosome are critical for developing wheat varieties with enhanced FHB resistance.

Chromosome 2B

QTL for FHB resistance on chromosome 2B have been extensively studied. Li et al. (2017a, b) and Gilsinger et al. (2005) reported significant QTL associated with resistance, which were further characterized by Liu et al. (2007); Wang et al. (2023). These studies reveal a diverse set of QTL that contribute to resistance, supported by high-density SNP mapping and meta-analysis (Sari et al. 2018). The characterization of these QTL provides valuable information for improving resistance through genetic selection and breeding.

Chromosome 7B

Chromosome 7B has been identified as a key region for FHB resistance. Wang et al. (2019); Schmolke et al. (2005) reported significant QTL on this chromosome, which were further characterized by studies such as those by Liu et al. (2019). These findings, including high-density SNP mapping by Mellers et al. (2020), highlight the importance of chromosome 7B in developing resistant wheat varieties. The characterization of QTL on this chromosome offers promising targets for enhancing FHB resistance.

Chromosome 7D

Chromosome 7D has also been associated with significant QTL for FHB resistance. Ren et al. (2019); Cativelli et al. (2013) identified key QTL on this chromosome, with further characterization provided by McCartney et al. (2016). These studies, along with those by Ren et al. (2019) have enhanced our understanding of the genetic factors contributing to resistance. The mapping and validation of QTL on chromosome 7D are crucial for developing wheat varieties with improved FHB resistance.

Conclusions

Rapid climate changes pose significant challenges to cereal crops worldwide, including Pakistan. Continued exploration of diverse genetic backgrounds and refinement of resistance mechanisms are crucial for developing wheat cultivars with improved resistance to Fusarium head blight. This study advances our understanding on the genetic architecture underlying FHB resistance in a diverse panel of spring wheat from Pakistan, highlighting the potential of integrated breeding strategies that incorporate marker-assisted selection and facilitate genomic selection in local breeding programs. However, we acknowledge obvious limitations in our study. The GWAS was conducted under controlled greenhouse conditions due to the currently low and inconsistent incidence of FHB in field settings across Pakistan, which limited our ability to assess resistance under natural infection pressures. Moreover, while our results offer important preliminary insights, further validation under field conditions is essential to confirm the effectiveness of the identified loci across environments. The identified loci represent minor-effect QTLs, as evidenced by their modest R^2 values and non-significant allelic effects in t-tests. The use of a more relaxed

significance threshold was employed to capture these minor-effect loci, which might otherwise be overlooked by more stringent corrections (e.g., Bonferroni) that can be overly conservative for polygenic traits. Although individually small, these minor-effect loci may collectively contribute to durable resistance and can be valuable targets when pyramided through genomic selection. Despite these constraints, this study provides a foundational dataset that can guide future multi-environment studies and supports the long-term goal of genome-informed breeding for FHB resistance in Pakistan.

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Author contributions All authors contributed to the study conception and design. The study was conceptualized by Rafi Ullah, Fahim Ullah Khan, Inam Ullah, Valentina Spanic, and Katarina Sunic Budimir. Material preparation, data collection, and recording were performed by Valentina Spanic and Katarina Sunic Budimir, while the experiments were conducted by Rafi Ullah, Valentina Spanic, and Katarina Sunic Budimir. Data analysis was carried out by Fahim Ullah Khan and Attiq ur Rehman. The first draft of the manuscript was written by Rafi Ullah and Fahim Ullah Khan and was finally revised by Attiq ur Rehman. All authors reviewed and approved the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no conflict of interests.

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