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Unraveling the genetic architecture of stripe rust resistance in ICARDA spring wheat

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ABSTRACT

Stripe rust, also known as yellow rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is among the most destructive fungal diseases affecting global wheat productivity. Identifying genetic loci associated with *Pst* resistance is crucial for developing durable *Pst*-resistant wheat varieties. This study aimed to discover genetic markers linked to *Pst*-resistance in wheat using a 15 K single-nucleotide polymorphism (SNP) array. Field screenings were conducted over two years (2018 and 2019) on a panel of 245 wheat breeding lines developed by the International Center for Agricultural Research in the Dry Areas (ICARDA) at the Kulumsa Agricultural Research Center in Ethiopia. Importantly, 36 breeding lines exhibited consistent immunity or resistance across both growing seasons. Genome-wide association studies (GWAS) identified 34 marker-trait associations (MTAs) across 10 loci that surpassed the significance threshold. Half of these SNP markers were located on chromosome 7B, while the remaining were distributed across chromosomes 1B, 2B, 4B, 5 A, and 6B. Many identified quantitative trait loci (QTLs) were in close proximity to known *Pst* resistance genes/QTLs, suggesting they correspond to the same genetic regions. Additionally, three QTLs—*EWYY5A.2, EWYY6B.1, and EWYY7B.3*—were notably distant from any of previously identified *Pst* resistance genes, emerging as potential novel loci from this study. These QTLs represent promising candidates for marker-assisted selection, facilitating the development of wheat cultivars with enhanced resistance to *Pst.* Additionally, this study recommends incorporating the 36 consistently resistant lines into national and international wheat breeding programs to enhance *Pst* disease management efforts.

1. Introduction

Wheat stripe/yellow rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* (*Pst*), is one of the major constraints limiting wheat productivity and quality around the world (Chen et al., 2014; Tadesse et al., 2017; Khanfri et al., 2018; Bhavani et al., 2021; Bhavani et al., 2022). In recent years, wheat production has faced a growing challenge with the emergence of new and virulent strains of the *Pst* pathogen (Afzal et al., 2024). Climate change worsens the spread of disease by altering pathogen dynamics and host susceptibility conditions (Bouvet et al., 2022; Zhang et al., 2022; Shahin et al., 2024; Župunski et al., 2024). Furthermore, this challenge is compounded by the narrow genetic base of current cultivars (Muleta et al., 2017a), which limits genetic diversity and increases susceptibility to pathogen adaptation. Many cultivars share similar resistance genes, which makes them susceptible to rapidly evolving pathogens. Consequently, the breakdown of resistance genes poses a significant threat to wheat production. To date, disease has been reported in more than 60 countries, including North and East Africa (Muleta et al., 2017a; Meyer et al., 2021), Asia (Sharma et al., 2016; Wu et al., 2021), South America (El Solh, 2012), Australia (Wellings, 2007;

Abbreviations: ANOVA, Analysis of variance; BLUP, Best linear unbiased prediction; CI, Coefficient of infection; DS, Disease severity; GWAS, Genome-wide association study; ICARDA, International Center for Agricultural Research in the Dry Areas; KARC, Kulumsa Agricultural Research Center; IR, Infection response; LD, Linkage disequilibrium; MAF, Minor allele frequency; MAS, Marker-assisted selection; MCMC, Markov chain Monte Carlo; MLM, Mixed Linear Model; MTA, Marker-trait association; PC, Principal of components; PCA, Principal component analysis; Pst, Wheat stem rust fungus *Puccinia striiformis Westend.* f. sp. *tritici,* Eriks; PIC, Polymorphism information content; PV, Phenotypic variance; QQ, Quantile-quantile; QTL, Quantitative trait locus; SNP, Single nucleotide polymorphism..

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Milus et al., 2009), North America (Markell and Milus, 2008; Wan, 2010), Europe (Bhattacharya, 2017), and other parts of the world (Savary et al., 2019; Mapuranga et al., 2022).

This issue is especially evident in Ethiopia, where *Pst* epidemics have significantly impacted crop yields (Dawana et al., 2024; Mekonnen et al., 2024), resulting in more than a 10 % decline and approximately $94 million in annual losses (Aslake and Sintayehu, 2022). The recurrent outbreaks have led to the collapse of many well-known and widely cultivated wheat varieties. For example, Laketch, the semi-dwarf wheat variety, in 1977 (Gebre-Mariam et al., 1991) and Dashen, a popular high-yielding variety with the *Yr9* gene, in 1988 were damaged by the newly emerged *Pst* races (Badebo and Bayu, 1991). In 2010, a major *Pst* epidemic affecting nearly 600,000 ha of wheat (Patpour et al., 2016; Tolemariam et al., 2018) led to a significant loss in total wheat production in Ethiopia (Meyer et al., 2021). The epidemic was caused primarily by the race *PstS6,* leading to the breakdown of dominant wheat varieties such as Galema (HAR 604) and Kubsa (HAR 1685) with the *Yr27* gene (Tadesse et al., 2017; Meyer et al., 2021). Another notable *Pst* epidemic occurred in 2016, primarily caused by the *PstS11* race. This epidemic resulted in an estimated loss of 65 million USD, damaging two widely cultivated wheat varieties, Ogolcho and Dandaa (Meyer et al., 2021).

Genomic-assisted breeding has emerged as a powerful approach to enhance breeding and selection efforts in crop plants (Alemu et al., 2024). Genome-wide association study (GWAS) is one of the genomic-assisted breeding methods used to discover genomic regions associated with various traits of interest in wheat and other crop plants. Over the past two decades, GWAS has successfully identified genomic regions linked to important agronomic traits in wheat, including resistance to pests and diseases, tolerance to abiotic stresses and for improving grain yield and quality (Muleta et al., 2017a; Ogbonnaya et al., 2017; Mathew et al., 2019; Ward et al., 2019; Chaurasia et al., 2021; Saini et al., 2022; Shewabez et al., 2022). GWAS aimed at identifying genetic loci related to *Pst* resistance began in 2007 with the pioneering work of Crossa et al. 2007Crossa et al., 2007. Since then, several GWAS studies have been conducted using diverse genetic resources to uncover additional genomic regions associated with *Pst* resistance (Zegeye et al., 2014a; Maccaferri et al., 2015; Muleta et al., 2017a; Kumar et al., 2020; Li et al., 2020a; El Messoadi et al., 2022). Wheat is particularly suitable for GWAS study due to the abundant availability of high-quality genome-wide DNA markers (Jamil et al., 2020; Rahman et al., 2020) that exhibits significant levels of linkage disequilibrium (LD) with causative QTLs/genes (Chao et al., 2010). To date, researchers have identified over 83 resistance genes/alleles (*Yr1*–*Yr83*) that confer resistance to *Pst* (Mcintosh et al., 2013; Li et al., 2020a, 2020b; Baranwal, 2022). However, achieving long-term and durable resistance against evolving pathogen strains remains a significant challenge. This highlights the necessity for ongoing research and breeding efforts in this field.

To address this issue, it is essential to identify and incorporate diverse genetic markers in wheat to confer durable resistance against this evolving threat. In this study, a diverse collection of wheat breeding lines from around the world underwent evaluation for their resistance to *Pst* in Ethiopia. The research employed GWAS to identify markers associated with resistance against *Pst* disease.

2. Materials and methods

2.1. Plant material and disease evaluation

In this study, a panel of 245 bread wheat (*Triticum aestivum*) breeding lines developed by ICARDA were evaluated. Their origin, pedigree, and experimental design are provided in **Table S1**. The field experiment was conducted at Kulumsa agricultural research centre in South-Eastern Ethiopia (8°2′ N, 39°10′ E, 2200 *m.a.s.l*) during the main cropping seasons of 2018 and 2019. The mean maximum and minimum temperatures were 23.2 °C and 10 °C, respectively, and the mean annual precipitation was 823.1 mm. This region has a favourable condition for *Pst* infection and is recognized as a centre of excellence to screen resistant wheat genotypes in the East African region.

Field evaluation was conducted with an augmented experimental design along with five known varieties used as checks (Digelu, Kubssa, Hidasse, Honqolo, and Ogolcho). These checks were replicated across five blocks, while the 245 wheat genotypes were evaluated without replication. Disease evaluation was conducted assessing the disease severity (DS) and infection response (IR) of genotypes. Each line was evaluated three times per year, and the score with highest susceptibility —typically the third score— were used for the current study. Disease severity was measured using the modified Cobb scale which quantifies the percentage of the flag leaf affected by rust pustules with the values of 0 %, 5 %, 10 %, 20 %, 40 %, 60 %, 80 %, and 100 % (Peterson et al., 2011). The infection response (IR) of genotypes against *Pst* was categorized into six different classifications. Immune (I) genotypes exhibited no yellow rust infection. Resistant (R) genotypes showed no yellow rust pustules or necrosis on the leaf. Moderately Resistant (MR) genotypes displayed small and tiny pustules with minimal necrosis on the leaf. Intermediate (M) genotypes were characterized by an intermediate level of pustules. Moderately Susceptible (MS) genotypes had a moderate level of pustules with no necrosis but displayed observable chlorosis on the leaf. Finally, Susceptible (S) genotypes were identified by fresh, bulky pustules accompanied by necrosis and chlorotic areas on the leaf surface. Coefficient of infection was calculated by multiplying the DS by a constant value of each IR; immune = 0.0, *R* = 0.2, RMR = 0.3, MR = 0.4, MRMS = 0.6, MS = 0.8, MSS = 0. 9, and S = 1.0.

2.2. Statistical analysis of phenotypic data

Analysis of variance (ANOVA) was performed on DS (disease severity), IR (infection response, and CI (coefficient of infection) performed using the *nlme* package (version 3.1.0) https://github.com/cran/nlme in R environment. The analysis was conducted considering the variables for DS, IR, and CI in relation to wheat line, year, and lines by year interactions (Morales et al., 2021; Shewabez et al., 2022). The adjusted mean values as best linear unbiased predictions (BLUPs) and correlations between phenotypic parameters over two years was calculated using a mixed linear model in the *lme4* package in R environment (https://github.com/cran/lme4) (Shewabez et al., 2022). Broad-sense heritability (H^2) across years was calculated using the formula:

$$H\hat{}2 = \sigma\hat{}2\,G/[\sigma\hat{}2\,G + ((\sigma\hat{}2\,E)/y) + (\sigma\hat{}2\,G \times (\sigma\hat{}2\,E)/y) + ((\sigma\hat{}2\ \text{error})/y)]$$

where σ^2G is the genotypic variance, σ^2E is the environmental (year-location combination) variance, $\sigma^2G \times E$ is the genotype by environment (GxE) interaction variance, σ^2error is the residual error variance and y is the number of years.

2.3. Genotyping

DNA was extracted from the leaf samples of one-week seedlings following the protocol described by Allen et al. (2006). Single nucleotide polymorphism (SNP) genotyping was performed using the Illumina's iSelect 15 K SNP wheat array by TraitGenetics in Gatersleben, Germany. The array produced a total of 13,006 SNP markers. Markers were further screened with a minor allele frequency (MAF) > 5 % and < 10 % missing values per individual genotypes to ensure data quality. After screening, a total of 9523 quality SNP markers and 245 lines were used for all subsequent statistical analyses.

2.4. Genome-wide association study

The genome-wide association study (GWAS) was conducted using the mixed linear model (MLM) in TASSEL 5.2 software to identify SNP markers significantly associated with resistance to *Pst* infection. The

MLM is one of the powerful single-locus based GWAS models with a capability to incorporate confounding factors such as population structure and kinship similarities and able to control the false-negative results. The applied MLM GWAS model can be explained with the formula:

$$y = X\alpha + Q\delta + K\mu + e$$

where y represents the phenotypic values, X represents SNP marker genotypes, α is a vector of fixed effects resulting from the genotype, Q represents the population structure, δ is a vector of fixed effects resulting from the population structure, K represents the relative kinship matrix, μ is a vector of random effects from kinship and e is a vector of residuals. To determine the significance of the GWAS results, a threshold value $-\log_{10}^{p}$ ($P \leq 0.001$) was applied. An exploratory threshold of $-log_{10}p \geq 3$ ($P \leq 0.001$) was employed to identify significantly linked SNP markers in the current GWAS analysis following previous studies (Alemu et al., 2021) (**Table S3**).

3. Results

3.1. Phenotypic evaluation

The 245 evaluated wheat lines exhibited significant variation in disease severity (DS) and infection response (IR) across two field trials. In 2018, DS ranged from 0 % to 50 %, averaging 14 %, with 78 % of lines classified as resistant, 15 % as moderately resistant, and 7 % as susceptible. Most lines (56.7 %) scored IR values between 0.4 and 0.6, indicating moderate resistance, while 29.3 % showed immunity or resistance, and 14 % were moderately susceptible or susceptible. Coefficient of infection (CI) scores were primarily low, with two-thirds of lines scoring ≤5 and an average CI of 8.

In 2019, wetter conditions led to higher disease reactions, with mean DS, IR, and CI values of 22 %, 0.5, and 19, respectively. Approximately 25 % of lines displayed immunity or resistance, nearly half exhibited moderate resistance, and the remaining 25 % were moderately susceptible or susceptible. Across both seasons, 121 lines (48 %) were classified as resistant based on DS (≤15 %), while 151 lines (60 %) were resistant according to CI (≤15). Notably, 36 lines (14.16 %) showed complete immunity to *Pst* with IR scores of 0 to 2. Despite increased disease severity in 2019, resistance patterns were consistent across years, underscoring the potential of these lines for breeding resistant varieties (Fig. 1 **and** Table 1).

3.2. Phenotypic analysis and heritability

The analysis of variance (ANOVA) result revealed high significant differences ($P < 0.0001$) among studied genotypes for all three applied parameters including disease severity (DS), infection response (IR), and coefficient of infection (CI) related to *Pst* infection. Pearson's correlation coefficients between DS, IR and CI between the two seasons were 0.72,

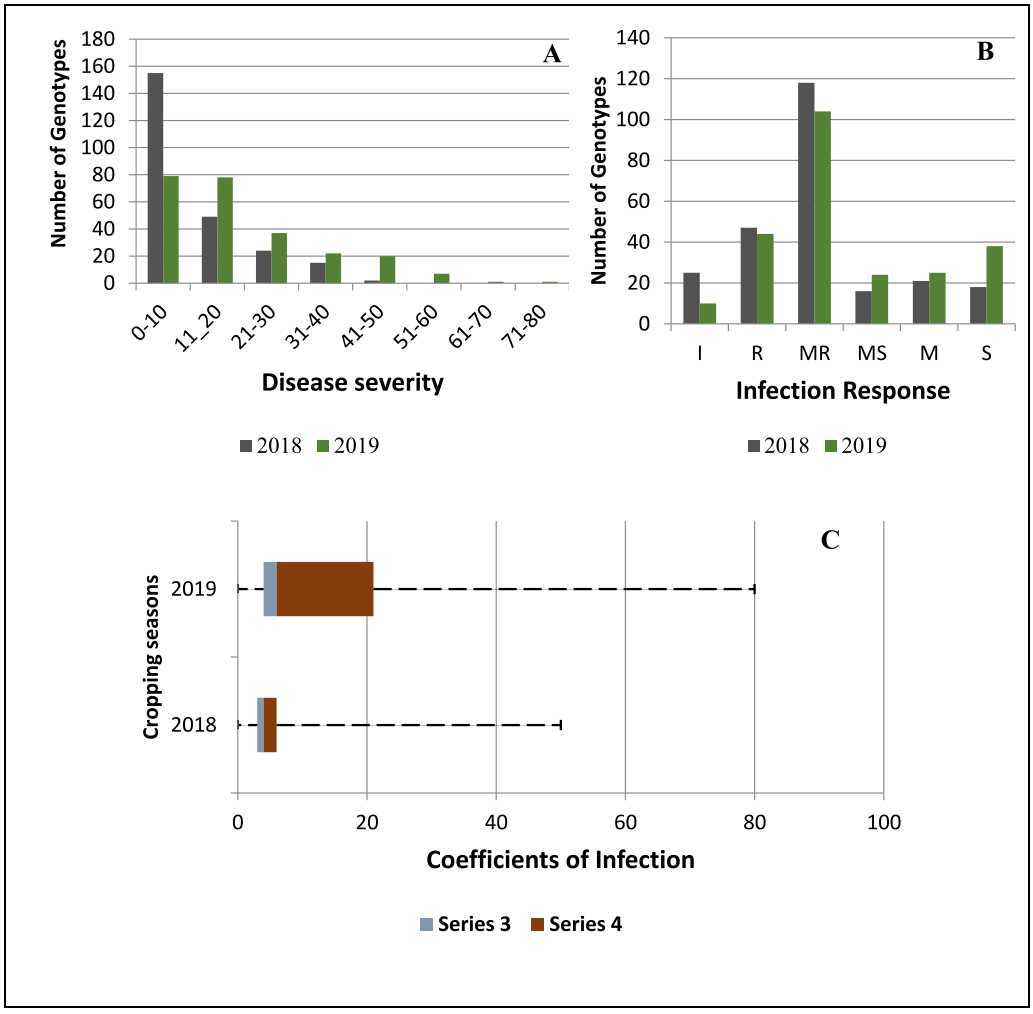

Fig. 1. Phenotypic distribution of 245 wheat lines against stripe rust under natural disease pressure as infection response (IR), disease severity (DS) and coefficient of infection (CI) in corresponding two environments (2018 and 2019). (A) Frequency of genotypes for DS in percentage; (B) frequency of genotypes for IT; (C) Frequency of genotypes for CI on the scale of 0–100. Each environment is represented with different colours as indicated by the colour legend.

Table 1
Mean response, variance component estimates and heritability for infection response (IR), disease severity (DS), and coefficient of infection (CI) variables.

Parameters	DS (%)	IR (1–0)	CI
Range	0–80	0–1	0–80
Mean	18.1	0.46	13.0
σ^2_G	330.3***	*1.6567* ***	516.22***
σ^2_E	48.4*	*0.10776* ***	64.12*
σ^2_{GxE}	67.5**	*0.03463*^{ns}	77.68*
σ^2 error	30.0^{ns}	*0.04549*	44.23
H	0.82	0.93	0.84
r	0.72	0.84	0.74

where σ^2G is the genotypic variance, σ^2E is the environmental (year-location combination).
variance, σ^2G × E is the genotype by environment (GxE) interaction variance, σ^2error is the.
residual error variance and y is the number of years; H^2 is heritability; r, Pearson's correlation coefficients among between two seasons.

0.84, and 0.74, respectively, all with *p*-values less than 0.0001, indicating strong positive relationships and high statistical significance. The broad-sense heritability (H^2) estimates from the variance components of DS, IR, and CI across the seasons were 84 %, 71 %, and 86 %, respectively (Table 1). The relatively high H^2 estimate indicate that the current disease evaluation is appropriate for conducting further GWAS analyses to explore more genomic resources for *Pst* resistance.

3.3. Genome-wide association analysis

The Genome-Wide Association Study (GWAS) was conducted using (Best Linear Unbiased Prediction (BLUP) values of coefficient of infection along with the SNP markers. To improve the accuracy of the analysis and control false-positives the mixed linear model (MLM) model was employed, integrating the population structure as principal components (PCs) and kinship matrix. The model performance was assessed through a quantile-quantile (Q-Q) plot, which indicated that the *p*-values from the MLM functions closely aligned with the expected distribution. This graphical evidence indicates that the MLM model provides an acceptable control for false positives in the GWAS analysis (Fig. 2B). In addition, the marker-trait associations with a threshold of -log10 (*P*-value) ≥ 3 is reported as identified MTAS (Fig. 2A).

The current study has identified 34 significant MTAs associated with *Pst* resistance grouped into 10 QTLs. These QTLs were further categorized into two groups based on the significance thresholds of MTAs: major QTLs (−log (*P*) ≥ 4) and nominal QTLs -log (*P*) values of ≥3). Among the 10 identified QTLs, the two adjacent QTLs, *EWYY7B.1* and EWYY7B.2, exhibited the highest significance. In the second category, eight QTLs (*EWYY1B.1*, *EWYY2B.1, EWYY2B.2, EWYY4B.2, EWYY5 A.2, EWYY6B.1, EWYY6B.2, and EWYY7B.3*) were classified as nominal QTLs (Table 2). Detailed information regarding the explained phenotypic variance, marker positions and references to previously reported *Pst* resistance genes and QTLs is provided in Table 2. Additionally, suggestive QTLs with a relatively low threshold value of -log (*P*) ≥ 2.5, encompassing 62 SNPs were identified. The comprehensive information about these suggestive QTLs is provided in **Table S2.**

The identified MTAs were distributed across six chromosomes including 5 A, 1B, 2B, 4B, 6B, and 7B. Chromosomes 5 A, 1B, and 4B each contained one QTL associated with *Pst* resistance. In contrast, chromosomes 2B and 6B each had two QTLs, while chromosome 7B featured three loci linked to *Pst* resistance (Fig. 3 **and** Table 2).

On chromosome 5 A, locus *EWYY5A.2* was identified comprising three co-localized SNP markers (*Tdurum_contig50175_875, BS00041063_51, Tdurum_contig10843_745*) at 93.23 centimorgans (cM). This QTL explained 6.62 % of the total of the observed phenotypic variation.

On chromosome 1B, with only a single SNP marker *Kukri_c92979_195* and designated as QTL *EWYY1B.1* (27.62 cM) explained 5.2 % of the total phenotypic variation.

On chromosome 4B, the QTL *EWYY4B.2* comprised four SNP markers including *Excalibur_c27948_1073*, *RAC875_c2456_849, RAC875_c39226_131, BS00003421_51*. The SNP markers have the genetic map position in the range of 71.37 cM to 75.65 cM and accounted for 5.4 % to 7.0 % of the total phenotypic variation, with an average of 7.7 %. (Table 2 **and** Fig. 3).

On chromosome 2B, two loci were associated with *Pst* resistance (Table 2 **and** Fig. 3). The markers *RAC875_c26469_480* and *RAC875_rep_c109207_706* were included in the QTL *EWYY2B.1* and collectively explained 5.7 % of the total phenotypic variation observed in the current population. The other identified QTL on this chromosome, *EWYY2B.2*, comprising the SNP marker *Kukri_c55909_1109* at 141.48 cM which accounted for 7.45 % of the total phenotypic variation.

On chromosome 6B, two QTLs (*EWYY6B.1* and *EWYY6B.2*) were identified, each associated with significantly linked SNPs. The QTL *EWYY6B.1* comprised three SNPs located on from 64.08 cM (*wsnp_Ex_c17667_26408733*) to 71.76 cM (*GENE-4183_1109*). The second QTL *EWYY6B.2* comprised markers *wsnp_CAP11_c1432_806102*, *wsnp_Ex_c1276_2445537* and *Kukri_c12602_861*. This QTL is situated at the genetic map position between 73.24 cM (wsnp_*CAP11_c1432_806102*) to 75.35 cM (*Kukri_c12602_861*) and accounts an average 5.93 % of the total phenotypic variation (Table 2**)**.

On chromosome 7B, the three QTLs *EWYY7B.1*, *EWYY7B.2* and *EWYY7B.3*, with SNP markers were significantly associated with *Pst* resistance. Notably, *EWYY7B.1* and *EWYY7B.2* were designated as major QTLs with SNPs surpassed the significant threshold of (≥ 4) *EWYY7B.1* found in between 63.09 cM (*wsnp_BF200891B_Ta_2_1*) and 71.33 cM (*wsnp_Ex_c10565_17249813*) accounted for an average phenotypic variation of 7.7 %. Adjacent to this QTL, another QTL *EWYY7B.2* was identified comprising three SNPs.

(*Excalibur_c17078_400, Tdurum_contig81318_116* and *wsnp_Ku_c217 52_31528824*) and contributed to an average phenotypic variation of 7.1 %. The third QTL identified on the same chromosome was *EWYY7B.3* with marker *BS00022045_5*1at 89.64 cM and accounted for 6.3 % of the total phenotypic variation (Table 2 **and** Fig. 3).

4. Discussion

Puccinia striiformis f. sp. *tritici* (*Pst*), the pathogen responsible for wheat stripe rust, is a significant global threat to wheat production (Schwessinger et al., 2019). The emergence of diverse strains complicates disease management, emphasizing the need for innovative approaches in breeding and monitoring (Shahin, 2020; Bouvet et al., 2022; Li et al., 2023). An effective strategy involves exploring new genomic resources through genome-wide association studies (GWAS) to identify and integrate quantitative trait loci (QTL) and genes associated with resistance to *Pst*. Recent studies have identified multiple resistance genes and loci critical for developing resistant wheat cultivars. Key findings include resistance genes identified in Chinese wheat landraces by Qiao et al. (2024), fourteen SNP markers associated with *Pst* resistance by El Messoadi et al. (2022), and a meta-QTL analysis by Kumar et al. (2023) revealed the complexity of resistance mechanisms. These findings provide valuable tools for breeders, yet the problem demands further research and genetic diversity in breeding strategies to ensure sustainable wheat production.

GWAS have been applied to identify novel QTLs associated with *Pst* resistance in Ethiopia wheat panels. For instance, Zegeye et al. (2014a, 2014b) conducted a GWAS on 181 synthetic hexaploid wheats, identifying 27 SNPs linked to seedling resistance and 38 SNPs associated with adult plant resistance to *Pst*. Six genomic regions were consistently associated with resistance, with novel QTLs found on chromosomes 1AS, 3DL, 6DS, and 7AL. Additionally, Atsbeha et al. (2023) conducted a GWAS on a panel of 178 genotypes, identifying significant QTLs for *Pst* resistance, including 12 novel loci. Similarly, Muleta et al. (2017a,

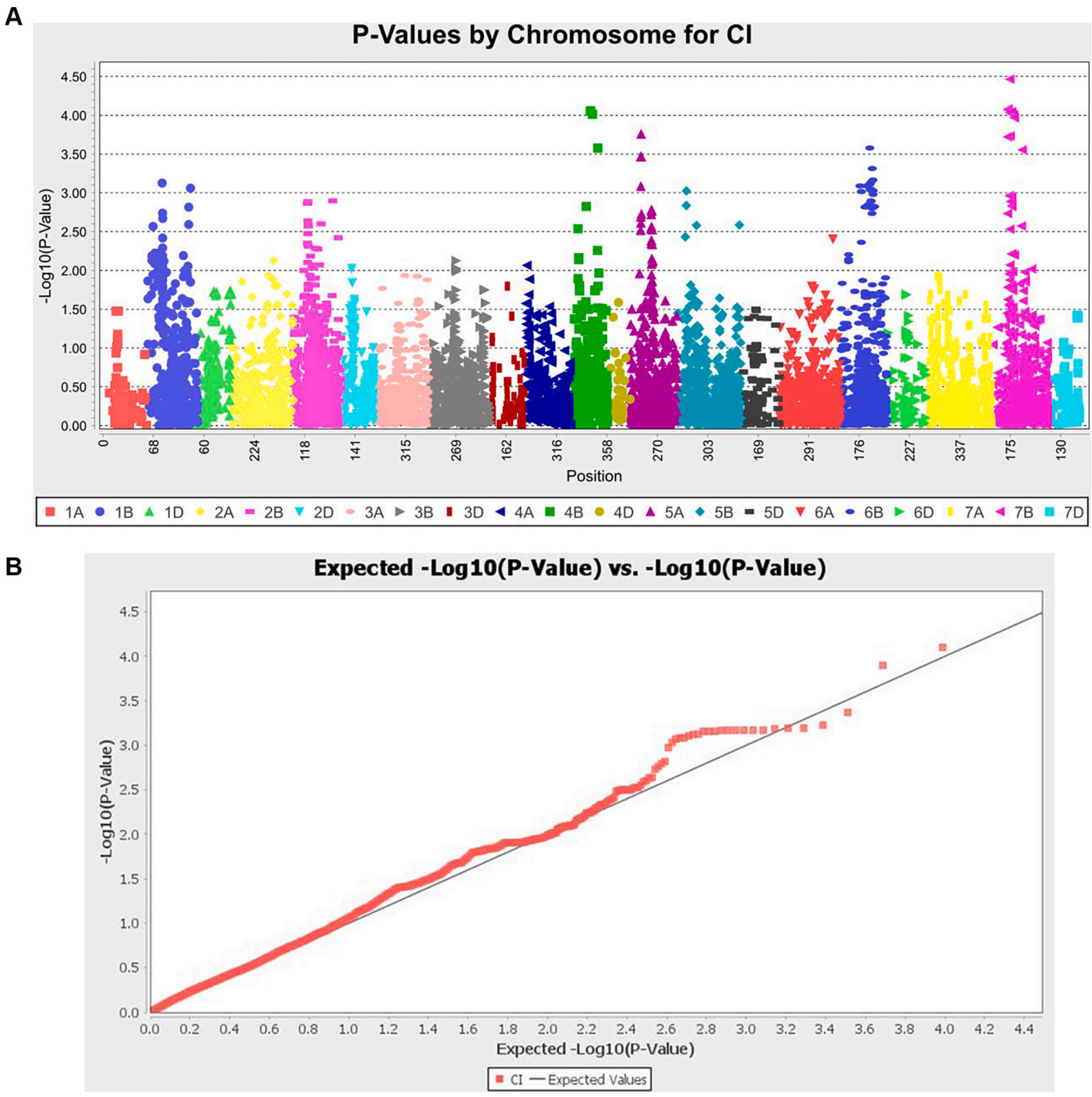

Fig. 2. Manhattan plot and Q-Q-plots of genome-wide association scan for stripe rust resistance in a panel of 245 wheat breeding lines using the mixed linear model (MLM) model. Each dot represents a single SNP. 2 A- is Manhattan plots of the –log 10 (*P*-value) values versus genomic distances of the SNPs significantly associated with wheat stripe rust resistance in wheat at FDR thresholds with *P* values of 0.05 above which the markers were strongly associated with stripe rust resistance. 2B-Q-Q plots of GWAS results using MLM model. The plots show the observed *p*-values (*p*) for the association between CI and each tested marker expressed as –log 10 (*P*-value) of *p* (y-axis) plotted against $-\log_{10} P$ of the expected p-values (x-axis) under the null hypothesis of no association for the analyses.

2017b) used multi-environment field trials and seedling resistance screening to reveal several marker-trait associations. Despite these significant advancements, fully understanding the genetic basis of wheat resistance to *Pst* remains a critical challenge. In the current study, GWAS was applied to identify SNP markers linked to *Pst* resistance in a set of 245 spring wheat lines. The findings showed that most of the lines displayed resistance or moderate resistance to *Pst*. Additionally, the study revealed several genes/QTLs associated with resistance to *Pst*, which had been previously identified by different researchers. Furthermore, this study identified three potentially novel genomic regions linked to resistance against *Pst*. This discovery contributes valuable insights into the genetic characteristics of these germplasms and its potential for enhancing the resistance of future wheat varieties against *Pst*.

4.1. Assessing environmental and genetic factors influencing wheat stripe disease severity in Ethiopia

The severity and spread of *Pst* disease are strongly influenced by environmental factors such as temperature, humidity, and rainfall. To enable effective management of wheat stripe rust, it is crucial to assess

Table 2
Summary of the marker-trait associations detected in the 245 wheat breeding lines for stripe rust resistance.

Trait	QTL	Marker ID	Marker Name	Alleles	C	Pos[a]	p	R2	*Yr/QTL*	%	Reference
CI									*QYr.cau-*		
	EWYY1B.1	IWB48324	Kukri_c92979_195	C/T	1B	27.62	3.340569	5.163	*1BS_AQ24788–53*	15.8	(Lukaszewski, 2000)
CI	*EWYY2B.1*	IWB55936	RAC875_c26469_480	C/T	2B	76.7	3.280868	6.48	*QYr.ucw-2B_UC1110*	40.6	El Solh, 2012
CI		IWB61862	RAC875_rep_c109207_706	C/T	2B	78.99	3.176148	4.859		40.6	
CI	*EWYY2B.2*	IWB46560	Kukri_c55909_1109	T/C	2B	141.48	3.807739	7.457	*Yr43, Yr53*	74.9	(Xu et al., 2013)
CI	*EWYY4B.2*	IWB24647	Excalibur_c27948_1073	C/T	4B	71.37	3.929556	7.683	*Yr62*	58.1	(Lu et al., 2014)
CI		IWB55675	RAC875_c2456_849	G/A	4B	71.46	3.918329	7.66		58.1	
CI		IWB57491	RAC875_c39226_131	A/G	4B	72.14	3.907244	7.638		58.1	
CI		IWB5827	BS00003421_51	C/T	4B	75.65	3.595919	7.019		61.5	
CI					5						
	EWYY5A.2	IWB72051	Tdurum_contig50175_875	A/G	A	93.23	3.461778	6.745		62.7	**This study**
CI					5						
		IWB8258	BS00041063_51	G/A	A	93.23	3.163018	6.185		62.7	
CI					5						
		IWB66780	Tdurum_contig10843_745	C/T	A	93.23	3.163018	6.185		62.7	
CI	*EWYY6B.1*	IWA2244	wsnp_Ex_c17667_26408733	A/G	6B	64.08	3	5.811		50.2	**This study**
CI		IWB31427	Excalibur_rep_c94584_98	C/T	6B	69.94	3.48071	6.776		50.2	
CI		IWB33815	GENE-4183_1109	C/T	6B	71.76	3.065522	5.944		50.2	
CI									*QYr.ucw*-6B		(Maccaferri et al.,
	EWYY6B.2	IWA670	wsnp_CAP11_c1432_806102	T/C	6B	73.24	3.03323	5.879	(*IWA7257*)	57.4	2015)
CI		IWA1679	wsnp_Ex_c1276_2445537	T/C	6B	73.93	3.118598	6.068		57.4	
CI		IWB40857	Kukri_c12602_861	G/A	6B	75.35	3.084785	5.982		57.4	
CI	*EWYY7B.1*	IWA418	wsnp_BF200891B_Ta_2_1	T/C	7B	63.09	3.628507	7.088	*Yr39*	33.4	(Lin, 2007)
CI		IWB3531	BobWhite_c46772_564	G/A	7B	64.59	4.034968	7.929			
CI		IWB54284	RAC875_c1638_165	G/A	7B	65.44	4.028302	7.884			
CI		IWA1543	wsnp_Ex_c11860_19030807	T/C	7B	67.5	4.404724	8.67		12.1	
CI		IWB58816	RAC875_c52266_76	T/C	7B	69.39	3.912787	7.649			
CI		IWB38584	Ku_c17257_926	A/G	7B	69.93	3.921145	7.697			
CI		IWB57902	RAC875_c43108_1021	G/A	7B	69.93	3.912787	7.649			
CI		IWB39827	Ku_c68626_1054	G/A	7B	69.93	3.912609	7.677			
CI		IWB50896	Ra_c11164_740	A/G	7B	71.33	3.916497	7.687			
CI		IWB34834	IAAV4126	G/A	7B	71.33	3.914281	7.681			
CI		IWB52695	Ra_c7974_1192	T/C	7B	71.33	3.913498	7.685			
CI		IWA311	wsnp_BE498662B_Ta_2_1	T/C	7B	71.33	3.912787	7.649			
CI		IWA1352	wsnp_Ex_c10565_17249813	T/C	7B	71.33	3.912787	7.649			
CI	*EWYY7B.2*	IWB22908	Excalibur_c17078_400	G/A	7B	73.39	3.930591	7.733	Yr39	39.1	(Lin, 2007)
CI		IWB73494	Tdurum_contig81318_116	C/T	7B	73.39	3.909107	7.657			
CI		IWA6717	wsnp_Ku_c21752_31528824	A/G	7B	73.79	4.003423	7.835			
CI	*EWYY7B.3*	IWB6876	BS00022045_51	T/C	7B	89.64	3.249414	6.311		47.5	**This study**

QTLs, quantitative trait loci; SNPs, single nucleotide polymorphism; C, chromosome position; Pos, marker's genetic position mapped in the wheat 90KSNP consensus map scale: centimorgan; %, percentage of the total length of the chromosomes in the consensus map (Wang et al., 2014); R^2, phenotypic variance explained by the markers.

natural disease pressure in regions where the disease is prevalent. In line with this, this study focuses on southeastern Ethiopia, recognized as a hotspot for *Pst* infection (Tilahun Hadis, 2019), making it an ideal location for evaluating disease dynamics and management strategies.

The study's phenotypic analysis reveals significant variation and high heritability in disease severity (DS), infection response (IR), and coefficient of infection (CI), indicating a strong genetic basis that supports the effectiveness of GWAS. High broad-sense heritability confirms that much of the observed variation is genetically driven. Similar to these findings, research on *Pst* indicates that genetic factors largely influence disease resistance, allowing GWAS to identify robust resistance loci (Li et al., 2020a; Mu et al., 2020). ANOVA results indicate that genetic factors significantly impact DS, IR, and CI, while genotype-by-environment interactions for DS and CI underscore the need for multi-environment trials, as environmental variability can affect resistance traits. In contrast, IR's high stability across the two years aligns with findings in wheat, where certain infection-related parameters demonstrate consistency across environments, enhancing their reliability for selection. Furthermore, the study's identification of resistant genotypes with strong correlations across years supports the persistence of resistance traits, which is crucial for effective long-term disease management (Table 1).

4.2. Identification of key QTLs for wheat stripe rust resistance through GWAS

The genome-wide association study (GWAS) method for identifying marker-trait associations (MTAs) is a powerful approach for identifying specific genomic regions linked to a trait of interest. In this study, 10 QTLs associated with *Pst* resistance were identified. These QTLs were classified into three groups based on their statistical significance threshold values following the criteria established by Alemu et al. (2021). These categories included major QTLs ($-\log(p) \geq 4$), nominal QTLs ($-\log(p) \geq 3$), and suggestive QTLs ($-\log(p) \geq 2.5$). Furthermore, these QTLs were categorized based on previously reported genes and QTLs associated with *Pst* resistance (Mcintosh et al., 2016; Maccaferri et al., 2015; Mcintosh et al., 2016; Kumar et al., 2020; Li et al., 2020b). The first category comprised QTLs located near previously identified *Pst* resistance genes. The second group included QTLs located at loci co-located with previously reported QTLs associated with *Pst* resistance. Nevertheless, the precise genes responsible for *Pst* resistance in these regions remain undetermined. The third, and the most importance category comprised genomic regions containing newly discovered QTLs in this study. These loci were distinctly distant from previously identified *Pst* resistance genes and QTLs.

4.3. Comparing QTLs to known genes with Pst resistance

Among the ten identified QTLs in this study, the four QTLs

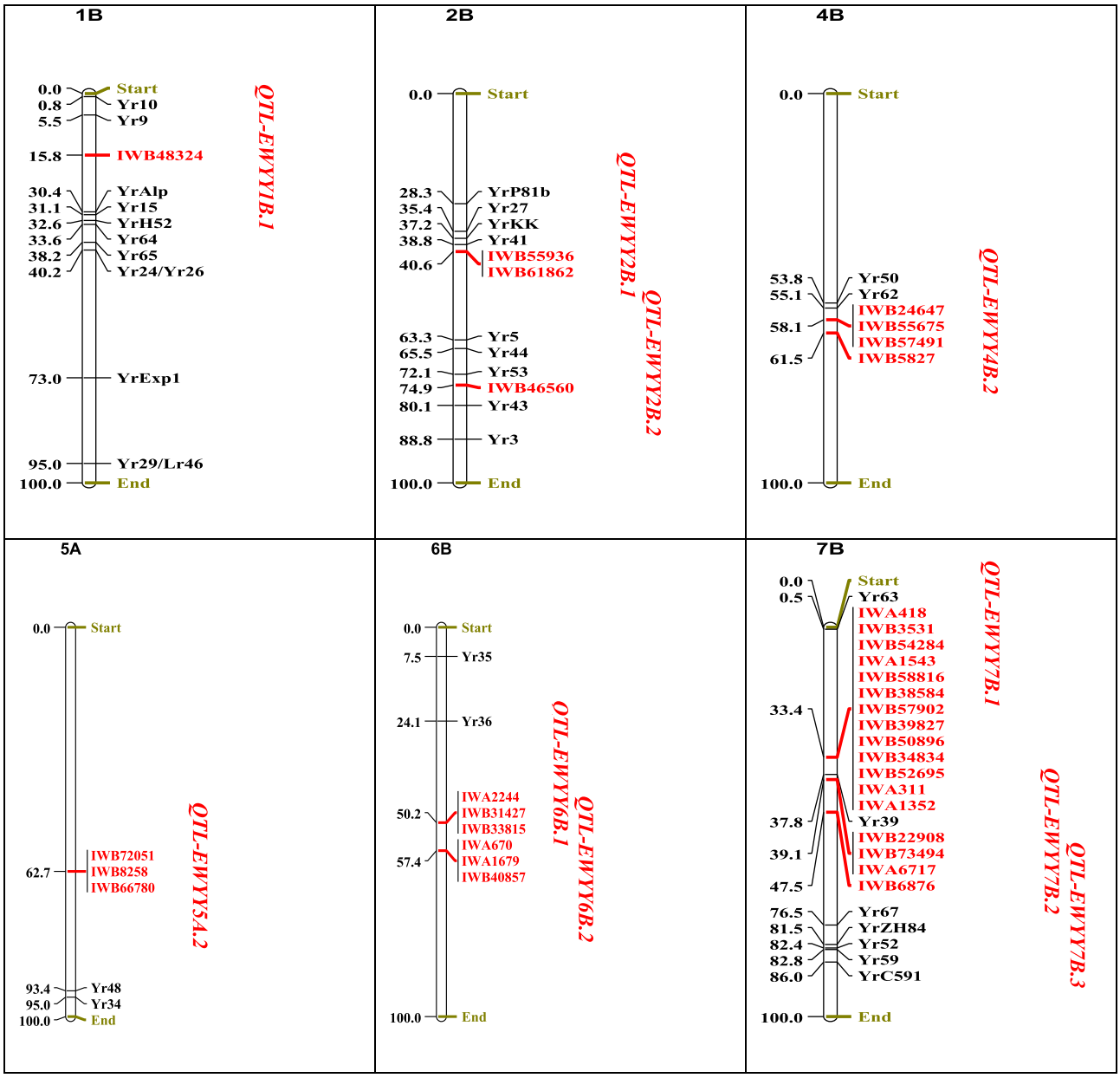

Fig. 3. Map of significant MTAs and QTLs showing 245 elite breeding lines of spring wheat and their corresponding resistance genes of stripe rust. The map positions are indicated on the left-hand side are as a percentage of the total length of the chromosomes in the re-scaled distances on the 90 K consensus map (Wang et al., 2014) while the single-nucleotide polymorphism (SNP) markers and Quantitative trait loci (QTLs) are shown in line to the right-hand side of each figure. The genetic map was constructed using MapChart 2.32 software (Voorrips, 2002).

EWYY2B.2, EWYY4B.2, EWYY7B.1, and *EWYY7B.2* were located within less than one-tenth of the chromosome length to previously identified *Pst* resistance genes (Fig. 3, Table 2**).** The close proximity of these genes to specific QTLs suggests the possibility that these QTLs might represent the same gene, which is essential for breeding programs targeting *Pst* control.

Five known *Pst* resistance genes including *Yr3, Yr5, YR43, Yr44* and *Yr53* have been identified on the long arm of chromosome 2B within a range of 62.1 % to 86.8 % of chromosome length (Xu et al., 2013; McGrann et al., 2014; Mcintosh et al., 2016). The *Yr43/Yr53* and *Yr3* genes are in close proximity to the currently identified *EWYY2B.2* QTL (142.5 cM, 74.9 %, Table 2). This suggests that the wheat germplasms carrying the *EWYY2B.2* QTL likely harbor at least one of these three genes. Similarly, on chromosome 5B, two known *Pst* resistance genes (*Yr19* and *Yr47)* and a candidate gene (*YrExp2)* have been identified (Mcintosh et al., 2013). In the same chromosome QTL *EWYY5B.1* positioned from 126.3 cM to 129.2 cM (Table 2) was identified in this study. This QTL is in close proximity to the previously designated partial *Pst* resistance gene *YrExp2*. As such, it is highly likely that this region represents the same genetic locus since no other *Pst* resistance genes have been mapped nearby Furthermore, on the long arm of chromosome 4B, the QTL *EWYY4B.2* was identified in close proximity to the previously known adult plant resistance gene *Yr62 (*Liu et al., 2013*)*.

The chromosome 7B region is known to harbor several *Pst* resistance genes, including five permanently registered ones —*Yr63, Yr39, Yr67, Yr52,* and *Yr59*— along with two temporarily registered *Pst* resistance genes—*YrC591* and *YrZH84* (Lin, 2007; Mcintosh et al., 2013, 2016). The current study identified two adjacent QTLs, *EWYY7B.1* and *EWYY7B.2.1* located at 126.3 cM - 129.2 cM (Table 2), which are in close proximity to the known *Yr39* gene, indicating a strong likelihood of these genes being present in associated wheat germplasms.

Notably, QTL *EWYY7B.1* exhibited the highest average phenotypic

Gen	CI	IWB54284	IWA1543	IWB58816	IWB38584	IWB57902	IWB39827	IWB50896	IWB34834	IWB52695	IWA311	IWA1352	IWA418
RP003	0	GG	TT	TT	AA	GG	GG	AA	GG	TT	TT	TT	TT
RP029	0	GG	TT	TT	AA	GG	GG	AA	GG	TT	TT	TT	CC
RP036	0	GG	TT	TT	AA	GG	GG	AA	GG	TT	TT	TT	TT
RP150	0	GG	TT	TT	AA	GG	GG	AA	GG	TT	TT	TT	CC
RP242	0	GG	TT	TT	AA	GG	GG	AA	GG	TT	TT	TT	CC
RP249	0	GG	TT	TT	AA	GG	GG	AA	GG	TT	TT	TT	CC
RP153	2.5	GG	TT	TT	AA	GG	GG	AA	GG	TT	CC	TT	TT
RP186	2.5	GG	CC	TT	AA	GG	GG	AA	GG	TT	CC	TT	CC
RP013	5	GG	TT	TT	AA	GG	GG	AA	GG	TT	TT	TT	CC
RP108	42.5	AA	CC	CC	GG	AA	AA	GG	AA	CC	CC	CC	CC
RP088	45	AA	CC	CC	GG	AA	AA	GG	AA	CC	TT	CC	CC
RP116	45	GG	TT	CC	GG	AA	AA	GG	AA	CC	CC	CC	CC
RP120	45	GG	TT	TT	AA	GG	GG	AA	GG	TT	TT	TT	TT
RP146	45	AA	CC	CC	GG	AA	AA	GG	AA	CC	CC	CC	CC
RP165	45	GG	CC	TT	AA	GG	GG	AA	GG	TT	CC	TT	CC
RP174	45	AA	CC	CC	GG	AA	AA	GG	AA	CC	CC	CC	CC
RP138	50	AA	CC	CC	GG	AA	AA	GG	AA	CC	TT	CC	CC
RP226	50	GG	TT	TT	AA	GG	GG	AA	GG	TT	TT	TT	TT
RP189	52.5	GG	CC	TT	AA	GG	GG	AA	GG	TT	TT	TT	TT
RP144	60	GG	TT	CC	GG	AA	AA	GG	AA	CC	CC	CC	TT

Fig. 4. Allelic distribution of QTL *EWYY7B.1* across twelve snps against stripe rust. A quantitative trait locus (QTL) labelled *EWYY7B.1* across twelve significant SNPs associated with *Pst* resistance in top 10 genotype and top susceptible genotype to stripe rust. Green Cells: Represent resistance genotypes. Red Cells: Represent susceptible genotypes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

variation, accounting for 7.7 % of the total. Consequently, *EWYY7B.1* was selected to show allelic distribution among the top ten resistant and the top ten susceptible genotypes. The figure illustrates distinct allele frequency patterns, with resistant genotypes predominantly exhibiting resistant alleles (GG, TT, AA), while susceptible genotypes primarily display susceptibility alleles (AA, CC, TT) across the selected SNPs within *EWYY7B.1*(Fig. 4).

4.4. Comparing with previously identified QTLs

The majority of the currently identified QTLs were in close proximity to the previously reported QTLs associated with *Pst* resistance. Three of the ten identified QTLs (*EWYY1B.1, EWYY2B.1,* and *EWYY6B.2*) were co-located in previously discovered genomic regions with *Pst* resistance. Specifically, the QTLs *EWYY1B.1* and *EWYY1B.2 are* co-located with the locus *QYr.cau1BS_AQ2478853*, reported by (Quan et al., 2013) at the short arm of chromosome 1B The other *EWYY2B.1* QTL positioned at 40.6 to 42.7 cM is nearby the previously reported QTL *QYr.ucw2-B_UC1110* (Lowe et al., 2011) located on 39.8 to 49.1 cM.

4.5. Novel QTLs identified on the current study

This study identified three potentially novel QTLs associated with *Pst* resistance in wheat through a comparative analysis of prior research on *Pst* resistance genes/QTLs. These potentially newly identified QTLs include *EWYY5 A.2* on the short arm of chromosome 5 A, *EWYY6B.1* on the long arm of chromosome 6B, and *EWYY7B.3* on the short arm of chromosome 7B (Table 2).

The QTL *EWYY5 A.2,* comprising three SNPs (*Tdurum_contig50175_875, BS00041063_51, and Tdurum_contig10843_745*), is located on chromosome 5 A at 93.23 cM accounting 6.1 % of the overall phenotypic variation (Table 2). So far, the short arm of chromosome 5 A has only been catalogued with a single *Pst* resistance gene *Yr34* (synonym *Yr48*) located within a distal region (Qureshi et al., 2018). Given the substantial distance between these genes and *EWYY5A.2* (62.7 cM), it is highly likely that this QTL represents a new and previously undiscovered association with *Pst* resistance. The *EWYY6B.1* QTL is located on the long arm of chromosome 6B spanning from 64.0 cM to 71.76 cM. Within this region, *Yr36* (Uauy et al., 2005) is found at positions ranging from 0 to 25.1 cM. Considering the distance between the gene and our identified QTL, it is highly possible that *EWYY6B.1* could potentially a novel genetic resource. Similarly, no *Pst* resistance genes/QTL was identified so far nearby to EWYY7B.3 which is on chromosome arm 7BS at 89.64 cM.

5. Conclusions

In this study, we identified several known genes and QTLs associated with resistance to wheat stripe rust disease and discovered three potentially novel genomic regions linked to this resistance. Given the significant global impact of wheat stripe rust outbreaks in recent years, integrating these resistance genes and QTLs into elite breeding lines is a critical strategy to combat this disease effectively. These findings offer valuable insights for wheat breeders, providing a robust foundation for implementing marker-assisted selection to develop durable stripe rust-resistant wheat cultivars. To fully realize the potential of these discoveries, further research is recommended to validate the novel QTLs, ensuring their effective application in breeding programs.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The SNP dataset used in the current study is available in table S3.

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Authors contribution

ES, LM, and WT conceived and designed the study. WT designed the study and provided germplasms and the genotypic data. LM guided the project development. ES wrote the draft manuscript. ES and AA performed data analysis. LM and AA edited the manuscript. All authors read and approved of the final manuscript.

CRediT authorship contribution statement

Elias Shewabez: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laura Mugnai:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Wuletaw Tadesse:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Admas Alemu:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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