







IDENTIFICATION OF QTL CONFERRING ADULT PLANT RESISTANCE TO WHEAT STRIPE RUST IN BREAD WHEAT LANDRACE BWLR-2347

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ABSTRACT

Stripe rust is among the most destructive foliar diseases affecting wheat globally. The identification of novel adult plant resistance loci represents a critical strategy for mitigating the substantial yield losses attributed to stripe rust across diverse regions worldwide. Wheat landraces possess untapped genetic resources for abiotic and biotic stresses including diseases like stripe rust. This study was conducted to identify the genetic basis of adult plant resistance response in bread wheat landrace BWLR-2347 conserved at ICARDA gene bank. The mapping population of 178 F₂ individuals was genotyped with high quality genotype-by-sequencing derived SNPs. The phenotypic disease assessment was carried out in F_{2:3} families derived from the cross between resistant bread wheat landrace BWLR-2347 and susceptible Avocet “S” in the field under artificial inoculation with a mixture of stripe rust races. Seven QTLs were identified for resistance to YR at the adult plant growth stage and mapped on five chromosomes. The QTLs were detected on the 1B, 2A, 2B, 2D, and 5A regions. The phenotypic variance explained by an individual QTL ranged from 2.01% to 5.59%. The study validated the six previously identified QTL and reported a novel QTL at chromosome 5A. The information obtained from the study will be helpful in wheat breeding programs towards the development of stripe rust resistant cultivars.

Keywords: DArT, QTL mapping, Stripe rust, Türkiye, Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) remains a crucial global food source, providing approximately 20% of human dietary calories and protein (Miner et al., 2022). Recent estimates suggest that wheat production must increase by 69 million ha by 2050 to meet growing food security demands (Bahar et al., 2020). This fundamental crop faces numerous climatic stresses, but rusts are the most damaging wheat disease worldwide. Wheat stripe rust, also known as yellow rust (Yr), is caused by the pathogen *Puccinia striiformis* f.sp. *tritici* (*Pst*) is the most devastating foliar disease for global wheat production (Wellings, 2011). Stripe rust mostly spread in cooler and moisture environments (0°C to 23°C) that cause global yield loss up to 1% (Savary et al., 2019) with a damage loss of more than 1 billion US dollars annually (Chen et al., 2021). Out of total wheat growing area of Turkey 25% mainly affect with stripe rust with 1-5% crop loss and epidemic occurs in 2 out of 5 year (Chen, 2020). This pathogen overcomes its resistance by producing new races through genetic

variation and it also spreads over distance through air that cause the epidemic in relatively short periods. The most effective strategy to control this disease is to develop disease-resistant varieties, which is an environmentally friendly and economical approach as it limits the use of fungicides (Chen and Kang, 2017; Akcura et al., 2017).

Two major types of resistance prominently characterized to control the spread of *Yr*: all-stage resistance (race specific or overall resistance) and adult plant stage resistance (race non-specific or partial resistance) (Chen, 2005; Habib et al., 2020a). All-stage resistance is usually controlled by a single gene and can be detected at the seedling stage and is expressed at all developmental stages of plant, but it readily overcome due to emergence of new races and that cultivars become susceptible. Adult plant stage also known as high temperature adult plant (HTAP) resistance is durable as controlled by minor and effective multiple loci that expression depends upon growth stages of plant development and temperature (Chen, 2013; Zhou et al.,

2014). Therefore, the best approach is to combine the durable HTAP with the all-stage resistance through gene pyramiding that utilize the molecular marker to map the desired gene in the germplasm and its selection through marker assisted selection. Quantitative trait loci (QTL), genomic regions containing genes that influence quantitatively inherited traits, have become powerful tools in understanding complex disease resistance mechanisms in wheat. Modern genotyping approaches such as Diversity Arrays Technology (DArT), a microarray-based technique for DNA polymorphism analysis, and genotype-by-sequencing (GBS), a next-generation sequencing method for discovering genetic variants, have revolutionized our ability to identify and map these resistance loci. To date almost 80 stripe rust resistance genes and >300 QTLs have been reported in wheat (Li et al., 2018; Chen, 2020; Mourad et al., 2021). Recently, simple sequence repeats (SSR), DArT and single nucleotide polymorphism (SNP) markers have been widely used to estimate the genetic diversity and to map the QTLs in the wheat genome (Habib et al., 2020a; Hussain et al., 2022; Kocak et al., 2022). DArT markers have been widely used for QTL mapping in wheat against different traits due to its economical convenience and high genome coverage (Jighly et al., 2015; Ahmed et al., 2021).

For effective utilization of genetic resource in breeding strategy, the extent of variation and correlation is prerequisite (Habib et al., 2020b) that can be depicts at genomic level using molecular markers. More genes need to be identified with major effect that show durability of resistance at HTAP (Zhou et al., 2014). The objective of this study is to characterize and map the QTLs/genes conferring the durable resistance to HTAP resistance to stripe rust in landrace BWLR 2347.

MATERIALS AND METHODS

Plant material

Seeds of the resistant bread wheat landrace parent BWLR 2347 (6571-landrace) were selected from the ICARDA gene bank based on its performance for adult plant resistance to stripe rust at Regional Cereal Rust Research Centre (RCRRC), in Izmir, Turkey. The landrace was crossed with stripe rust universal susceptible cultivar Avocet S. The mapping population was developed from cross of single plants of the susceptible 'Avocet S' (AvS) variety as the male parent and the resistant wheat landrace 'BWLR 2347' as the female parent. Individual F₁ plants were selfed to produce the F₂ segregation population. The F₂ mapping population, consisting of 240 individual plants, was used for genetic map construction and phenotypic evaluation. F₂ derived F₃ (F_{2:3}) were used for adult plant phenotypic assessment of stripe rust.

DNA extraction and genotyping

Genomic DNA was extracted from fresh leaves collected from individual F₂ plants at 10-day old seedlings stage using a modified CTAB (cetyltrimethylammonium bromide) method (Hoisington et al., 1994). The seedling leaves were collected in labeled eppendorf tubes and stored in an Ultra freezer at -80°C for subsequent DNA extraction.

Leaf samples were ground using a tissue lyser (Tissue Lyser II from QIAGEN) until a fine powder was obtained. 0.1g of the powdered leaf samples were used for DNA extraction using the CTAB method (Doyle and Doyle, 1990). The extracted DNA was dissolved in 100µl tris-EDTA (TE) buffer. The samples were analyzed on 1% agarose gel for the purity test and quantified with a spectrophotometer (NanoDrop ND1000). The DNA samples were stored at -80°C.

From the initial 240 F₂ plants, 178 lines were successfully genotyped and used for mapping. The reduction in population size was due to several factors: (i) DNA quality requirements for GBS analysis eliminated 35 samples, (ii) 15 plants did not survive to produce adequate tissue for DNA extraction, and (iii) 12 samples failed quality control during the genotyping process. The final population size of 178 individuals provides sufficient statistical power for preliminary QTL detection.

The extracted DNA samples of the F₂ individual plants and two parental lines were sent to Diversity Arrays Technology Pty Ltd. (Canberra, Australia, <http://www.DiversityArrays.com/>) for genotyping using the genotype by sequencing (GBS) method. The genotypic data obtained for 178 lines including the parents were filtered for markers with >10% missing data and with <0.1% minor allele frequency. The results obtained from 1115 polymorphic SNP markers were used to construct the genetic linkage map.

Phenotypic evaluation of adult plant resistance

The field experiments were carried out at the RCRRC during cropping season 2020. Fifty seeds from each F_{2:3} accessions were planted in a 1-meter row with 30 cm spacing between the rows. To ensure sufficient inoculum production for disease infection, a mixture of the universally susceptible varieties 'Morocco', 'Seri 82', and 'Avocet S' along with the locally susceptible varieties 'Bolani', 'Basribey' (also derived from the CIMMYT cross 'Kauz'), and 'Cumhuriyet 75', 'Kunduru', 'Kasibey', and 'Gonen' was planted as spreader after every 20 rows, as well as the spreader rows bordering the nurseries. The experiments were managed as per the standard local agronomic practices during the crop season.

PstS2 and *Warrior (PstS7)* pathotypes collected from previous years and preserved at RCRRC were multiplied using susceptible variety Avocet S. Freshly collected urediniospores were used for field inoculations. The F_{2:3} accessions along with the spreader rows bordering the experiments were artificially sprayed with a mixture of the two races in talcum powder using a backpack sprayer at seedling, tillering, and booting stages. The field was irrigated through a mist irrigation system.

Field scoring started when disease severity reached 100% on the susceptible checks, 'Morocco' and 'Avocet S'. Because of conducive environmental conditions during January- February, the onset of the stripe rust (under artificial inoculation) usually starts at early February and reach full disease severity in susceptible genotypes by mid-

February, when the plants are generally at tillering stage. This is a unique condition for the evaluation of resistance in wheat germplasms at the regional rust phenotyping platform in Izmir. Adult-plant responses were recorded three times at 10-day intervals for the major infection types Resistant (R), Moderately resistant (MR), Moderately Susceptible (MS), and Susceptible (S) (Roelfs et al., 1992), and the disease severities (0-100%) following the Modified Cobb's Scale (Peterson et al., 1948). All three recordings were averaged and the Coefficients of Infection (CI) were calculated for infection types and disease severities following Saari and Wilcoxson (1974).

Map construction and QTL mapping

The QTL mapping strategy utilizing F₂ genotype and F_{2:3} phenotype data follows established procedures in genetic mapping of disease resistance (Xu et al., 2017). This approach combines the advantage of precise genotyping at the F₂ generation with reliable phenotypic evaluation using F_{2:3} families. The use of F_{2:3} families allows multiple plants per line to be evaluated, reducing environmental variance and providing more accurate phenotypic data compared to single F₂ plants. The phenotypic means of F_{2:3} families were used as trait values for their corresponding F₂ plants in QTL analysis. The composite interval mapping (CIM) method with the Kosambi mapping function was used for the detection of QTLs by Windows IciMapping v4.1. The threshold value for the logarithm of odds (LOD) score was determined through permutation testing (1000 iterations) at an experiment-wise error rate of 0.05, resulting in a population-specific threshold of 2.5. This empirically derived threshold accounts for multiple testing and

provides more accurate control of false positives compared to arbitrary universal thresholds (Churchill and Doerge, 1994). Genetic maps with QTLs were drawn using MapChart v.2.32 software. For the markers with the same positions, only one single nucleotide polymorphism (SNP) maker was selected for the map.

RESULTS

Inheritance of YR resistance

Phenotypic data indicating the genetic variance to adult plant response in the BWLR 2347/AvS population varied from resistant to susceptible (CI= 0-9) in a field study at RCRRRC-Izmir, Turkey. All the F₁ plants of BWLR 2347 were resistant to rust when tested at field conditions (CI=0) whereas all plants AvS were susceptible to stripe rust infection (CI=9). The 11 F₁ plants from BWLR 2347/AvS were resistant producing mild uredinia (CI=3-4). That suggest the resistance gene in BWLR was partially dominant (Table 1). The 240 F₂ population was divided into 197 resistant (RR: Rr) plants and 43 susceptible (rr) which fit a segregation ratio of 3R:1S ($\chi^2= 6.4$; P=0.011). The segregating 178 lines of F₂ derived F₃ population (F_{2:3}) the 70 plants were homozygous resistant (RR) (0-3) whereas 81 plants originating from F₂ plants were heterozygous representing the segregating alleles (Rr) (CI=4-6) and 27 plants were homozygous susceptible (rr) (CI=7-9). The segregating F_{2:3} lines fitted the expected ratio of 1 resistant:2 segregating:1 susceptible ratio with chi-square value $\chi^2= 22.2$, P=0.00001 (Table 1). The analysis entails the presence of 1 dominant stripe rust resistance genes in the BWLR 2347 landrace.

Table 1. Phenotypic segregation ratios and chi-square analysis of BWLR 2347 x AvS parental cross and F_{2:3} hybrids for adult plant resistance to stripe rust.

Parents and Populations	Observed number of plants or lines ^(a)			Expected ratio ^(b)			
	Resistance	Segregation	Susceptible	Total	Res:Seg:Sus	χ^2 ^(c)	P-value
BWLR 2347	All		0				
Avocet S	0		All				
F ₁	11			11			
F ₂	197		43	240	3:1	6.4	0.011
F _{2:3}	70	81	27	178	1:2:1	22.2	0.00001

^(a) The F₂ ratios are for resistant (CI= 0-4) and susceptible (CI=7-9) plants.

^(b) The F_{2:3} ratios are for homozygous Resistant, Segregating, and homozygous Susceptible lines.

^(c) χ^2 0.05=3.84

Identification of QTL for adult plant resistance to stripe rust in the F_{2:3} population

Phenotypic data of stripe rust along with the genotypic data of 1115 SNP markers were subjected to one linkage group using software QTL IciMapping V4.1. One QTL *QYr.RCRRRC.2D-1* was mapped on chromosome 2D by composite interval mapping (CIM) at 79 cM position flanked with SNP227-1318441 and SNP 220-1209547. This QTL explained 5.59% phenotypic variance with an LOD score of 7.98 (Table 2, Figure 1). A total of 7 QTLs were identified for resistance to YR at the adult plant growth stage using Interval mapping (IM) method. These

seven QTLs were mapped on five chromosomes 1B, 2A, 2B, 2D, and 5A. One QTL *QYr.RCRRRC.1B-1* is present at chromosome 1B flanked with SNP95-982151 and SNP93-306479 explaining the 2.67% phenotypic variance with LOD value of 3.57 (Table 2, Figure 1). Three QTLs were detected at chromosome 2A explaining the phenotypic variance as 3.61%, 4.75% and 5.03%, respectively (Table 2). At chromosome 2B, one QTL *QYr.RCRRRC.2B-1* present at 934 cM position flanked by SNP178-1316732 and SNP171-3948116. This QTL elucidate 4.17% phenotypic variance with 4.97 LOD score. Highest phenotypic variance explained by QTL *QYr.RCRRRC.2D-1*

present at 2D chromosome i.e 5.59% the same QTL was identified through both mapping procedure IM and CIM. One QTL QYr.RCRRC.5A-1 mapped on chromosome 5A with LOD score 2.68 and phenotypic variance of 2.01%. The QTL was flanked with SNP611-11204643 and SNP610-1084102. The QTL that explained phenotypic

variance of more than 10% is generally considered as major QTL therefore in the study all the QTLs detected were minor QTLs. The phenotypic variance explained by an individual QTL ranged from 2.01% to 5.59%. and the LOD scores of identified QTLs for YR were in the range of 2.68 to 7.98 (Figure 2).

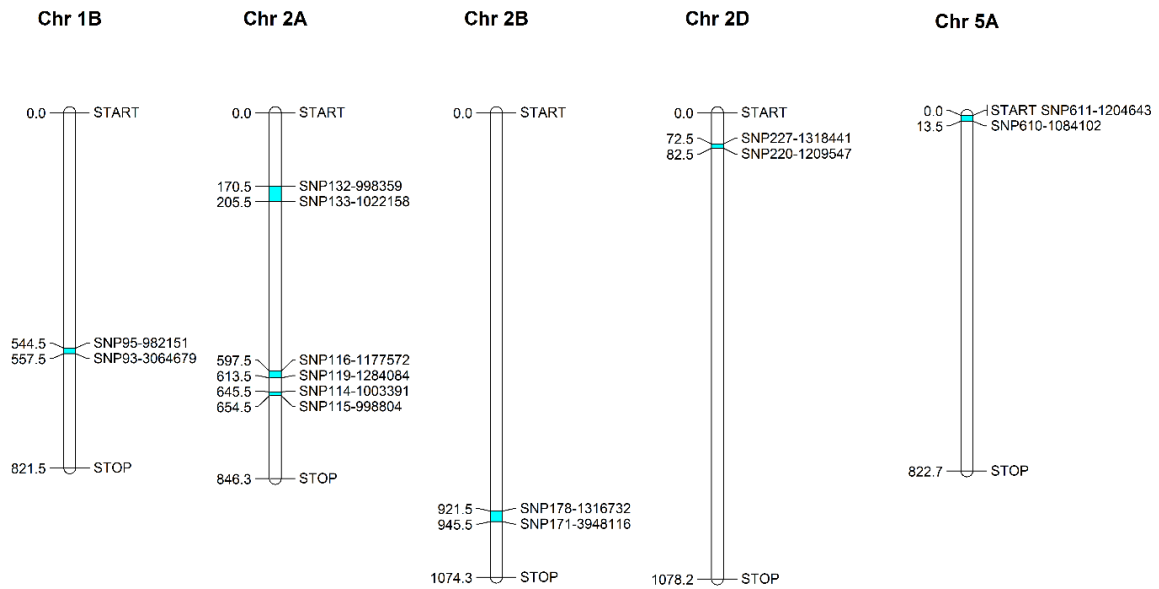


Figure 1. Genetic linkage map showing positions of QTLs conferring adult plant resistance to stripe rust in the $F_{2:3}$ population derived from BWLR 2347/AvS. Chromosome numbers are indicated at the top of each linkage group. Genetic distances (cM) are shown on the left side of each chromosome. The flanking markers are shown on the right side of each chromosome.

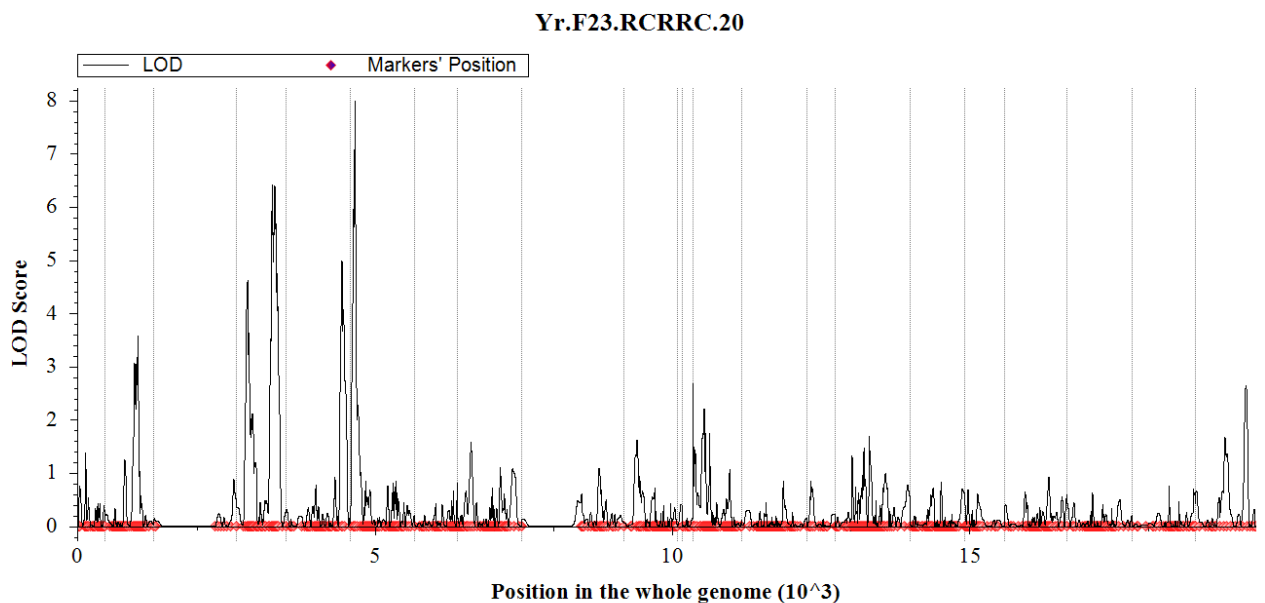


Figure 2. Genome-wide scan of $F_{2:3}$ population with LOD scores on y-axis and genome size on x-axis every single vertical line interval represents a chromosome.

Table 2. Quantitative trait loci for disease resistance to stripe rust at adult plant stage.

QTL Name	Chr	Position	Left Marker	Right Marker	LOD	PVE (%)	Add.	Left CI	Right CI	QTL/gene	Reference
<i>QYr.RCRRC.1B-1</i>	1B	549	SNP95-982151	SNP93-3064679	3.5755	2.671	-0.5912	544.5	557.5	Yr29/Lr46	William et al., (2003)
<i>QYr.RCRRC.2A-1</i>	2A	193	SNP132-998359	SNP133-1022158	4.6107	3.6132	-0.6915	170.5	205.5	Yr17	Helguera et al., (2003)
<i>QYr.RCRRC.2A-2</i>	2A	651	SNP114-1003391	SNP115-998804	6.4022	4.7497	-0.7975	645.5	654.5	Yr32	Eriksen et al., (2004)
<i>QYr.RCRRC.2A-3</i>	2A	606	SNP116-1177572	SNP119-1284084	6.4128	5.0361	-0.8122	597.5	613.5	QYr.ucw-2AS_PI610750	Lowe et al., (2011)
<i>QYr.RCRRC.2B-1</i>	2B	934	SNP178-1316732	SNP171-3948116	4.9792	4.19	-0.743	921.5	945.5	QYr-2B_Attila, QYrlu.cau-2BS1_Luke	Rosewarne et al., (2008); Guo et al., (2008)
<i>QYr.RCRRC.2D-1</i>	2D	79	SNP227-1318441	SNP220-1209547	7.9803	5.5949	-0.8562	72.5	82.5	QYr.caas-2DS_Libellula, QYr.wpg-2D.1 (IWA1939)	Lu et al., (2009); Naruoka et al., (2015)
<i>QYr.RCRRC.2D-1</i>	5A	0	SNP611-1204643	SNP610-1084102	2.6854	2.015	-0.5181	0	13.5	<i>Novel</i>	

CIM: Composite interval mapping; LOD: Logarithm of odds score; PVE: Percentage of phenotypic variance explained by individual QTL; Add: Additive effect of resistance allele; CI: Confidence interval

DISCUSSION

The frequent outbreaks of stripe rust epidemics in many countries seriously threaten wheat production and threaten food security. Growing disease resistance wheat cultivars, is the most feasible method to abate the stripe rust disease that developed from gene pyramiding and marker assisted selection (Mourad et al., 2021). In present study, we characterized the stripe rust resistance in wheat landrace BWLR 2347 as adult plant resistance source and mapped seven QTLs covering the five genomic regions. The integrated map constructed by Bulli et al., (2016) was used to compare the significant SNPs detected in the study with previously published *Yr* genes and QTL. From all the identified seven QTLs, the four were in the same genomic regions as previously described for *Yr* resistance genes. Whereas three identified QTLs were found in proximity of previously reported YR resistance QTLs.

Chromosome 1B

A minor QTL *QYr.RCRRC.1B-1* (SNP95-982151) was identified in association with adult plant resistance to stripe rust on chromosome 1B. The region overlapped the previously reported *Yr29* gene (William et al., 2003). This moderate adult plant resistance gene is linked with another leaf rust resistance gene *Lr46* which is highly effective for LR resistance. The presence of *Yr29* gene in the same set of landraces has been reported previously by Tehseen et al. (2021). Therefore, it is likely that SNP95-982151 is tagging *Yr29* gene.

Chromosome 2A

Three QTLs *QYr.RCRRC.2A-1* (SNP132-998359), *QYr.RCRRC.2A-1* (SNP114-1003391), and *QYr.RCRRC.2A-3* (SNP116-1177572) were identified on chromosome 2A, which carries several *Yr* resistant genes. (Helguera et al., 2003; Eriksen et al., 2004; McIntosh et al., 2017). The stripe rust resistant gene *Yr17* lies within the confidence interval of SNP132-998359. *Yr17* resistance gene is linked with *Lr37* and *Sr38* a leaf rust and stem rust resistance respectively. Although rust pathogens with virulence to *Yr17* has been reported in many parts of the world, the combination of these resistance genes has proven effective against a wide range of cereal rust races (Kolmer et al., 2009). SNP114-1003391 and SNP116-1177572 were detected in close proximity with a *Yr* resistance gene *Yr32* and a QTL *QYr.ucw-2AS_PI610750*. The resistance gene *Yr32* confers resistance to stripe rust at seedling and all stage resistance and is also within the proximity of several other stripe rust QTLs (Boukhatem et al., 2002; Eriksen et al., 2004; Mallard et al., 2005; Bansal et al., 2014). Since SNP114-1003391 lies in the same vicinity as *Yr32* and other QTL it is accepted that the resistance conferred by SNP114-1003391 is due to *Yr32* gene. A previously reported QTL *QYr.ucw-2AS_PI610750* and SNP116-1177572 overlap and, therefore are considered same both confer resistance to stripe rust at adult plant stage.

Chromosome 2B

The QTL region identified in the study on chromosome 2B was in the proximity of previously reported QTLs on

the long arm of chromosome with moderate resistance and conferring slow rusting QTL (Guo et al., 2008; Rosewarne et al., 2008). The QTL *QYr.lu.cau-2BS1_Luke* was identified to confer high temperature adult plant stage since our experiment did not undergo any temperature treatment therefore further studies are necessary to confirm the relationship however, based on genetic map distances and phenotypic evaluation the region detected in the study is the same as previously reported.

Chromosome 2D

The *QYr.RCRRC.2D-1* region on chromosome 2D was detected in both CIM and IM methods. This region overlaps with the previously reported QTL regions for adult plant resistance to stripe rust (Lu et al., 2009; Naruoka et al., 2015). The earlier reported QTLs were detected in an F₃ mapping population derived from Italian common wheat cultivars Libellula and Strampelli and a diversity panel of 402 winter wheat accessions. The QTL detected in the current study explained 7.98% of the phenotypic variance, similar to the previously reported QTLs. Furthermore, all these QTLs showed minor effect for resistance; therefore, it is suggested that this region on chromosome 2D exhibits adult plant resistance to stripe rust. When accompanied by other minor QTLs, it could prove to be a good source of horizontal resistance against stripe rust.

Chromosome 5A

An adult plant stripe rust resistance loci *IWA4767_APR* at the long arm of chromosome 5A has been reported Zegeye et al. (2014), however, the *IWA4767_APR* was identified at 113cm which is far away from the QTL detected in the current study at 0-13.5cm. Both QTL conferred stripe rust resistance at the adult plant stage and were detected in hexaploid wheat landraces however the distance between the two QTLs is more than 100cm. Therefore, both QTLs were suggested different. There was no other previously reported in this region thus making it a novel QTL for adult plant stripe rust resistance.

While segregation analysis in the F_{2:3} population suggested a qualitative inheritance pattern controlled by a single dominant gene (Table 1), our QTL mapping revealed a more complex quantitative inheritance involving multiple minor-effect loci. This apparent discrepancy between inheritance patterns deserves careful consideration. The phenotypic segregation suggesting single-gene inheritance may reflect a complex genetic architecture where multiple linked QTLs segregate together, creating the appearance of simpler inheritance patterns (Periyannan et al., 2013). The resolution limitations of our mapping population may have prevented the separation of closely linked loci that could collectively behave as a single primary effect locus (Kertho et al., 2015; Ellis et al., 2014).

The nature of adult plant resistance itself may contribute to this complexity. Adult plant resistance typically shows intricate gene-by-environment interactions, where field conditions during phenotyping can influence the expression of resistance genes differently than controlled conditions (Boyd et al., 2013). This environmental interaction could

potentially mask or enhance certain QTL effects, leading to detecting multiple minor QTLs rather than a single major locus. Furthermore, the detected QTLs may participate in epistatic interactions that create threshold effects in resistance expression, resulting in more discrete phenotypic categories than typically expected for quantitative inheritance (Krattinger et al., 2016; Moore et al., 2015). Similar phenomena have been reported in other wheat disease resistance studies (Niks et al., 2015; Santra et al., 2008).

Our findings align with the growing understanding that durable adult plant resistance often results from the cumulative action of multiple partial resistance genes rather than single major effect loci (Figueroa et al., 2018; Klymiuk et al., 2018).

Identifying multiple minor-effect QTLs in BWLR-2347 has essential implications for wheat breeding programs. While individual QTLs explain relatively small proportions of phenotypic variance (2.01-5.59%), their combined effect could provide durable resistance through QTL pyramiding strategies (Rosewarne et al., 2013). These QTLs can be incorporated into elite breeding lines using marker-assisted selection, particularly utilizing the SNP markers flanking each QTL region identified in this study. The novel QTL on chromosome 5A (*QYr.RCRR.C.5A-1*) represents a previously unreported source of resistance that could complement existing resistance genes. The co-location of several identified QTLs with previously reported *Yr* genes suggests the potential for developing multi-line resistance strategies.

CONCLUSIONS

This study used the wheat stripe rust resistance source BWLR 2347 to map the QTLs using GBS. Six QTLs were identified on 1B, 2A, 2B and 2D that are validated with previously identified QTLs and *Yr* genes. The SNP identified on the chromosome 5A was recognized as a novel QTL from the study and can be exploited in wheat breeding programs toward the development of stripe rust resistant cultivars. The newly identified and the previously validated QTLs will be a valuable source of resistance to adult plant stripe rust resistance that can be used for gene pyramiding to enhance the resistant source in the wheat cultivars.

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