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Molecular mapping of QTLs for stripe rust resistance in bread wheat (*Triticum aestivum* L.)

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Abstract

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, poses a major threat to global wheat production, adversely impacting grain yield, quality, and nutritional value. Identifying quantitative trait loci (QTLs) associated with resistance is essential for wheat breeders aiming to develop rust-resistant varieties through molecular breeding strategies. To unravel the genetic basis of resistance, a mapping population of 212 recombinant inbred lines (F_8), derived from a cross between resistant parent (W8627) and the susceptible parent (PBW343), was evaluated against the most prevalent and virulent pathotypes at both the seedling and adult plant stages over two consecutive years (2021–22 and 2022–23). The area under the disease progression curve (AUDPC) and analysis of variance revealed high variability among the RILs across both years. For QTL mapping, genotyping was conducted using a comprehensive approach that combined a 3.9 K Diversity Arrays Technology (DArT) array with SSR markers. This effort resulted in the identification of 602 DArT markers and 23 SSR markers, which were used to construct a robust integrated linkage map comprising 21 linkage groups which led to the identification of two seedling resistance QTLs, *QYr.iwbr.3BS.1* (3B) and *QYr.iwbr.6DS.1* (6D), as well as two consistent QTLs; *QYr.iwbr.2BL.1* (2B) showing the phenotypic variance of 9.67% with a LOD score 4.51 and *QYr.iwbr.6DS.2* (6D) explaining 7.33% phenotypic variance with LOD score 3.61 for adult plant resistance. Further, in silico analysis revealed putative candidate genes, including those encoding Inositol transporter-1 and protein transport protein sec16, which hold promise for future functional validation and deployment in rust resistance breeding.

Keywords *Triticum aestivum*, Stripe rust, QTL, DArT markers, Linkage map, Seedling and adult plant resistance

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Introduction

Wheat is a staple crop that feeds billions of people worldwide, making its production critical for global food security. However, wheat production faces significant threats from various diseases, with stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, being one of the most devastating. This disease not only reduces grain yield but also affects the quality and nutritional value of the wheat, posing a serious challenge to farmers. Wheat stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) has been explicitly known for inducing substantial loss in wheat cultivars throughout the world. Yellow rust (YR) infection notably affects wheat leaves, interrupting photosynthesis, which results in reduced light absorption and radiation use efficiency, ultimately leading to decreased yields. Moreover, it can also affect the glumes, lemma, and palea of the wheat ear, especially during moderate to severe epidemics, resulting in significant loss in crop yield and quality and hence threatening food security [9, 65].

YR is highly prevalent in India, particularly affecting the sub-mountainous regions of Punjab, Haryana, and Western Uttar Pradesh, which are important wheat-growing areas in the country. Significant outbreaks of stripe rust were observed during 2006 and 2012–2013, resulting in considerable crop losses [43, 46, 51]. PBW343 gained prominence as a major wheat variety due to its ability to thrive in diverse environments, leading to its widespread cultivation for more than a decade. The highly virulent pathotype '78S84' against gene *Yr27* rendered PBW343 susceptible to yellow rust [46, 55]. The *Yr15* was previously recognised as a key gene conferring resistance to yellow rust [54]. However, recent findings from the Borlaug Global Rust Institute (BGRI) indicate that this resistance has been compromised particularly across Northern and Central Europe [6]. The main cause of stripe rust epidemics is the occurrence of several virulent pathotypes. New pathotypes continue to emerge in nature due to their ability to mutate and sexually recombine. To date, among the 140 pathotypes recognized globally, India has documented more than 28 pathotypes of stripe rust [5, 45].

Although fungicides and appropriate agronomical practices aid in controlling rust infection, genetic resistance is still one of the best and environmentally safe means for minimizing losses [66]. Resistance to cereal rusts is classified into two types: Seedling resistance also referred to as all stage resistance (ASR), counters one or few *Pst* races. Seedling resistance genes are expressed at seedling as well as adult plant stages and are characterized by a strong to moderate immune response. Adult plant resistance (APR) or quantitative resistance combats more than one race of pathogens is very well documented. Due to its polygenic nature, APR provides durable resistance,

with each gene having a partial impact. APR genes slow down the life cycle of pathogen sporulation and loss of genetic diversity [19]. To date, there are 87 resistance genes have been discovered of which few are effective at the adult plant stage [36, 57, 70]. Therefore, there is increasing emphasis on discovering and characterizing novel genes, particularly APR genes, in breeding endeavors. A few APR genes governing resistance to yellow rust such as *Yr18* [7, 27], *Yr36* [12] and *Yr46* [38] have been cloned. The extensive use of the *Yr18* with two to three additional slow-rusting genes was carried out in the CIMMYT wheat breeding program which has provided durable resistance against yellow rust [9, 58].

QTL mapping for the identification of genomic regions associated with stripe rust resistance becomes critical. Along with the thorough quantitative trait loci (QTL) research, marker systems have advanced quickly, with older maps being characterized using restriction fragment length polymorphisms. Simple sequence repeats (SSR) are frequently used to link maps with various marker types and to create consensus among various maps to assess genetic diversity within populations because they have the advantages of co-dominance, locus specificity, and high polymorphism [56]. Single nucleotide polymorphisms (SNPs) are being utilized for genotyping due to their high genome coverage. However, DArT marker systems offer a robust and versatile toolset for genetic analysis and breeding applications due to their comprehensive genome coverage, cost-effectiveness, and high throughput capabilities [22, 52]. More than 440 *Pst* resistance QTLs on 21 wheat chromosomes have been discovered and summarized but only a few of them are effective and hence a little utility in gene deployment ([29, 49, 60]).

The present study was designed with the objective i) to identify the QTLs conferring seedling and adult plant resistance to stripe rust against prevalent pathotypes in India, using recombinant inbred lines (RILs) derived from W8627 x PBW343, and ii) to look deeper into the method of identifying potential candidate genes to assist in further validation and gene cloning experiments.

Material and methods

Plant material

The plant material consisted of a mapping population of 212 F₈ RILs developed via single seed descent from a cross between W8627 (resistant parent) and PBW343 (susceptible parent) were used for phenotypic and genotypic analysis. W8627 is a multiple disease-resistant wheat genetic stock that possesses resistance against stripe rust [62]. While PBW343 was developed from Attila sib (Nord Desprez/VG9144//Kalyansona/Bluebird/3/Yaco/4/Veery#5) at CIMMYT, Mexico. It was released for general cultivation under timely sown

irrigated conditions in the entire North Western Plain Zone (NWPZ), including Punjab, in 1995. Both parents differed in terms of yield traits and disease resistance. The RILs along with parents were planted in randomized block design with two replications during two crop seasons 2021–22 and 2022–23 at ICAR- IIWBR, Karnal. Susceptible check wheat variety "Agra Local" was sown after every 20 rows of the test material in order to determine the homogeneity of infection across the field. Infector rows were planted on the periphery of the experimental material which consisted of a mixture of susceptible varieties: Agra Local, Kharchia, WL711 and C306.

Seedling reaction test (SRT)

Wheat plants were raised in aluminium bread pan/trays with a sterilized mixture of fine loam and farmyard manure in a 3:1 ratio. Each tray, 29 × 12 × 7 cm, contained 10 rows of seedlings including a susceptible control variety (Agra Local). The greenhouse temperature was maintained at 22 ± 2 °C with optimum conditions for normal growth of seedlings. The seedlings were inoculated at single leaf stage (12–14 days) with uredospores of the four most virulent pathotypes 110S119, 238S119, 110S84 and 78S84, by spraying the inoculum suspended in a non-phytotoxic paraffinic oil (Soltrol 170). Subsequently, the seedlings were moved to a greenhouse where they were dusted with elemental sulphur powder of Sulfur to prevent powdery mildew infection. Greenhouse conditions were carefully regulated, maintaining a photoperiod of 16 h of light and 8 h of darkness, with temperature kept at 15 ± 1 °C [45], to encourage healthy plant growth and optimize stripe rust infection. The stripe rust resistance was assessed by scoring the host response for seedling infection 14 days after inoculation using 0–9 disease rating scale [37], wherein (i) 0 = immune = no uredinia or other macroscopic sign of infection, (ii) 0_n = nearly immune = no uredinia, but hypersensitive necrotic or chlorotic flecks present, (iii) 1 = highly resistant = small uredinia surrounded by necrosis, (iv) 2 = moderately resistant = small to medium uredinia surrounded by chlorosis or necrosis, green island may be surrounded by chlorotic or necrotic border, (v) 3 = moderately susceptible = medium-sized uredinia that may be associated with chlorosis, (vi) 3+, 4 = susceptible = large uredinia without chlorosis). Infection types 0–3 were classified as resistant, 4–5 as moderately resistant, 6–7 as moderately susceptible and 8–9 as susceptible. for QTL analysis.

Adult plant resistance (APR)

The RIL population was also evaluated for APR to stripe rust using a mixture of pathotypes during the years 2021–22 and 2022–23. The inoculum was procured from ICAR- IIWBR Regional Station, Flowerdale, Shimla. The inoculum was prepared with spores and talc in a ratio of

1:100 by suspending it in an aqueous solution comprising a few drops of Tween-20. The inoculum of uredospore of *Pst* was sprayed at regular intervals to induce the stripe rust epidemics in the field. Starting at the end of December and continuing every other day until stripe rust developed on the vulnerable checks, the spray inoculations were applied in the evening using an ultralow volume sprayer. The stripe rust was tracked weekly beginning in the last week of January and ending in the first week of March following the modified Cobb's Scale [42]. The stripe rust resistance was assessed by grading host responses as: (i) I: No infection or Immune (0.0), (ii) R: Resistant: necrotic areas with or without minute uredia (0.2), (iii) MR: Moderately resistant: Small uredia surrounded by necrotic areas (0.4), (iv) X: Intermediate: variable size uredia, some with necrosis or chlorosis (0.6), (v) MS: Moderately susceptible: medium uredia with no chlorosis (0.8), and (vi) S: Susceptible: Large uredia, no necrosis or chlorosis (1.0). The coefficient of infection (CI) was calculated by multiplying disease severity (DS) and constant values of infection type (IT) based on Immune = 0, R = 0.2, MR = 0.4, M = 0.6, MS = 0.8, S = 1 [59]. AUDPC [41], was calculated as follows: $AUDPC = D^{1/2}(Y_1 + Y_k) + Y_2 + Y_3 + \dots + Y_k - 1$ where, D = Time interval (days between each two successive readings), (Y₁ + Y_k) = Sum of first and last disease scores. (Y₂ + Y₃ + ... + Y_{k-1}) = Sum of all in between disease scores.

Phenotypic data analysis

To estimate the genetic and environmental effects of RILs analysis of variance was performed using 'Agricolae' package in R Studio (R version 4.3.2). Frequency distribution curves were generated using 'ggplot' package. In addition, the mean values of Average Coefficient of Infection (ACI) of both years were used to evaluate the genetic effects and detect QTL.

DNA extraction and PCR analysis

DNA extraction from plant tissue was performed by using CTAB method [50]. Briefly, freshly collected leaf samples from two-week-old seedlings were ground to a fine powder using liquid nitrogen followed by incubation in 700 ml of pre-heated CTAB buffer at 65 °C for 1 h. Chloroform-isoamyl alcohol (24:1) was added (Volume equals to CTAB buffer used) to each sample followed by centrifugation at 12,000 rpm for 10 min at room temperature. The chilled isopropanol was added to the supernatant collected in fresh eppendorf tube. The DNA was pelleted down by performing centrifugation at 12,000 rpm for 10 min at 4 °C. The pellets were air-dried and dissolved in 100 µL of nuclease-free water. RNA contamination if any, was removed with RNase-A treatment.

The qualitative analysis of DNA was done with 0.8% agarose gel electrophoresis (Bio-Rad, USA).

DNA amplification was performed using ABI Applied Biosystems 9902 Veriti PCR thermocycler in a total volume of 10 μ L containing 10 mM Tris-HCl, pH 7.5, 50 mM MgCl₂, 0.2 mM dNTP, 25 ng of each primer, 0.75 U of Taq polymerase, and 50 ng of genomic DNA as the template. After an initial denaturing step for 5 min at 94 °C, 35 cycles were performed for 45 s at 94 °C (denaturation), 50–61 °C (annealing) for 45 s, and 72 °C for 1 min (elongation), with a final extension at 72 °C for 10 min. The amplified PCR products were run on 3% agarose low EEO (HIMEDIA) in 1X TBE buffer solution using GeNei MAXI SUB XXL SYSTEM electrophoresis unit, at 120 voltages for 2.20 h. The polymorphic bands were visualized under UV light using Alphamager[®]EP (Alpha Innotech. Ltd.) and photographed to score visually.

DArT genotyping and data filtering (DArT)

Genotyping was done using a marker panel comprising of 3,897 DArT SNP markers distributed across 21 bread wheat (*Triticum aestivum* L.) chromosomes. This panel is primarily derived from a large genotyping-by-sequencing (GBS) dataset and a microarray dataset (90 K iSelect SNP genotyping array) [53, 61]. The genotypic data so obtained was subjected to stringent filtering using the dartR package in RStudio. The marker data was filtered out for which the reproducibility (strictly repeatability) is less than a specified threshold, say threshold = 0.99 for which the call rate (rate of non-missing values) is less than a specified threshold, say threshold = 0.95 for missing data and > 30% heterozygosity [14, 21]. Finally, the markers were screened for parental polymorphism.

Linkage map construction and QTL analysis

Out of 3,897 DArT markers, a total of 602 DArT markers were used to construct a linkage map for the RIL population (Table S1). IciMapping v4.2 [63] was used to construct the linkage map and QTL analysis. The Kosambi mapping function was used to construct linkage groups, using a threshold logarithm of odds (LOD) score of 3.0 [26]. The marker order was set using the 2-opt algorithm within each linkage group, and a window size of 5 cM was maintained for rippling. The inclusive composite interval additive functionality mapping (ICIM-ADD) method was used to map QTLs [31]. The walking speed parameter of each step for the genome-wide scan was set at 1.0 cM, and for all significant QTLs, the critical LOD values were defined at 3.0 to increase the reliability and accuracy of QTL detection. The significance thresholds were calculated using 1,000 permutations, with a type I error of 0.01 and genome-wide error rates of 0.10. Then, the QTLs were localized on the respective chromosomes.

The QTLs detected in two environments were considered stable QTLs. To obtain the high-density linkage map and further narrow down the flanking intervals of target loci, 23 genome-wide SSR markers (Table S2) [68] distributed on 21 pairs of wheat chromosomes were integrated with DArT markers.

In silico analysis

The potential candidate genes and their molecular functions were identified by analyzing the flanking marker sequences for stable QTLs by utilizing the RefSeq v2.1 wheat genome assembly from the International Wheat Genome Sequencing Consortium (IWGSC) by utilizing BLASTN function of BLAST program integrated into the Ensembl Plant database (<https://plants.ensembl.org/index.html>). The functions of putative candidate genes were validated by thorough analysis of published literature.

Results

Seedling reaction test

The results displayed the stripe rust severity against 4 distinct pathotypes in the order: 238S119 > 110S119 > 110S84 > 78S84. The resistant parent (W8627) showed high resistance against 78S84 and 110S84 (IT0-1) but it showed susceptible infection type against 238S119 and 110S119 (IT 8–9) while for PBW343 (susceptible parent) and Agra Local (susceptible check) IT (8–9) was observed for all pathotypes (Table S3).

Field evaluation

Stripe rust response of both the parents and the RIL population were evaluated during the adult plant stage for the years 2021–22 and 2022–23. The findings indicate that W8627 consistently displayed a resistant response (with ACI values of 3.0 and 2.7, and AUDPC values of 55 and 50) over the two consecutive seasons. Whereas, wheat genotype PBW343 exhibited a moderately susceptible response (with ACI values of 40 and 60, and AUDPC values of 435 and 540) during the same periods of 2021–22 and 2022–23, respectively. While mean values showed for resistant and susceptible parents, respectively (ACI = 2.85, 50 and AUDPC = 52.5, 487.5). For the RILs, mean value of the AUDPC ranging from 0 to 880.4. The average AUDPC values among recombinant inbred lines (RILs) over the days after inoculation for both the years clearly depicted in Fig. 1 indicating that AUDPC increases steadily with days after inoculation in both years, but the consistently higher and steeper line revealing the stronger disease pressure in 2022–23, providing better discrimination among genotypes for quantitative resistance. The susceptible check (Agra Local) expressed the average coefficient of infection up to 80. Normal distribution of ACI data was observed among parents and RILs (Fig. 2) indicating the suitability of RIL population

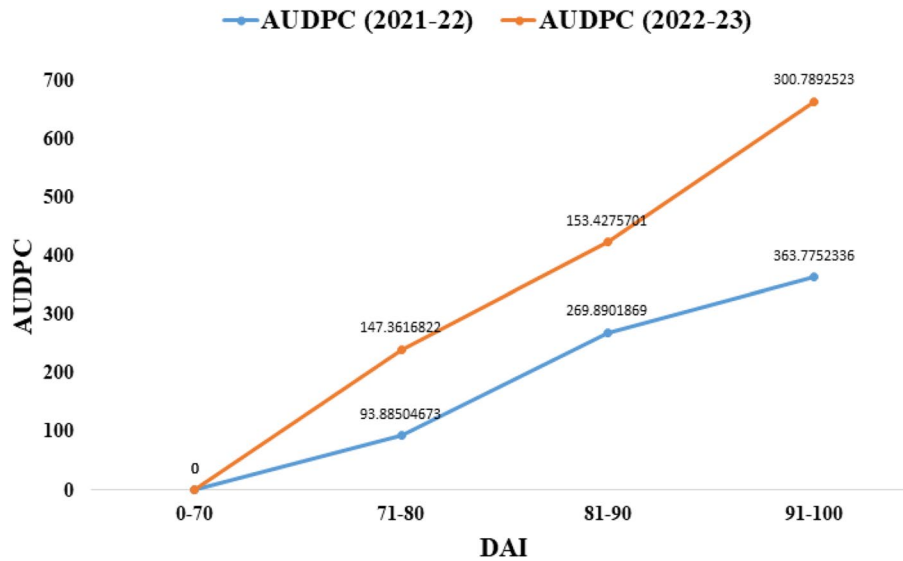


Fig. 1 Area under the disease progression curve (AUDPC) for the crop season 2021–22 and 2022–23

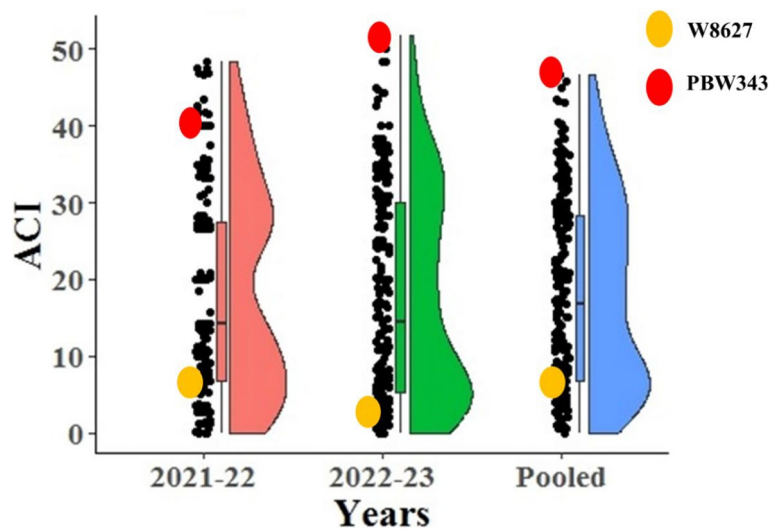


Fig. 2 Frequency distribution of the Average Coefficient of Infection (ACI) for RILs and parent lines across the 2021–22 and 2022–23 crop seasons, including pooled data

Table 1 Variance components of disease severity (DS) scores of 212 RILs for the crop season (2021–22& 2022–23)

Source of variation	df	Mean square	F- value
RILs	211	905.05568396***	43.64158
Replicates/Environment	1	9.036816038	0.435753
Error	211	20.73838002	

***P<0.001

for the QTL mapping purpose In addition, the disease severity of RILs and parents is indicated in the supplementary figure S1. Further, the results exhibited the presence of wide variability among RILs for stripe rust resistance as evidenced by significant genotypic, environmental, and genotype-by-environment variances ($P < 0.01$) (Table 1).

Data filtering and construction of linkage map

After following the standard data filtering procedure, a total of 1,500 marker post-filter SNP repeatability and less markers were filtered for SNP call rate and none of the markers was filtered based on heterozygosity (Fig. 3A & B). A total of 1420 DArT markers were filtered out and were further screened for parental polymorphism. Only 602 DArT markers were found to be polymorphic on both the parents and RILs which were utilized for the construction of linkage map. The total length of the genetic map was 1395.52 cM, with a mean marker/bin interval of 0.46 cM. The linkage group size of chromosome 5D was the longest (144.23 cM), and it was the shortest for 4D (4.0 cM). Linkage group 2A had 91 markers with the length of 125.43 cM (0.73 markers/

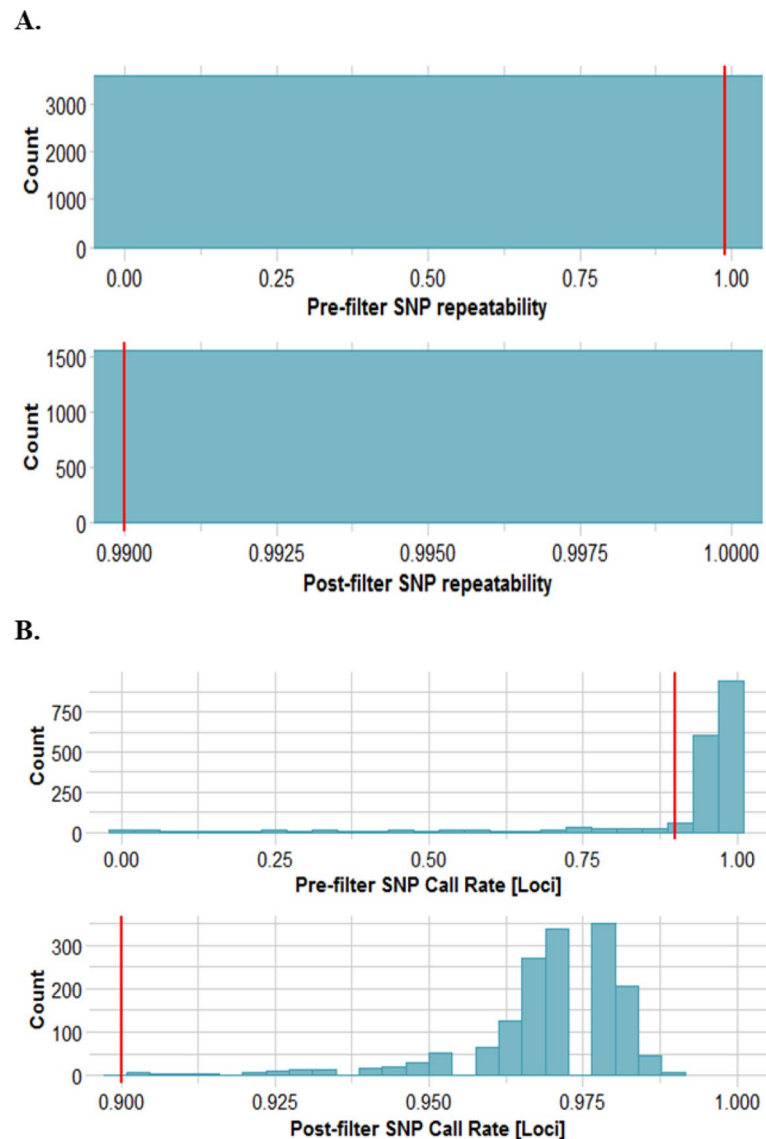


Fig. 3 Filtering of DArT SNPs based on the following criteria: **(A)** SNP repeatability and **(B)** SNP call rate

cM). In the wheat genome, the A sub-genome exhibited the highest number of markers (283, 47%), while the D sub-genome displayed the lowest (83, 13.78%). Among the seven homologous groups of wheat, the fifth homology group had the highest number of SNPs (110, 18.27%), while the first homology group had the least (54, 8.97%) (Fig. 4A, B and C). The final genetic map included 21 linkage groups corresponding to 21 chromosomes.

QTL analysis

For SRT, no QTLs were identified for stripe rust resistance with DArT markers. Moreover, we explored the possibility of an integrated linkage map comprising 602 DArT and 23 SSR markers. This approach led to the identification of two QTLs conferring resistance to the pathotype '78S84', associated with seedling resistance on

chromosome 3B and 6D, designated as *QYr.iwbr.3BS.1* and *QYr.iwbr.6DS.1*, respectively. These QTLs had exhibited LOD scores of 7.6 and 8.1, explaining phenotypic variations of 0.69% and 0.82%, respectively. The QTLs were delimited by molecular markers TaDArTAG002817& wmc693 for *QYr.iwbr.3BS.1* and TaDArTAG009545& TaDArTAG009594 for *QYr.iwbr.6DS.1* (Fig. 5 and Table 2).

In the case of adult plant resistance, utilizing this linkage map constructed with 602 DArT markers, two stable QTLs were identified on chromosome 2B and 6D over two consecutive seasons (2021–22 and 2022–23). Further, QTL analysis revealed that a new QTL mapped on chromosome 2B, designated as *QYr.iwbr.2BL.1*, was flanked by two DArT SNPs (TaDArTAG000521& TaDArTAG007479) with an estimated LOD score of 4.5

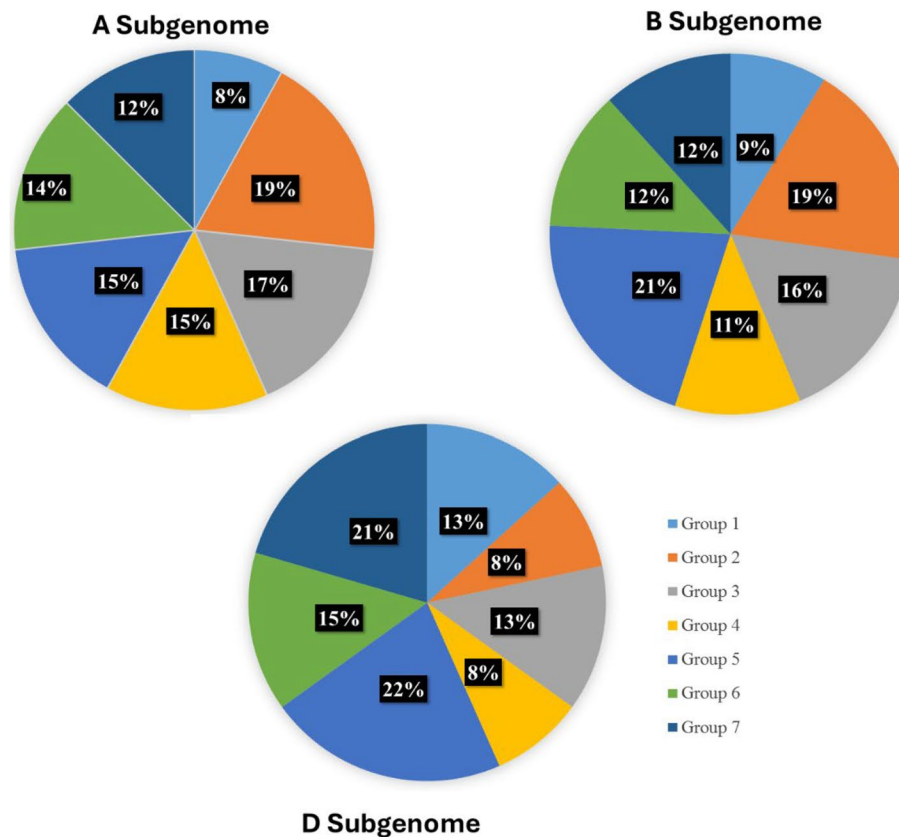


Fig. 4 Distribution of DArT SNPs across the three wheat sub-genomes (A, B, and D), each consisting of seven linkage groups

explaining 9.67% of the phenotypic variance. Later, the DArT markers were integrated with SSR markers for the enrichment of this QTL region identified on chromosome 2B. The DArT markers TaDArTAG000521 & TaDArTAG0007479 were flanked by two SSR markers (*cfa2278* and *efd70*) (Fig. 5b).

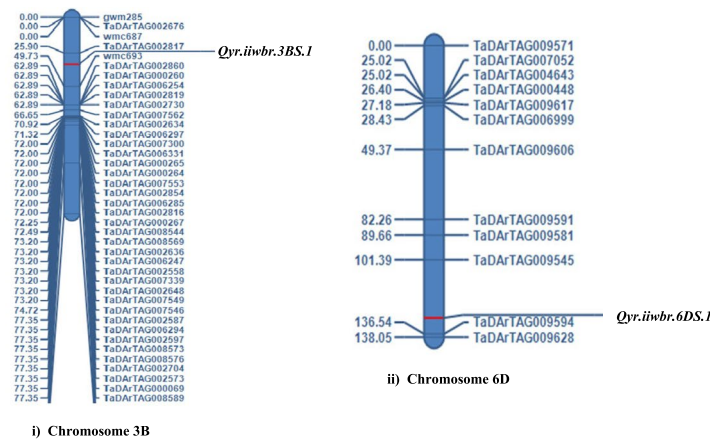
Another QTL i.e. *QYr.iwbr.6DS.2* was located at 0–12.5 cM distance and was delimited by two markers (TaDArTAG0009571 & TaDArTAG0007052) with estimated LOD score value of 3.61 explaining 7.33% phenotypic variance (Fig. 5b and Table 2).

Putative candidate gene identification

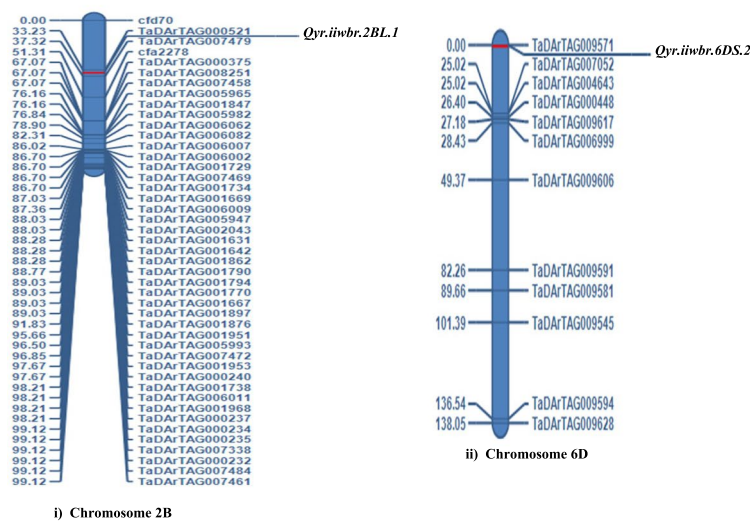
The putative candidate genes were identified for stable QTLs with high phenotypic variation through in silico analysis represented in Table S4. The QTL, i.e., *QYr.iwbr.2BL.1*, located at 525,519,604–525,519,754 Mb encodes for Inositol Transporter 1 (TraesCS2B02G368200). The QTL, i.e., *QYr.iwbr.6DS.2*, located at 8,661,722–8,668,626 Mb encodes Protein transport protein *sec16* (TraesCS6D02G022300.1).

Discussion

Biotic stresses, leads to substantial yield losses in wheat globally. These pathogens interfere with the plant's normal metabolic functions by extracting nutrients, damaging tissues, and disrupting photosynthesis and water balance. Resistance to biotic stresses involves pattern recognition receptors detecting PAMPs for PAMP-triggered immunity, followed by effector-triggered immunity where resistance genes recognize pathogen effectors. Management includes resistance gene pyramiding, systemic acquired resistance activation, and biological/chemical treatments. Molecular breeding tools are of great importance develop durable resistance while maintaining productivity. Stripe rust being the most devastating disease causes potential yield losses. Therefore, the genetic dissection of such traits by utilizing QTL through marker-based breeding schemes will be meaningful in designing breeding strategies through MAS [23, 25, 28]. The majority of rust resistance genes discovered are from wild relatives due to which an undesired linkage drag linked to the resistance locus prevents their direct application in breeding programs. Further, compared to APR genes, the durability of seedling resistance genes is lower. Plant breeders therefore implied a great deal of interest in molecular investigations in superior genetic backgrounds



a: Identified QTLs for seedling stage resistance and their genomic locations: i) QTL on chromosome 3B i.e. *QYr.iwbr.3BS.1*, ii) QTL on chromosome 6D i.e. *QYr.iwbr.6DS.1*.



b: Identified QTLs for adult plant stage and their genomic locations: i) QTL on chromosome 2B i.e. *QYr.iwbr.2BL.1* and; ii) QTL on chromosome 6D i.e. *QYr.iwbr.6DS.2*.

Fig. 5 a Identified QTLs for seedling stage resistance and their genomic locations: i) QTL on chromosome 3B i.e. *QYr.iwbr.3BS.1*, ii) QTL on chromosome 6D i.e. *QYr.iwbr.6DS.1*. b Identified QTLs for adult plant stage and their genomic locations: i) QTL on chromosome 2B i.e. *QYr.iwbr.2BL.1* and; ii) QTL on chromosome 6D i.e. *QYr.iwbr.6DS.2*

Table 2 QTLs identified for stripe rust resistance in W8627 x PBW343 derived RIL population

Trait	Chr	Pos	Left marker	Right marker	LOD	PVE (%)	Add	Left CI	Right CI
SRT	3B	34	TaDarTAG002817	wmc693	7.6	0.82	-2.92	33.5	34.5
SRT	6D	129	TaDarTAG009545	TaDarTAG009594	8.1	0.69	-2.87	128.5	129.5
APR	2B	63	TaDarTAG007479	TaDarTAG000521	4.56	9.67	4.49	61.5	64
APR	6D	0	TaDarTAG009571	TaDarTAG007052	3.61	7.33	3.72	0	12.5

to prevent linkage drag and resistance breakdown. Germplasm lines including landraces are the primary source of genes and a valuable source of resistance, until now very few landraces have been used in wheat breeding programs. A key source of resistance genes are germplasm lines, which include landraces. However, relatively

few landraces have been employed in wheat breeding efforts to date [3, 39, 43]. Identifying quantitative trait loci (QTLs) for disease resistance plays a crucial role in advancing molecular plant breeding. It aids in characterizing germplasm and developing new varieties. Molecular markers are valuable tools for identifying

QTLs associated with traits that are controlled by multiple genes with different levels of heritability. These traits are often influenced by a variety of environmental factors across generations. Even though a great number of genes have been identified to confer resistance against stripe rust during the crop season or at the adult plant stage, the pathogen's rapid and ongoing evolution in various regions of the world has rendered many of these genes ineffective, resulting in us with a small number of effective *Yr* genes.

We could not find any QTLs for ASR using DArT SNP markers in the present study. However, we identified 2 QTLs that confer resistance at all stages against the 78S84 pathotype by combining the map with SSR markers, it was found that a QTL on chromosome 3B overlaps with the marker interval TaDArTAG002817 and wmc693 at 34 cM. Another QTL on chromosome 6D is delimited by TaDArTAG009545 and TaDArTAG009594 markers at 129 cM, accounting for 0.69% and 0.82% of the phenotypic variance, respectively. The study did not identify any QTLs associated with races 238S119, 110S119 and 110S84 due to their recent evolution and high virulence. At the seedling stage, SRT QTLs explained only a slight proportion of the phenotypic variance, consistent with polygenic control by numerous loci of modest effect. These minor-effect QTLs likely remained undetected in the individual maps owing to low marker density and local gaps, hence, compromising the resolution of linked loci. By integrating datasets into a higher-density, consensus map with enhanced coverage and collinearity, thereby refining QTL intervals, diminishing residual variance, enhancing statistical power in underrepresented regions to reveal subtle SRT QTLs of modest additive effect [17, 33]. Previous research also indicated the presence of a QTL on chromosome 6D within the marker interval Bcd1510ksuD27 [8]. Additionally, QTLs on chromosome 3B have previously been documented to confer resistance to stripe rust in mature plants and seedlings [20]. To our knowledge, many *Yr* genes have previously been employed for stripe rust resistance, still there is only one report comprehending the utilization of QTLs viz., *QYr.nafu-2BL* and *QYr.nafu-3BS* for the transfer of stripe rust resistance in wheat varieties. A few *Yr* genes including *Yr5*, *Yr10*, *Yr15*, *Yrsp*, *Yr47*, *Yr57* and *Yr63* are still effective in India [44, 55].

Studies suggested that identifying the same or closely linked Quantitative Trait Loci (QTL) across multiple studies, involving diverse genetic backgrounds and/or environmental conditions, strengthens the reliability of pinpointing genomic regions for resistance breeding. Such genomic regions have the potential to confer relatively long-term and broad-spectrum resistance. In the current study, two stable QTLs were identified for adult plant stage. One was located on chromosome 2B,

within the marker interval TaDArTAG000521—TaDArTAG007479, with a LOD score value of 4.5 and a phenotypic variance explaining (PVE) of 9.67% for adult plant resistance (Table S5). This region aligns with previously identified QTLs reported by Palliard et al. [40], further supporting the presence of a QRC. In the present study, 2 stable QTLs were identified i) on chromosome 2B (*QYr.iwbr.2BL.1*) with marker interval TaDArTAG000521 & TaDArTAG007479 with an estimated LOD score value of 4.5 explaining PVE 9.67% for adult plant resistance. Subsequently, DArT markers were integrated with SSR markers to enrich this QTL region. Notably, the DArT markers TaDArTAG000521 & TaDArTAG007479 were flanked by two SSR markers, *cfa2278* and *efd70*. *QYr.iwbr.2BL.1* resides within a previously reported QTL-rich region on chromosome 2B. However, its distinct peak position and unique flanking markers in our mapping population support its designation as a novel QTL within this genomic interval. We have revised the text to clarify that this locus represents a newly identified QTL situated in a recognized resistance hotspot. The novelty of our finding lies in its specific flanking markers, its demonstrated stability across our genetic background, and the identification of candidate genes within the underlying interval. Identification of candidate gene within this interval adds a new layer of insight, facilitating targeted molecular studies and marker assisted selection. ii) Another QTL identified for adult plant resistance was located on chromosome 6D at position 129 cM explaining 7.334% of phenotypic variance. Chromosome 2B serve as a key reservoir for formally designated rust resistance genes, including *Yr5*, *Yr7*, *Yr27*, *Yr31*, *YrSP*, *Yr43*, *Yr44*, *Yr53* and the recently described *Yr72* together with numerous QTL clusters for stripe rust resistance, highlighting the chromosome. Recently, some stable QTLs were also identified by Kokhmetova et al. [25] on 2B chromosome for adult plant resistance in two different RIL populations (Avocet and Anza). A total of 65 QTLs are located on chromosome 2B [20, 29] viz., *QYrid.ui-2B.1* in IDO444 [34], *QYr.sgi-2B.1* in Kariega [48], *QYrlu.cau-2BS1* and *QYrlu.cau-2BS2* in Luke [16] *QYrlo.wpg-2BS* in Louise [10], *QYr2B* in Opata 85 [47], and *QYr.cim-2BS* in Francolin [30] mapped at 73.6, 100.8, 172.7, 47.6, 1.2, 20.6, 100.8, and 166.5 Mb physical positions, respectively. In the current population and marker density, the confidence intervals of the 2B QTL are still too broad to distinguish between closely linked genes and truly pleiotropic loci, the 2B region is now explicitly highlighted as a candidate interval where future high-resolution mapping, fine-mapping, and functional validation could clarify whether the observed effects arise from linkage blocks or single pleiotropic genes. Furthermore, previously, few stripe rust QTLs were identified on 6D chromosomes at different locations [2, 4, 8, 13, 69]. Gene interactions among selected

wheat resistance genes, particularly for stripe rust, are crucial for enhancing the durability and effectiveness of resistance. Combining major all-stage resistance (ASR) genes with adult plant resistance (APR) genes often produces additive or epistatic effects that strengthen defense responses. For example, pyramiding *Yr30* and *Yr17* leads to significantly improved resistance, exemplifying an epistatic effect, while gene pairs such as *Yr29+Yr5* and *Yr26+Yr30* also show enhanced disease resistance [1]. Notably, APR genes including *Yr29*, *Yr30*, and *Yr48* boost the performance of ASR genes like *Yr5*, *Yr15*, *Yr17*, *Yr64*, and *Yr65*, promoting durable and broad-spectrum resistance.

Moreover, incorporating stable and moderately effective QTLs such as those identified on chromosomes 2B and 6D enhances resistance durability across varied environments. These QTLs are particularly valuable in improving resistance against evolving pathogen races, making them key resources for crop improvement programs. Overall, these interactions and QTL integrations emphasize the importance of strategic gene combinations and the deployment of multiple resistance loci to achieve long-lasting protection against rapidly evolving stripe rust pathogens.

Furthermore, we aim to look deeper into the method of identifying potential candidate genes using computational methods for identifying stable QTLs with significant phenotypic variability. For instance, *QYr.iwbr.2BL.1* encodes for Inositol Transporter 1 (TraesCS2B02G368200) which plays an important role in combating biotic and abiotic stresses [17]. Inositol transporters play a pivotal role in the transport of vital metabolites, sugars, lipids and minerals. Balanced inositol transport is essential for maintaining cell wall integrity and proper signaling during pathogen attack, which helps activate defense responses and may restrict fungal invasion.

Inositol is involved in the biosynthesis of sugars which are the crucial component of cell walls in plants. INT1 regulates intracellular myo-inositol homeostasis, which feeds into cell wall biosynthesis (via pectin and hemicellulose precursors), phosphoinositide-based signaling [18] and the production of inositol-derived second messengers. Enhanced inositol flux could therefore reinforce cell wall integrity at infection sites and modulate Ca^{2+} /phosphoinositide signaling and hormone crosstalk (e.g. salicylic acid and jasmonic acid), all of which are central to defense against biotrophic fungi such as *Pst* as depicted in supplementary figure S2A. There is one report comprehending the upregulation of TaMIPS2 (*D-myoinositol-3-phosphate synthase 2*) transcript in wheat seedlings under heat stress [24]. In a recent study, some stable QTLs like *QLR-APR-2B* (Anza population) and *QYR-APR-2B.2* (Avocet population) were identified which

encodes NBS domains and hence governs a potential role in stripe rust resistance. While other stable QTLs such as *QYR-APR-4D.2* (Anza population) and *QYR-APR-4D.1* (Avocet population) encoding *zinc finger C2H2 superfamily* which is another leading player among the different class of R-genes in imparting disease resistance [15]. The knockdown of the wheat Ser/Thr kinase gene, also recognized as TaPsIPK1, *Puccinia striiformis*-induced protein kinase 1, confers broad-spectrum resistance to *Pst* races. On the other hand, there are various transcription factors in wheat that are hosting a crucial role in governing disease resistance [20]. For instance, the NAC domain superfamily encoded by the QTL, *QYR-APR-4D.1* (TraesCS4D02G316800.1) [67] aids in conferring stripe rust resistance. Wang et al. [64] suggested the role of TaWRKY70 in YR resistance, particularly during the seedling stage. Furthermore, transgenic overexpression of barley WRKY genes, recognized as, HvWRKY6 and HvWRKY70, provides resistance to stripe rust [32]. As a result, consistent QTLs encoding the same putative genes could be the potential genomic locations for further validation. Another QTL, *QYr.iwbr.6DS.2* encoded protein transport protein sec16 which regulates COPII mediated trafficking in plants [35]. Sec16 is a key determinant of transitional ER organization reported in yeast (*S. cerevisiae*). It has a role in ER protein export by defining the ER region for COPII assembly at the ER exit sites (ERES) [11]. The protein transport protein Sec16 is involved in COPII-mediated trafficking, plays an important role in ER protein export by defining the ER region for COPII assembly at the ER exit sites (ERES) which may suggest the rapid and coordinated secretion of defense-related proteins, enzymes, and immune receptors to the cell surface. Sec16 could alter the defence cargo export dynamics and contribute to quantitative differences in rust resistance as indicated in the supplementary figure S2B. To our understanding, there have been no previous reports on the presence of this protein in wheat. There is potential for further investigation into these newly discovered putative candidate genes.

Conclusion

The present study revealed significant variability in stripe rust resistance among the RIL population, with some lines showing complete immunity- surpassing even the resistance parent, W8627- highlights their potential for direct use in breeding. Two new QTLs for seedling-stage resistance were identified, offering opportunities for pyramiding with APR genes to achieve durable resistance. A stable APR QTL, *QYr.iwbr.2BL.1*, associated with the inositol transporter 1 gene, and another QTL, *QYr.iwbr.6DS.2*, linked to the protein transport protein Sec16, present promising targets for further functional validation. Characterizing these candidate genes

will strengthen their utility in rust resistance breeding programs. Future validation efforts will combine KASP marker development, integrated genotyping-phenotyping, and detailed functional checks to confirm candidate gene roles and maximize durable resistance breeding.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-025-07981-x>.

Supplementary Material 1: Figure S1. Maximum disease severity (MDS%) among the RILs and parents across the 2021–22 and 2022–23 crop seasons, including pooled data. Figure S2. Schematic representation of biological functions and mechanism of action in plant defense against fungal pathogens; A) Role and mechanism of action of INT1 in plant defense; B) Role and mechanism of action of Sec16 in plant defense

Supplementary Material 2: Table S1. Polymorphic DArT markers and their genomic location. Table S2. Genome wide SSR markers, their genomic location, melting temperature and sequence information. Table S3. Rust severity among parents and 212 RILs at seedling stage. Table S4. Identified putative candidate genes for adult plant resistance. Table S5. List of QTLs reported in this study and previous studies on chromosome 2B, 3B and 6D

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Authors' contributions

RS: Performed field and lab experiments, Data analysis, Writing original draft; SK: APR data analysis, review and editing; PP: SRT data analysis; Review and editing; UK: Supervision, review and editing; SY: Review and editing, OPG: Data recording SRT, review and editing, SU: Dart facility; IS: RILs development and editing, RT: Funding, Supervision, review and editing, PS: Conceptualization, Supervision, formal data analysis, review and editing. All authors read and approved the final manuscript.

Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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