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Genetic dissection of leaf rust resistance in a diversity panel of tetraploid wheat (*Triticum turgidum*)

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Abstract

Background Leaf rust, caused by *Puccinia triticina* Eriks (*Pt*) is a major threat to wheat cultivation worldwide. The rapid evolution of this pathogen has led to the emergence of new virulent strains that can overcome the resistance of commonly cultivated wheat varieties. To address this threat, continuous monitoring of leaf rust pathotypes is conducted in wheat-growing regions across the world. This approach helps prioritize the development and deployment of resistant cultivars, as well as the implementation of other effective control measures against the prevailing races. The key wheat leaf rust pathotypes in India include 77–5 (121R63-1), 77–6 (121R55-1), 77–9 (121R60-1), 12–5 (29R45), and 104 (17R23). Among these pathotypes, 77–5 (121R63-1) and 77–9 (121R60-1) are the most prevalent since 2016. As virulent pathotypes continue to evolve and adapt, there is an urgent need to continually explore the vast germplasm repositories of wheat and its related species to identify novel genetic resources and genes that confer resistance to these evolving leaf rust pathotypes. Therefore, the present study aims to identify genes and genomic regions responsible for leaf rust resistance against prevalent pathotypes in India, focusing on a subset of the Global Durum Wheat Panel, which includes genotypes from various tetraploid wheat species.

Results This study revealed wide variation in seedling-stage resistance among 189 tetraploid wheat accessions against five prevalent leaf rust pathotypes in India namely, 77–5 (121R63-1), 77–6 (121R55-1), 77–9 (121R60-1), 12–5 (29R45) and 104 (17R23). Approximately 45% of the population exhibited immune/highly resistant to moderately resistant responses to pathotypes 77–5, 77–6 and 104, while around 23–27% showed similar levels of resistance to pathotypes 77–9 and 12–5. A genome-wide association study using six multi-locus models identified 88 significantly associated quantitative trait nucleotides (QTNs) across the five leaf rust pathotypes. Among these, 22 QTNs were considered reliable, including four for pathotype 77–5, six for 12–5, three for 77–9, seven for 104, and two for 77–6. Among the 22 reliable QTNs, 10 coincided with the rust resistance regions reported in previous studies, whereas 12 appeared to be novel. Further investigations of the regions flanking all 88 QTNs revealed 300 genes, including 62 associated with disease resistance or defense responses. In silico expression analysis of these

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defense-related genes revealed two nucleotide-binding site–leucine-rich repeat genes: one on chromosome 6B (*TRITD6Bv1G224600*) near *QTN RAC875_c35430_373*, and another on chromosome 6A (*TRITD6Av1G225060*) in the vicinity of QTN Excalibur_c77841_224 with significantly higher levels of expression in the leaf-resistant genotype during the early hours of *Pt* infection. Therefore, these two genes could be potential candidates for resistance to leaf rust in tetraploid wheat germplasm.

Conclusions Our study provides a comprehensive understanding of the genetic basis underlying leaf rust resistance in a diverse tetraploid wheat germplasm panel. It has also revealed novel candidate genomic regions for leaf rust resistance. These genomic regions represent important targets for inclusion in marker-assisted breeding initiatives, aimed at fostering durable resistance against leaf rust disease.

Keywords *Durum* wheat, Leaf rust, *Puccinia triticina*, GWAS, *Lr* genes, Association mapping, Quantitative trait loci

Introduction

Wheat leaf rust, caused by *Puccinia triticina* Eriks, is the most prevalent of the three varieties of wheat rust and is one of the major limiting factors in wheat production [13, 68]. The fungal pathogen causing this disease thrives across diverse environmental conditions and is widespread in wheat-growing regions worldwide. It primarily affects leaves, leading to diminished photosynthesis, dehydration, and premature leaf shedding. Depending on the growth stage at which initial rust infections occur, susceptible wheat cultivars may suffer yield reductions ranging from 5 to 50% or more [26, 28, 66, 79]. Significant yield losses due to leaf rust have been reported across various countries. In China, leaf rust affects over half of the wheat-growing areas, with yield losses commonly ranging between 10 and 30% in commercial wheat fields [26]. Similarly, in Russia, leaf rust occurs every year, causing yield losses of up to 30–40% [51]. While bread wheat is generally more susceptible to leaf rust than durum wheat, several durum wheat genotypes display resistance to many prevalent *P. triticina* pathotypes affecting common wheat [1, 21]. However, in recent years, the emergence of new leaf rust pathotypes specific to durum wheat, coupled with the breakdown of resistance in various durum wheat growing regions, has posed a significant challenge to durum wheat breeders worldwide [1, 58]. Recently, a comprehensive genetic diversity analysis of leaf rust isolates from around the world, utilizing SNP markers, has revealed a distinct grouping of isolates infecting durum wheat and those infecting common wheat [33]. This suggests that a separate set of resistant genes may be needed for the pathotypes specifically infecting tetraploid wheat. Therefore, it is crucial to search for novel leaf rust-resistant genes within the tetraploid wheat germplasm pool to combat the prevailing leaf rust pathotypes affecting tetraploid wheat. Wheat rust can be managed by using fungicide treatments or by utilizing wheat varieties resistant to leaf rust. Among these methods, the development of resistant varieties is considered a cost-effective and environmentally sustainable approach for managing this disease. Therefore, the identification and deployment of rust resistance genes are key to effectively managing leaf rust in cultivated wheat varieties [23, 60]. To date, 83 leaf rust resistance (*Lr*) genes have been identified from wheat and its wild relatives. However, only ten of these genes have been cloned, namely, *Lr10* [14], *Lr21* [24], *Lr1* [11], *Lr34* [37], *Lr67* [53], *Lr22a* [73], *Lr9* [80], *Lr13* [22], *Lr14a* [36] and *Lr 42* [40]. Among the reported *Lr* genes, the majority confer resistance at the seedling stage. Nonetheless, a few adult plant resistance (APR) genes have also been identified, the most important of which are the race-specific genes *Lr12* and *Lr13* and the race-non-specific genes *Lr34* and *Lr67* [38]. The resistant genes have been identified from cultivated wheat, including *Triticum aestivum* and *Triticum turgidum,* as well as from their progenitors and other wild relative species such as *Aegilops tauschii*, *Aegilops speltoides*, *Aegilops neglecta*, *Aegilops peregrina* and *Aegilops markgrafii* [40, 47, 61, 73].

In recent years, with the availability of reference-scale genome assemblies and advanced genotyping technologies such as high-density SNP chips and next-generation sequencing-based approaches such as genotyping by sequencing (GBS), the identification of genes and allelic variants responsible for desirable traits has become easier in major food crops, including bread wheat and tetraploid wheat [15, 25, 29]. Unlike traditional quantitative trait locus (QTL) mapping, GWAS enables the identification of genes within natural populations such as germplasm collections providing a more accessible and comprehensive pathway for their discovery. Moreover, GWAS facilitates high-resolution mapping of traits as the genotypes within diversity panels often represent numerous historical recombination events that have accumulated over time. The present

study aims to evaluate the global tetraploid wheat germplasm collection for resistance to prevailing leaf rust pathotypes in India and identify the associated genes/genomic regions.

Materials and methods

Plant materials

The genetic material for this study consisted of 189 accessions, a subset of the Global Durum Panel (GDP), including both cultivated and wild tetraploid wheat species. The genotypes in the GDP subset included important tetraploid species such as *Triticum aethiopicum, Triticum carthlicum, Triticum durum, Triticum dicoccoides, Triticum dicoccum, Triticum turanicum,* and *Triticum polonicum*. These genotypes originated from 28 countries including Ethiopia, Turkey, Namibia, Iran, Spain, Italy, Israel, Lebanon, Russia, Georgia, Syria, Germany, The United Kingdom, Hungary, India, and others. The details of the GDP accessions included in this study are provided in Supplementary Table 1.

Leaf rust pathotypes

Pure inocula of important prevailing leaf rust pathotypes in India namely, 77–5 (121R63-1), 77–6 (121R55-1), 77–9 (121R60-1), 12–5 (29R45), and 104 (17R23) were sourced from the ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Shimla. These were multiplied and used to screen the response of tetraploid wheat accessions against leaf rust.

Multiplication of leaf rust pathotypes

The leaf rust pathotypes were multiplied and maintained on the wheat leaf rust susceptible cultivar 'Agra Local' under glasshouse conditions at the Indian Agricultural Research Institute (IARI), New Delhi. For this purpose, ten-day-old seedlings of 'Agra Local' were grown in pots under greenhouse conditions and then used for inoculation. The *P. triticina* uredospore was mixed with talcum powder and the dry mixture of powder was smeared manually on the leaf of each plant. To prevent cross-contamination, inoculated seedlings were kept for 48 h in a separate humid glass chamber before being moved to benches in the glasshouse with ambient humidity and light. At 12 days of inoculation, erumpent pustules profusely producing uredospores developed. The fresh inoculum was collected by dusting the plants and used for disease screening.

Screening of GDP genotypes for leaf rust reactions at the seedling stage

The phenotypic evaluation experiments were conducted under controlled glasshouse conditions during the main wheat seasons of 2022 and 2023 respectively at the Division of Genetics, IARI, New Delhi. The GDP accessions were sown in five sets in aluminum trays filled with soil and FYM at a ratio of 10:1 for screening against five different pathotypes. In each aluminum tray, a total of 10 genotypes including 9 GDP lines and one susceptible check (Agra Local) were sown with 10 seeds per line. The first leaves of 10-day-old seedlings of all the accessions were inoculated by hand with a homogeneous mixture of urediospores (rust spores were mixed with a drop of Tween 20), followed by incubation in a humid glass chamber for 48 h. These infected seedlings were then transferred to benches in a glasshouse where the temperature regime was maintained at 18–22 °C, along with ambient humidity and light levels. The infection types (ITs) for the disease were recorded 12–15 days after inoculation using a 0–4 scale [69]. In this scoring system, an infection type (IT) of '0' indicates no visible symptoms, while an IT of ';' represents hypersensitive flecks. An IT of '1' corresponds to small uredinia with necrosis, and an IT of '2' indicates small-to-medium-sized uredinia surrounded by chlorosis. An IT of '3' is characterized by medium-sized uredinia without chlorosis or necrosis, and an IT of '4' represents large uredinia without necrosis or chlorosis. Larger or smaller uredinia than typically associated with each IT were denoted by '+' and '–' respectively. Seedlings displaying ITs of 0–2+were classified as resistant, while seedlings with ITs of 3–4 were categorized as susceptible. Plants that displayed randomly scattered uredinia of varying sizes or a mesothetic response were deemed resistant and assigned an 'X' type of IT [19, 63].

Genotyping and SNP filtering

The GDP accessions included in this study were genotyped using the Illumina iSelect 90K SNP array [75]. A total of 42,520 polymorphic SNPs were identified in the GDP accessions. The genotyping data of the GDP panel was filtered based on various quality parameters and finally 15,144 SNP markers were retained. The SNP genotype data file was converted into HapMap format in TASSEL (Trait Analysis by Association, Evolution, and Linkage) software V.2.3.4 for subsequent analyses.

Population structure, phylogenetic relationships, and linkage disequilibrium

The population structure of the studied GDP germplasm subset was analyzed using the Bayesian inference program STRUCTURE 2.3.4 [56]. The STRUCTURE program is a model-based clustering method in which genotype data comprising unlinked markers are used to infer population structure. The input data comprise a

matrix where the rows represent data from individuals and columns express SNP loci. The putative number of subpopulations (K) in the GDP subset was determined using STRUCTURE v2.2 using 10,000 burn-in iterations followed by 10,000 MCMC for K-values ranging from K = 1 to 8. The kinship of the genotypes was determined using TASSEL v.2.3.4 software. The genetic relationships among GDP germplasm lines were assessed by constructing a phylogenetic tree using TASSEL v.2.3.4 software. Linkage disequilibrium (LD) was measured using the correlation (r^2) in frequency among allele pairs across a pair of markers by using TASSEL V.2.3.4 software. LD decay was observed and performed by plotting the r^2 (pairwise LD) values against the physical distance. The pattern of LD decay was determined by fitting a locally weighted polynomial regression (LOESS) curve to the physical distance where the LOESS curve intercepts the r^2 threshold value of 0.1.

Marker-trait association analysis

GWAS analysis was performed by six different multi-locus methods, including mrMLM [77], pLARmEB [82], ISIS EMBLASSO [70], FASTmrMLM [71], pKWmEB [62] and FASTmrEMMA [78] which are included in the R package mrMLM v4.0.2 [83]. A logarithm of the odds (LOD) score of ≥ 3.00 was set as a critical threshold for a significant association. The SNPs that were associated with the target traits in at least two methods were designated as reliable SNPs.

Identification of candidate genes

To identify potential candidate genes within the genomic regions significantly associated with leaf rust-resistance traits, the probe sequences of corresponding SNPs were searched against the *Triticum turgidum* genome assembly Svevo.v1 (genomic sequence) in the online web resource Ensemble Plants (https://plants.ensembl.org/Triticum *turgidum*/Tools/Blast).

In silico expression of candidate genes

The RNA sequencing data of the leaf rust-resistant wheat genotype were obtained for control and leaf rust-inoculated conditions after 6 h and 24 h (NCBI bioproject: PRJEB41456). The data were then subjected to quality control (QC) and trimmed using Trimmomatic to retain reads with a Q value of ≥ 20. The FPKM values of the candidate genes were then used to generate a heatmap using R software.

Results

Phenotyping

The screening experiments were performed under controlled glasshouse conditions during the main wheat cropping season of 2022 and 2023. The phenotypic responses (infection scores) of GDP genotypes recorded in both experiments are presented in Supplementary Tables 2 and 3. The GDP lines displayed different infection types (ITs) ranging from '0' to '4' along with the mesothetic response. On the basis of the degree of resistance and susceptibility, the disease reactions were categorized as immune, very resistant, moderately resistant, moderately susceptible, susceptible, highly susceptible, and heterogeneous for all the pathotypes (Table 1, Fig. 1). The percentage of accessions displaying an immune response against each rust-resistant pathotype varied widely, ranging from 2.5% for pathotype 77–9 to 26% for pathotype 77–5. When resistant and immune accessions were combined into a broad resistance category, the percentage of individuals showing resistance was notably higher against pathotypes 77–6 and 104. In other words, pathotypes 77–6 and 104 may be less virulent than pathotypes 77–5, 12–5, and 77–9. Furthermore, a total of 5

Table 1 Distribution of GDP accessions into different categories based on their average response to five Indian leaf rust pathotypes (77–5, 77–6, 77–9, 12–5 and 104) across two experiments conducted under glasshouse conditions during the main wheat cropping seasons of 2022 and 2023 respectively

S.No	Category	Reaction type[a]	GDP accessions (%) against various pathotypes				
			77–5	77–6	12–5	77–9	104
1	Immune	0;	5	26	3	2.5	24
2	Very resistant	;1–1 +	35	19.5	23.5	19.5	20.5
3	Moderately resistant	2	5	0	1	1.5	0
4	Moderately susceptible	3- to 3	26	27.5	39	50	23.5
5	Susceptible	3 + to 3 + +	1.5	9.5	0	0	21
6	Highly susceptible	4	0	0	0	0	0
7	Heterogeneous	X	27.5	17.5	33.5	19	11

[a] Reaction type recorded as per the scale described by Stakman et al. [69]

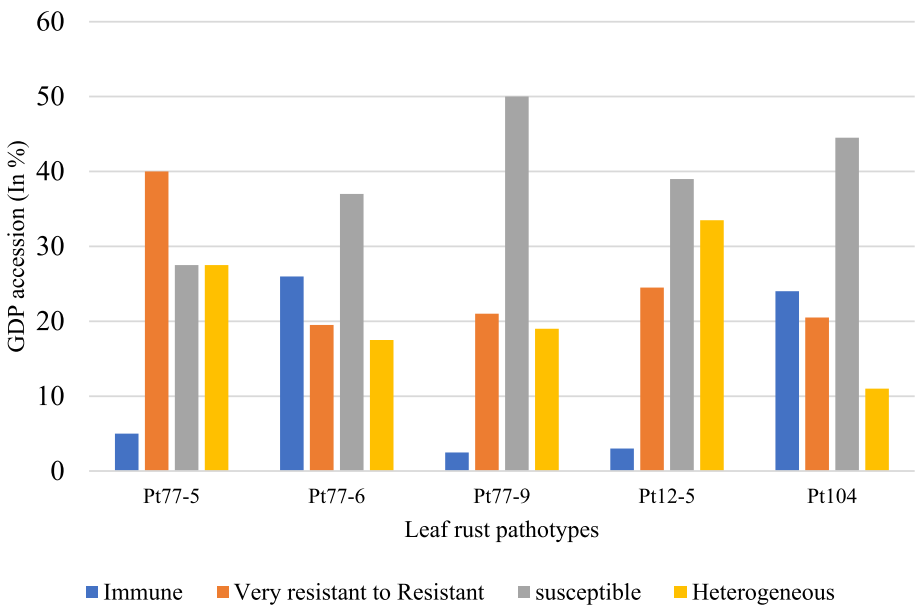

Fig. 1 Distribution of GDP accessions into immune, very resistant to resistant, susceptible and heterogeneous classes on the basis of screening against the five different pathotypes of leaf rust

accessions (GDP810, GDP845, GDP855, GDP921 and GDP941) were immune or highly resistant to all five pathotypes. Additionally, these five genotypes consistently exhibited an immune response or highly resistant ITs when challenged against the two most prevalent leaf rust pathotypes (77–5 and 77–9) in repeated controlled conditions experiments. The disease reaction scores for these five genotypes, along with those for the susceptible check (Agra Local) across four environmental conditions are provided in Supplementary Table 4.

Population structure, genetic diversity and linkage disequilibrium

The genotyping data of all 189 accessions consisted of information on 42,520 polymorphic SNPs. After several quality filtering steps, a total of 15,144 SNP markers were retained and used for population structure and other marker-based analyses. STRUCTURE software was used to analyze the population structure of the association panel (Fig. 2). By implementing Evanno's approach in the STRUCTURE HARVESTER, the optimum K was found to be three as the ΔK value was maximum at K = 3. Accordingly, the association panel was divided into three sub-populations (SPs) namely, SP1, SP2, and SP3 (Fig. 2A). Among the 189 accessions, 93 accessions were pure, 35 of which were (18.51%) grouped into SP1, 25 (13.22%) were grouped into SP2 and remaining 33 accessions (17.46%) were grouped into SP3 (Fig. 2B). A majority of the accessions ($n = 96$) were admixtures, of which 10 accessions (5.29%) were shared between SP1 and SP2, 15 accessions (7.96%) between SP1 and SP3, 33 accessions (17.46%) between SP2 and SP3, and 38 accessions (20.10%) were shared by all three subpopulations i.e., SP1, SP2 and SP3. Further, principal component analysis (PCA) was conducted to determine the principal components (PCs) that account for variations in the sample of genotypically different populations (Fig. 2C). There was minimal population stratification in the association panel (Fig. 2C). Furthermore, genetic relationships among the association panel genotypes was also analyzed using a neighbor-joining (NJ) tree (Fig. 2D). The entire association panel was divided into three clusters (1, 2, and 3) that broadly corresponded to the three subpoulations (SP-1, SP-2, and SP-3) respectively, derived from population structure analysis. Specifically, there were 62 genotypes in Cluster 1, 44 genotypes in Cluster 2, and 83 genotypes in Cluster 3.

LD decay analysis of the association panel revealed a LD block of 1.45 Mb for genome A and 1.04 Mb for genome B. However, when both the A and B subgenomes were considered together, the LD block size was 1.19 Mb (Fig. 3). The relatively short linkage disequilibrium (LD) decay distance observed in the association panel indicates a higher degree of genetic recombination within the population. This characteristic suggests that variants occur at reasonable intervals across the genome making this panel highly suitable for high-resolution mapping using GWAS to identify genetic variants associated with target traits.

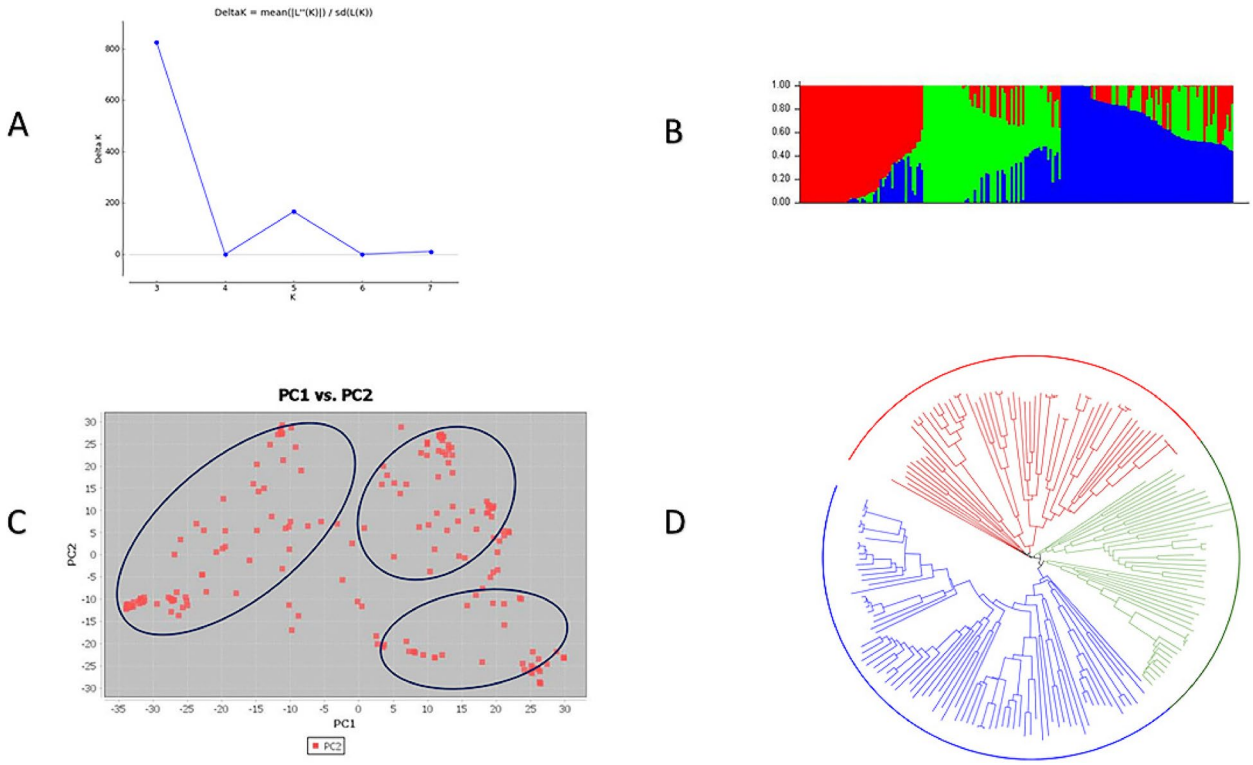

Fig. 2 Genetic diversity and population structure analyses in the association panel. **A** Delta K values for different numbers of populations assumed (K) in the STRUCTURE analysis. **B** Distribution of GDP subset accessions into three distinct subpopulations. The X- and Y-axis indicate GDP accessions and % membership to a genetic group, respectively. **C** PCA plot of first 2 components. **D** NJ phylogenetic tree based on the genetic relationships among GDP accessions

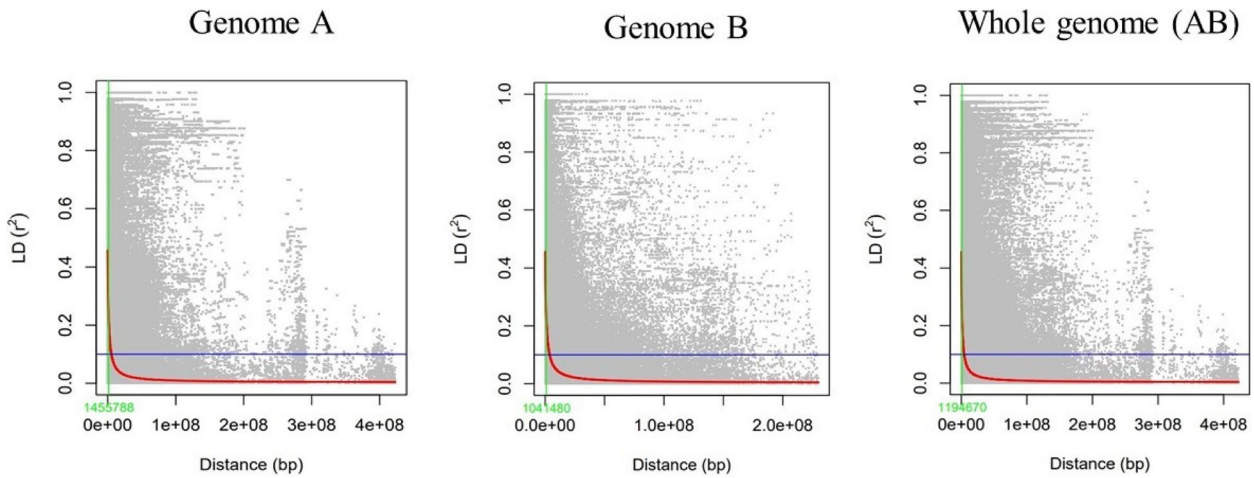

Fig. 3 LD decay patterns of the **A**, **B** and whole genomes (**AB**s) of 189 GDP accessions

Genome-wide association analysis

The study used six ML-GWAS models (mrMLM, FASTmrMLM, FASTmrEMMA, pLARmEB, ISIS EM-BLASSO, and pKWmEB) to identify genomic regions significantly associated with the response to leaf rust pathotypes. A total of 88 significant quantitative trait nucleotides (QTNs) were identified for 5 different leaf rust pathotypes (77–5, 12–5, 77–9, 104 and 77–6) with LOD score ≥ 3 (Supplementary Table 5). The distributions of these significant QTNs across different models were as follows:

FASTmrEMMA-8, mrMLM-13, FASTmrMLM-13, ISIS-EM-BLASSO-19, pKWmEB-17, and pLARmEB-18. The mrMLM was unable to detect any significant QTN for pathotype 77–6. Chromosome 6B contained the maximum number of QTNs (17), followed by chromosomes 3A (12), 3B (10), 1B and 6A (7), 1A, 4A and 5A (6), 5B and 7A (4), 6A and 7B (3), and chromosomes 4B, 2A and 2B containing only 1 QTN (Supplementary Table 5). Furthermore, out of these 88 QTNs, 22 were considered reliable, as they were consistently detected in two or more GWAS models (Table 2). The details of different QTNs/ genomic regions identified against each of the 5 pathotypes are provided below.

Pathotype 77–5

A total of 20 QTNs were associated with pathotype 77–5. Among these, 4 QTNs (*Q.77–5-1B*, *Q.77–5-3B.1*, *Q.77–5-3B.2*, and *Q.77–5-5B*) were identified using two or more GWAS models and were thus considered reliably associated genomic regions with this pathotype (Fig. 4). Furthermore, these reliable genomic regions were designated as major or minor QTNs depending on their percentage contribution to the trait. A genomic region was designated a major QTN if the phenotypic variance explained (PVE) by it was ≥ 10% in at least one GWAS model. The *Q.77–5-3B.1* (marker: BS00075879_51) on 3B was detected in 4 GWAS models i.e., mrMLM, ISIS EM-BLASSO, pLARmEB and FASTmrEMMA with LOD values ranging from 3.0–9.60, explaining 8.57–21.38% phenotypic variance (R^2). Among these 4 models, mrMLM and ISIS EM-BLASSO detected this QTN with values of 15.84% and 21.31% respectively.

Table 2 List of reliable QTNs identified for five-leaf rust pathotypes using different GWAS methods

S.NO	Pathotype	QTN	SNP Marker	Chr	Position	LOD Score (in range)	PVE (%)	GWAS method
1	77–5	*Q.77–5-1B*	Kukri_rep_c110309_129	1B	676,793,400	3.89–3.94	6.12–9.16	pKWmEB, FASTmrMLM
2	77–5	*Q.77–5-3B.1*	BS00075879_51	3B	823,777,171	3.0–9.60	8.57–21.38	mrMLM, ISIS EM-BLASSO, pLARmEB, FASTmrEMMA
3	77–5	*Q.77–5-3B.2*	RAC875_c69_1583	3B	747,126,734	3.58–4.56	4.95–7.45	mrMLM, pKWmEB
4	77–5	*Q.77–5-5B*	IAAV4830	5B	417,718,730	3.4–3.9	0–5.9	pLARmEB, mrMLM
5	12–5	*Q.12–5-3A.1*	BobWhite_c2868_183	3A	613,929,339	4.65–5.95	8.45–12.00	pKWmEB, FASTmrMLM, mrMLM,ISIS EM-BLASSO
6	12–5	*Q.12–5-3A.2*	Kukri_rep_c100057_198	3A	869,747	3.07–4.03	3.83–6.25	pKWmEB, FASTmrMLM, ISIS EM-BLASSO
7	12–5	*Q.12–5-5B*	wsnp_RFL_Contig1548_762547	5B	616,023,061	4.65–6.77	4.9–8.90	mrMLM, FASTmrMLM
8	12–5	*Q.12–5-6A*	wsnp_Ex_c31508_40288653	6A	347,612,974	3.42–5.0	4.55–5.20	pLARmEB, pKWmEB
9	12–5	*Q.12–5-6B.1*	wsnp_Ex_c34011_42398664	6B	439,368,191	3.3–6.4	2.65–7.8	pLARmEB, mrMLM, FASTmrMLM
10	12–5	*Q.12–5-6B.2*	Tdurum_contig54642_177	6B	659,808,359	3.3–4.6	3.5–5.95	pLARmEB, FASTmrEMMA
11	77–9	*Q.77–9-4A*	Excalibur_c38000_595	4A	573,831,833	3.35–6.42	5.33–8.17	FASTmrMLM, mrMLM, pKWmEB
12	77–9	*Q.77–9-5A*	GENE-3101_137	5A	421,138,304	3.30–4.64	3.48–5.29	ISIS EM-BLASSO, pKWmEB
13	77–9	*Q.77–9-6B*	Tdurum_contig54642_177	6B	659,808,359	3.85–4.64	5.78–9.07	FASTmrEMMA,ISIS EM-BLASSO, pKWmEB
14	104	*Q.104-3A*	BobWhite_c2868_183	3A	613,929,339	3.14–6.38	6.0–9.81	pLARmEB, pKWmEB
15	104	*Q.104-6A*	RAC875_c35430_373	6A	612,163,127	3.50–4.63	3.76–7.26	pKWmEB, ISIS EM-BLASSO
16	104	*Q.104-1B.1*	Ra_c349_1237	1B	9,054,547	4.24–4.56	5.95–10.98	mrMLM, FASTmrMLM
17	104	*Q.104-1B.2*	BS00064948_51	1B	675,361,064	3.43–4.09	3.94–7.057	pKWmEB,ISIS EM-BLASSO
18	104	*Q.104-6B.1*	wsnp_Ku_c1876_3666308	6B	532,595,022	4.57–7.58	6.98–12.29	pKWmEB,ISIS EM-BLASSO
19	104	*Q.104-6B.2*	Excalibur_c98849_278	6B	550,910,996	3.50–4.96	4.31–5.80	FASTmrMLM, pLARmEB
20	104	*Q.104-7B*	Tdurum_contig75127_589	7B	697,951,719	3.0–4.44	4.34–8.59	FASTmrEMMA, ISIS EM-BLASSO
21	77–6	*Q.77-6A*	Excalibur_c77841_224	6A	611,027,231	3.61–7.70	7.90–14.27	ISIS EM-BLASSO, pLARmEB, FASTmrMLM, pKWmEB, FASTmrEMMA
22	77–6	*Q.77-6B*	CAP11_c1087_327	6B	2,064,685	3.66–5.188	0–5.80	ISIS EM-BLASSO, pLARmEB

QTN quantitative trait nucleotide, *Chr* chromosome, *PVE* phenotypic variation explained

Pathotype 12–5

A total of 19 QTNs were associated with pathotype 12–5 (Fig. 5). Of these, six QTNs were detected in 2 or more GWAS models, spanning over five chromosomes 3A, 5B, 6A and 6B (*Q.12–5-3A.1*, *Q.12–5-3A.2*, *Q.12–5-5B*, *Q.12–5-6A*, *Q.12–5-6B.1* and *Q.12–5-6B.1)*. Among these only one QTN on chromosome 3A (*Q.12–5-3A.1*) was major with PVE ≥ 10%. The PVE for this QTN was estimated as 10.55% in the pKWmEB model and 12.0% in the mrMLM model.

Pathotype 77–9

A total of 15 QTNs were associated with pathotype 77–9. However, only three of them were consistent across ≥ 2 GWAS models (Fig. 6). These QTNs were located on chromosomes 4A, 5A and 6B and were detected at LODs ranging from 3.30–6.42 with PVEs ranging from

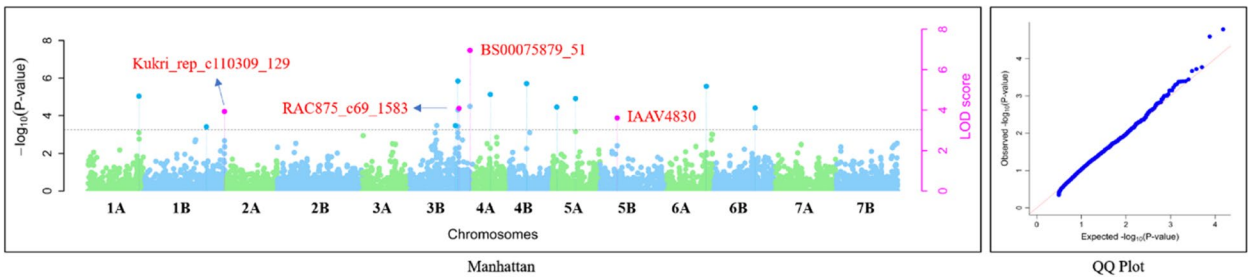

Fig. 4 Manhattan and Q-Q plots of GWAS for the pathotype 77–5 generated with mrMLM v4.0.2. Loci identified using multiple methods are indicated by pink dots on the Manhattan plot, whereas those detected by a single method are marked with dark blue dots. The remaining two colour dots in the Manhattan plot, light blue and green, represent SNP markers that are alternately distributed across 14 chromosomes of tetraploid wheat. The horizontal line represents a critical LOD score of 3.0

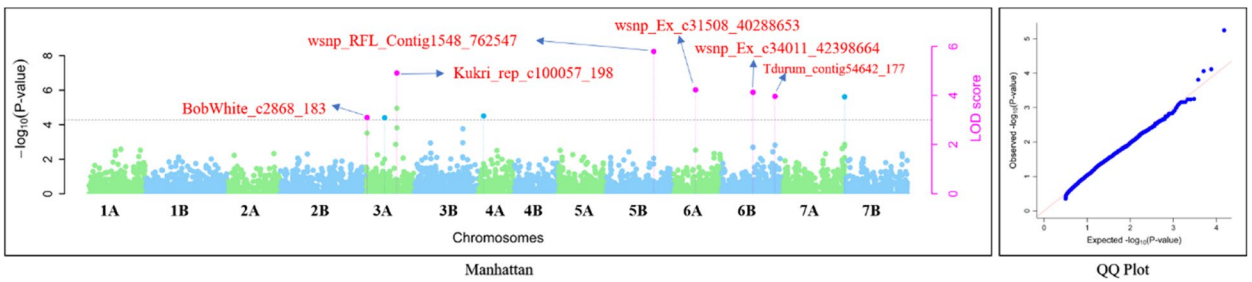

Fig. 5 Manhattan and Q-Q plots of the GWAS for pathotype 12–5, generated with mrMLM v4.0.2. Loci identified using multiple methods are indicated by pink dots on the Manhattan plot, whereas those detected by a single method are marked with dark blue dots. The remaining two colour dots in the Manhattan plot, light blue and green, represent SNP markers that are alternately distributed across 14 chromosomes of tetraploid wheat. The horizontal line represents a critical LOD score of 3.0

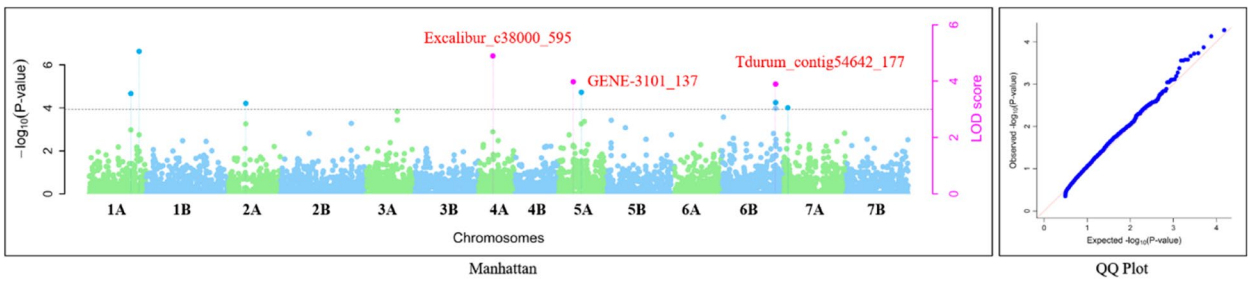

Fig. 6 Manhattan and Q-Q plots of the GWAS for pathotype 77–9, generated using mrMLM v4.0.2. Loci identified using multiple methods are indicated by pink dots on the Manhattan plot, whereas those detected by a single method are marked with dark blue dots. The remaining two dots in the Manhattan plot, light blue and green colour, correspond to SNP markers, alternatively present on 14 chromosomes of tetraploid wheat. The horizontal line represents a critical LOD score of 3.0

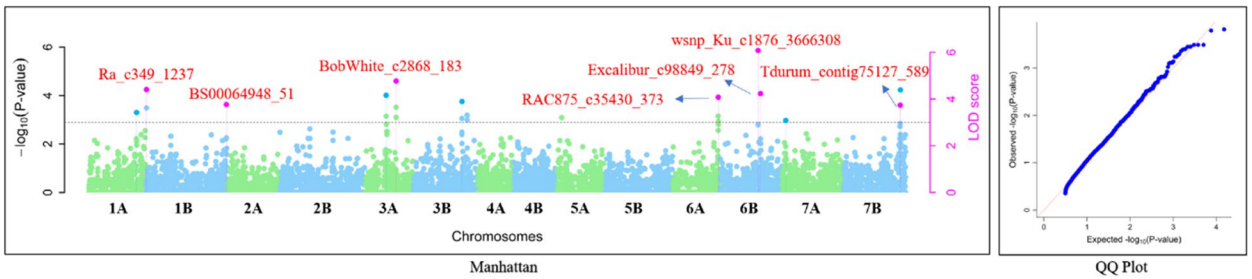

Fig. 7 Manhattan and Q-Q plots from the GWAS analysis for pathotype 104, generated with the mrMLM v4.0.2. Loci identified using multiple methods are indicated by pink dots on the Manhattan plot while those detected by a single method are marked with dark blue dots. The remaining two colour dots in the Manhattan plot, light blue and green, represent SNP markers that are alternately distributed across 14 chromosomes of tetraploid wheat. The horizontal line represents a critical LOD score of 3.0

3.48–9.07%. The pKWmEB model was found to be most effective for identifying loci associated with pathotype 77–9 as it could identify all the three associated QTNs consistently identified by one or more other models.

Pathotype 104

Association analysis revealed 15 QTNs associated with pathotype 104 (Fig. 7). The maximum number (7) of reliable QTNs were associated with this pathotype which were distributed across five chromosomes 1B, 3A, 6A, 6B and 7A (*Q.1041B.1*, *Q.104-1B,2, Q.104-3A, Q.104-6A, Q.104-6B.1, Q.104-6B.2* and *Q.104-7A*). Among these, two QTNs, *Q.104-1B.1* and *Q.104-6B* were classified as major, as both had a PVE ≥ 10% in at least one GWAS model. *Q.104-1B.1* was detected in two models, mrMLM and FASTmrMLM with PVE values of 5.95% and 10.98% respectively. Similarly, Q.104-6B was identified in the pKWmEB and ISIS EM-BLASSO models with PVE values of 6.98% and 12.29% respectively.

Pathotype 77–6

A total of 15 QTNs were identified to be associated with pathotype 77–6 (Fig. 8). Of these, two QTNs namely *QTN77-6-6A* and *QTN77-6-6B* were considered reliable. *QTN 77–6-6A*, located on chromosome 6A, stood out as the most reliable, having been detected by five different models including ISIS EM-BLASSO, pLARmEB, FASTmrMLM, pKWmEB, and FASTmrEMMA, with LOD scores ranging from 3.61–7.70. This QTN explained a phenotypic variance ranging from 7.90% to 14.27%, indicating its significant role as a major QTN governing the response of tetraploid wheat against pathotype 77–6. The results for this QTN were highly consistent across three GWAS models FASTmrMLM, ISIS EM-BLASSO, and pKWmEB which revealed a PVE of 13.22%, 14.27% and 14.53% respectively.

Furthermore, we examined the GWAS results to identify any common QTNs associated with two or more leaf rust pathotypes. Two such QTNs (markers) were identified. The marker Tdurum_contig54642_177 on chromosome 6B was detected for pathotypes 12–5 and 77–9 while another marker BobWhite_c2868_183 on chromosome 3A was associated with pathotypes 12–5, 77–6 and 104.

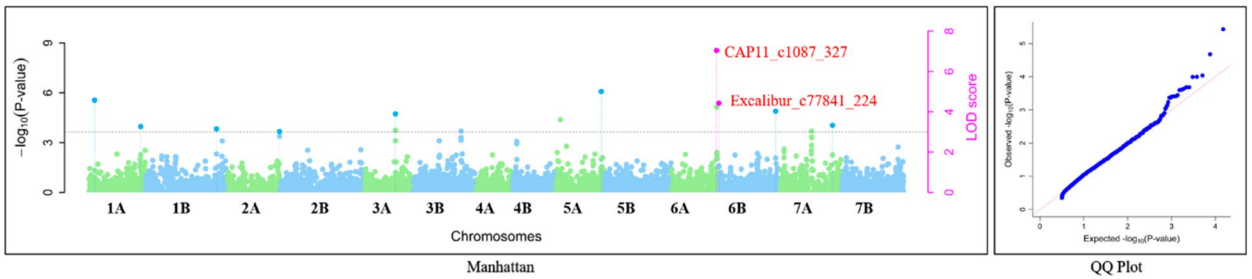

Fig. 8 Manhattan and Q-Q plots of the GWAS for pathotype 77–6, generated with mrMLM v4.0.2. Loci identified using multiple methods are indicated by pink dots on the Manhattan plot, while those detected by a single method are marked with dark blue dots. The remaining two colour dots in the Manhattan plot, light blue and green, represent SNP markers that are alternately distributed across 14 chromosomes of tetraploid wheat. The horizontal line represents a critical LOD score of 3.0

Identification of candidate genes and their *in-silico* expression analysis

To identify candidate leaf rust-resistant genes/transcripts in the identified genomic regions, the SNPs (probe sequences) associated with leaf rust resistance traits were searched against the *T. turgidum* genome assembly Svevo.v1 in the Ensembl Plants genome database. The genes present in the LD decay distance region, which is ~ 1 Mb on either side of the SNP, were considered putative candidates for rust resistance. Over 300 annotated genes were present in the LD decay region of 1 Mb surrounding the 22 reliable QTNs identified in the study (Supplementary Table 6). These genes included a total of 63 genes with defense-related functions, including R genes. A maximum of 19 R-genes were located in the 2 Mb region on chromosome 6B around the QTN *RAC875_c35430_373*. The classification of all 304 genes on the basis of their functional role revealed that the majority of them encode enzymes with functions such as kinases, antioxidant enzymes, and those involved in mitochondrial energy production (Fig. 9). Additionally, a few genes in the identified regions encode DNA-modifying enzymes such as histone acetyltransferases and cysteine methyltransferases, along with other cellular and metabolic enzymes. Genes encoding structural proteins, such as ribosomal proteins, membrane-embedded proteins such as V-ATPases, sugar transporters, and intracellular trafficking proteins such as SNARE proteins, were also identified. Six zinc ion-binding proteins with zinc-finger domains, which likely play a role in the transcriptional response of downstream effector genes, were also identified. Signaling proteins with WD40 repeats that function in plant immune signaling pathway [52], the START domain and the C2 domain are additional candidates identified in this study.

An in silico expression analysis of 63 R-genes identified from the remaining resistance-associated regions revealed that 50 of these *R*-genes were differentially expressed between rust pathogen-treated and control wheat plants at 6 and 24 h post-inoculation [9]. The gene *TRITD6Bv1G224600* located near the QTN RAC875_c35430_373 on chromosome 6B was significantly upregulated in the leaf-resistant genotype in response to leaf rust pathogen inoculation. Its expression was 52-fold and fold higher in inoculated plants than in control plants at 24 h and 6 h post-inoculation, respectively. Another *R*-gene, *TRITD6Av1G225060* on chromosome 6A, located adjacent to the QTN Excalibur_c77841_224, showed a 28-fold higher FPKM value in the inoculated plants than in control plants. A heatmap depicting the expression analysis of all 50 genes is shown in Fig. 10.

Discussion

Cultivated wheat faces challenges from various biotic factors such as rusts, smuts, bunts etc., which can cause considerable damage resulting in lower productivity. Among these, leaf rust in particular has become a major concern of wheat breeders globally in recent years, as new pathotypes of the pathogen have emerged, leading to the loss of resistance in many regions. The problem has

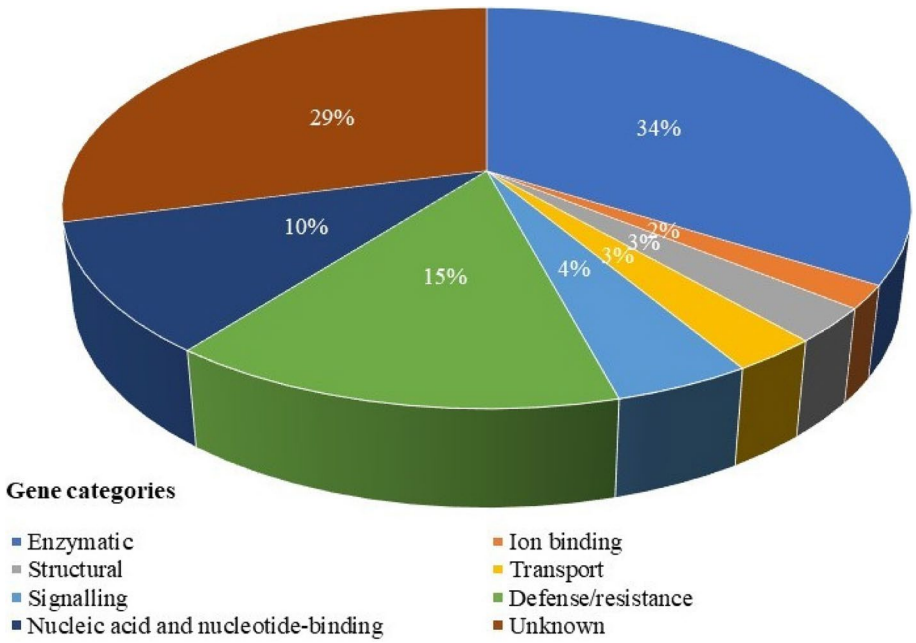

Fig. 9 Distribution of genes identified within the associated genomic regions into different functional classes

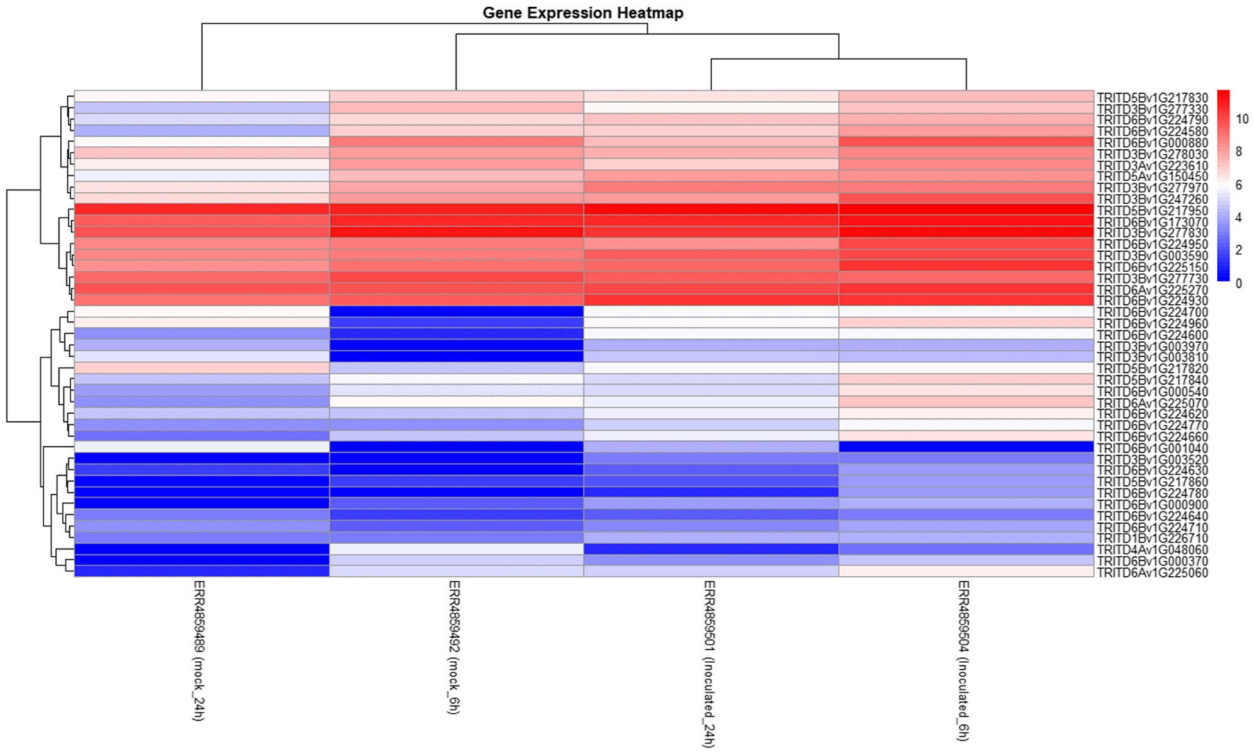

Fig. 10 Heatmap showing theexpression levels of defense-related genes in the leaf tissue transcriptome of wheat at 6 h and 24 h after infection with the *Pt* pathotype. The colour scale represents the relative expression level from low (blue) to high values (red). The conditions included mock and inoculated

been further aggravated by climate change, which may alter the pathogen's life cycle, potentially shifting pathogen populations and increasing virulence. The emergence of new *Pt* pathotypes has led to the resistance breakdown in many regions with wheat varieties often becoming susceptible within 4–5 years. For example, an evaluation of the *Pt* pathotypes population of India from 2016 to 2019 revealed that just two pathotypes 121R60-1 (77–9) and 121R63-1 (77–5) accounted for 79.46% of the population [6]. Many other studies on *Pt* variability have consistently shown that one or two races tend to dominate within a geographical region, causing severe damage to wheat crops [18, 2]. Additionally, variations in climatic conditions such as prolonged droughts, changes in rainfall patterns, and rising temperatures can also influence the population dynamics of *Pt* pathotypes. A comprehensive study conducted in Russia from 2001 to 2019 revealed a significant shift in *Pt* pathotype populations following two years of severe drought in 2010–2011 [20]. In addition to pathotype variability, the aggressiveness within a *Pt* pathotype may also change over time which can be significant drivers of evolution in pathogen populations. A recent study by Fontyn [17] reported that the decline of two dominant *Pt* pathotypes in France between 2005 and 2016 was primarily attributed to changes in their aggressiveness, which facilitated the evolution of new pathotypes. The continuous evolution of virulent pathotypes, coupled with changes in aggressiveness leads to the knockdown of common and broad-spectrum *Lr* genes underscoring the importance of identifying and transferring broad-spectrum leaf rust resistance genes.

Leaf rust management requires regular monitoring of pathotypes and identification of rust resistance genes from germplasm collections. Wheat breeders and pathologists have made consistent efforts to understand the genetic basis of host–pathogen interactions related to leaf rust. As a result, 83 leaf rust resistance genes have been identified and catalogued [4, 31, 38, 48]. The leaf rust resistance genes *Lr1*, *Lr3*, *Lr9*, *Lr10*, *Lr13*, *Lr14a*, *Lr17*, *Lr19* and *Lr23* are mainly associated with Indian wheat varieties. All of these have been rendered ineffective by the fast-altering leaf rust pathotypes. Even virulence has evolved against several alien-derived leaf rust resistance genes including *Lr9* [54], *Lr19* [8] and *Lr28* [7]. *Lr34,* an adult plant resistance gene that is not race-specific, is nonetheless useful in fighting this disease. The majority of the resistance genes are from hexaploid wheat and only a few resistance genes are known from tetraploid wheat including *Lr3, Lr14a*, *Lr23*, *Lr27+Lr31*, *Lr53*, *Lr61*, *Lr64*, *Lr72*, *Lr79*, *Lr83* and *LrCamayo* [31, 59,

60]. This calls for the need to explore the available global germplasm collection of tetraploid wheat including *T. durum* and the related subspecies *T. dicoccum* to identify new genes conferring resistance to *Pt* races.

Leaf rust resistance in global tetraploid wheat germplasm

In this study, we have evaluated tetraploid wheat germplasms from 28 different countries for resistance to 5 prevailing leaf rust pathotypes of India namely 77–5, 12–5, 77–9, 104 and 77–6. The large variation in the percentage of resistant genotypes against the five pathotypes suggested that genotypes in the global diversity panel have different genes or combinations of genes. Furthermore, the results also indicated that the GDP lines may possess major leaf rust resistance genes that are highly effective against the most prevalent pathotypes, while some of those lines are susceptible to pathotypes 77–9 (50% of the population showed susceptibility reactions) and 12–5 (40% of the population showed susceptibility reactions). The study revealed that only 2.5% of the accessions were immune to all the five studied pathotypes. The findings of the screening results are consistent with results from previous studies where only a small proportion of accessions have been identified as highly resistant to leaf rust pathotypes [46, 3]. Aoun et al. [3] evaluated a panel of durum lines against both hexaploid and durum-type leaf rust pathotypes. The results showed that the majority of the lines were resistant to hexaploid-type landraces, while only a small percentage exhibited resistance to durum-type races. Similarly, in yet another study, the evaluation of the Spanish core collection of tetraploid wheat against three *Pt* pathotypes prevalent in wheat-growing regions of Spain yielded comparable results [46]. In our study, the accessions classified into the class 'susceptible' were those whose infection type ranged from '3+' to '3++', although none of the accessions were 'highly susceptible'. A total of 1.73% to 4.62% of the accessions in this class were susceptible to all the pathotypes, but 21% of the accessions were susceptible to pathotype 104. Further, we observed that 24% to 39% of the population exhibited a moderately susceptible response to pathotypes 77–5, 12–5 and 104. However, a surprisingly large percentage of the population was moderately susceptible to 77–9. Overall, when we considered the different susceptible classes as one, an average of 27–50% of the lines belonged to the susceptible class with the highest number for pathotype 77–9 followed by pathotype 104. Further in our analysis, minor differences were observed in the disease scores of at least a few genotypes across two independent controlled condition experiments. This variation was anticipated due to the inherent complexity of disease development, which is influenced by factors such as host plant physiology and genetic factors. Additionally, slight differences in the quality and quantity of inoculum, even under controlled conditions, can contribute to these minor variations. A recent study characterized resistance in durum wheat varieties against leaf rust under climate change conditions, including elevated temperatures and CO2 levels, in a greenhouse setting. The findings revealed that the durum wheat response to infection was primarily driven by temperature [57]. The temperature sensitivity of *Lr* genes has already been reported for some tetraploid-origin genes, such as *Lr18* from *Triticum timopheevii* which is most effective at 18 °C and becomes completely ineffective when temperature exceeds 25 °C [10]. Similarly, *Lr14a,* another tetraploid-origin gene was highly effective at 15 °C [36]. On the other hand gene, *Lr23* identified from the durum wheat variety 'Gaza' is known to be effective at temperatures around 25 °C but becomes ineffective ≥ 30 °C [16] The temperature sensitivity of *Lr* genes underscore the need for a diverse pool of genes that can provide resistance at elevated temperatures or remain unaffected by temperature variations. This is particularly crucial in light of the anticipated rise in Earth's surface temperature due to climate change [50].

LD Decay

The extent and distribution of linkage disequilibrium in the genome define regions that are inherited together. Factors such as the recombination rate and the number of generations of recombination affect LD decay, thus defining the resolution of association mapping. Therefore, information on LD decay patterns is critical for assessing the number of SNP markers required for achieving a reasonable scale GWAS [74, 81].

LD in the global durum panel subset decayed at 1.45 Mb for the A genome, 1.04 Mb for the B genome, and 1.19 for the A and B genomes combined. The LD decay distance of the subset was relatively faster than those reported in previous studies [64, 72, 76]. Basi et al. [5] observed LD decay at a distance of 51.3 Mb in a collection of ICARDA lines. Similarly, in another study, the LD decay distance in a worldwide durum wheat panel was recorded as 11.8 Mb whereas it was 30.50 Mb in a set of durum lines from Argentina which was much higher [64]. The short LD decay distance observed in our study was expected as the genotypes in the durum subsets were highly diverse, representing 28 different countries. The accessions from each country might have accumulated historical recombination resulting in faster LD decay in the set.

Population structure and phylogenetic analysis

Population structure (stratification) in a group of individuals can be defined as differences in allele frequencies of its subgroups which may arise due to non-random

factors such as migration, mutation, etc. Genetic variants need not always be associated with a trait; nevertheless, sometimes variants may coincidentally correlate with a target trait because of population stratification. Therefore, structure in the association panel is considered a major confounding variable in marker traits association analysis and should be accounted for in GWAS analysis [42]. Population structure can influence LD and likewise LD-decay estimates in any population [74]. In our analysis, three subpopulations were observed in the GDP subsets. The maximum number of genotypes were clustered in SP-3 (83 genotypes). The genotypes in this cluster mainly represented countries such as Iran, Italy, Ethiopia, Georgia, Hungary, Russia, Syria, Portugal, and Lebanon. The distribution of the genotypes in the subpopulations was broadly according to their origin. A similar clustering pattern for the durum wheat genotypes was also observed in the phylogenetic tree drawn using the neighbor-joining method [42].

Identification of genomic regions controlling leaf rust resistance

GWAS is a powerful approach for identifying genomic regions controlling a target trait. Our study revealed that a total of 88 significant QTNs were associated with resistance to 5 different leaf rust pathotypes. The number of QTNs identified to be associated with the five pathotypes were as follows: 77–5 (20 QTNs), 12–5 (19 QTNs), 77–9 (15 QTNs), 104 (19 QTNs), and 77–6 (15 QTNs). These included 2 QTNs on chromosome 3B (at positions 823.77 Mb and 747.126 Mb) and one each on chromosome 1B (at 676.79 Mb) and 5B (at 417.71 Mb). Notably, chromosome 3B is known to harbor several leaf rust resistance genes including *Lr27+31, Lr53, Lr74, Lr77* and *Lr79* [12, 27, 35, 59]. *Lr27*, derived from *T. aestivum* Gatcher, has been mapped and found to be linked with *Sr2* [41], whereas *Lr74* is an adult plant resistance gene mapped in *T. aestivum* [35]. Among these genes, *Lr79* is notable because it is located on the long arm of chromosome 3B, whereas other seedling-stage resistance genes are located on the short arm. The identification of two genomic regions on the long arm of chromosome 3B suggests that at least one of these regions represents a novel locus for leaf rust resistance [27]. Furthermore, no leaf rust resistance genes from durum wheat have been reported on chromosomes 1B and 5B, suggesting that the QTNs identified for pathotype 77–5 on both these chromosomes may represent novel genomic regions associated with this trait.

Six QTNs are identified in response to pathotype 12–5. Specifically, one QTN was located on chromosome 5B, one on chromosome 6A and two QTNs each were found on chromosomes 6B and 3A. Notably, the QTNs identified on chromosome 6B coincided with previously reported leaf rust resistance genes. However, the QTNs identified on chromosomes 5B, 6A and 3A may represent novel leaf rust resistance genes or genomic regions as no QTLs for leaf rust resistance have been previously identified on these chromosomes. These findings suggest that these regions could harbor new genetic elements contributing to resistance against pathotype 12–5.

In response to pathotype 77–9, three significant SNPs were identified, namely, Excalibur_c38000_595 on chromosome 4A at 573.83 Mb, Tdurum_contig54642_177 on chromosome 6B at 659.00 Mb and GENE-3101_137 on chromosome 5A at 421.13 Mb. Among these SNPs, the one on chromosome 6B coincides with the previously reported leaf rust resistance gene *LrOft* from the durum cultivar Ofanto [84]. The other identified genomic regions appear to be novel and can be further explored either through a bi-parental approach or through a functional genomics approach to identify and clone genes for leaf rust resistance.

A total of seven QTNs: wsnp_Ku_c1876_3666308, Excalibur_c98849_278, RAC875_c35430_373, Ra_c349_1237, BS00064948_51, BobWhite_c2868_183, and Tdurum_contig75127_589 were identified for pathotype 104. Of these, two QTNs each were located on chromosomes 6B (at 532.59 Mb and 550.91 Mb) and 1B (at 90.54 Mb and 675.36 Mb). The remaining QTNs were located on chromosomes 6A (at 163 Mb), 3A (at 613.92 Mb) and 7B (at 697.95 Mb). The two QTNs on chromosome 6B appear to coincide with the previously known *LrOft* gene and are near to the QTNs identified for pathotype 12–5, suggesting that this is a common and functional genomic region for rust resistance. The QTN on the short arm of chromosome 1B (90.54 Mb) seems to coincide with *Lr75* [67]. On chromosome 7B, four leaf rust resistance genes have been identified and mapped: *Lr14a* (on 7BL) from *T. durum*, *Lr14b* (on 7BL) from *T. aestivum* 'Maria Escobar', *Lr68* from *T. aestivum* 'Parula', and *Lr72* (on 7BS) from *T. turgidum durum* 'Altar 84'. Therefore, the three QTNs located on 1B (on the long arm), 6A, and 3A appear to represent novel genomic regions for leaf rust resistance.

For pathotype 77–6, two SNPs were found (*Excalibur_c77841_224,* and *CAP11_c1087_327*) located on chromosomes 6A and 6B at positions 611.027 Mb and 2.06 Mb, with R2 values 4.3% to 8.4% respectively. To date, three leaf rust resistance genes *Lr56*, *Lr62*, and *Lr64* have been mapped on chromosome 6A. Interestingly none of these genes are derived from the durum germplasm. *Lr56* is derived from *Ae. sharonensis* and was identified by Marais et al., [45]. *Lr62* was derived from *A. neglecta* and was identified and mapped by Marais et al. [43]. Whereas, *Lr64* is of *T. dicoccoides* origin and

was identified and mapped by Kolmer et al. [30] in the Thatcher wheat line RL6149.

A common marker, *Tdurum_contig54642_177,* on chromosome 6B was detected for pathotypes 12–5 and 77–9. Another common marker BobWhite_c2868_183 on chromosome 3A was detected for pathotypes 12–5, 77–6 and 104. The leaf rust resistance genes *Lr63* from *T. monococcum* and *Lr66* from *Ae. speltoides* were located on the chromosome 3A [34, 44]). However, to date, no QTLs have been reported on chromosome 3A from durum or any tetraploid wheat germplasm, which suggests that the QTN identified in this study represents a novel locus. Additionally, 9 of these QTNs on chromosomes 5B (2), 6A (2), 7B (1), 4A (1), 5A (1) and one each on the long arms of chromosome 1B and 3B are novel regions identified in the current study.

Candidate gene annotation within 20 reliable QTNs/genomic regions revealed defense-related genes encoding diverse functional groups of proteins. The annotation of the associated genomic regions revealed 62 genes associated with disease resistance or defense responses. Among these, the expression of two genes belonging to the NBS-LRR family namely, *TRITD6Bv1G224600* located in the vicinity of the QTN RAC875_c35430_373, and *TRITD6Av1G225060* which is in proximity to the QTN Excalibur_c77841_224 was found to be upregulated in response to infection with leaf rust pathotypes, suggesting their potential involvement in conferring resistance to leaf rust. Several important leaf rust-resistance genes that encode for LRR family proteins including *Lr10* [14], *Lr21* [24], and *Lr1* [11] have already been identified. Therefore, additional LRR family genes identified from our study using a diverse tetraploid germplasm collection might also be involved in conferring resistance to emerging leaf rust pathotypes. The other groups of genes such as serine-threonine kinase, thioredoxin, and protein kinases are also known to confer resistance against diseases [14, 65]. Notably, in plant species with complex genomes, such as wheat, the presence of repetitive resistance gene sequences is common, as these have several regions with duplications. The presence of such genomic regions carrying many genes for resistance can be very important as they can provide durable and broad-spectrum resistance against several pathogens. The identified chromosomal region further needs to be explored to identify SSR and SNP-based markers, which are polymorphic between resistant and susceptible individuals and also cosegregate with the trait in biparental mapping populations or independent germplasm populations. If the gene/allele thus identified is found to be novel, then it can be introgressed into elite backgrounds by marker-assisted breeding.

Conclusion

This study evaluated a diverse tetraploid wheat germplasm panel to identify variations in resistance against prevailing leaf rust pathotypes in India. Many germplasm lines were immune/ resistant, offering an opportunity to discover novel genes and alleles conferring resistance to leaf rust. The novel leaf rust resistant germplasm identified in this study can be exploited in wheat breeding programs for the development of wheat varieties with durable resistance. Moreover, multilocus-based GWAS identified 22 reliable QTNs conferring resistance to five predominant leaf rust pathotypes in India. Additionally, two important candidate genes were identified from two of the reliably associated leaf rust-resistant genomic regions. These regions can potentially be exploited in wheat breeding programs to develop wheat varieties with durable resistance to leaf rust.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06330-2.

Supplementary Material 1: Supplementary Fig. 1. A) Representative leaf sample of the accessions showing resistant and susceptible reactions against leaf rust pathotype 77–5, 1 = GDP-810, 2 = GDP-845, 3 = GDP-855, 4 = GDP-921, 5 = GDP-941 and B) Representative leaf sample of the accessions showing resistant and susceptible reactions against leaf rust pathotype 77–9, 1 = GDP-810, 2 = GDP-845, 3 = GDP-855, 4 = GDP-921, 5 = GDP-941.

Supplementary Material 2: Supplementary Table 1. Details of GDP genotypes included in the study.

Supplementary Material 3: Supplementary Table 2. Infection scores of GDP accessions against five leaf rust pathotypes under the controlled glasshouse conditions (temperature 18–24 °C) during the main wheat season of the year 2022.

Supplementary Material 4: Supplementary Table 3. Infection scores of GDP accessions against five leaf rust pathotypes under controlled glasshouse conditions (temperature 18–24 °C) during the main wheat season of the year 2023.

Supplementary Material 5: Supplementary Table 4. The infection scores of five GDP genotypes with immune/ highly resistant response and the susceptible check against two leaf rust pathotypes across four environments.

Supplementary Material 6: Supplementary Table 5. Details of 88 QTNs identified for infection response to five-leaf rust pathotypes in GDP subset using different GWAS methods.

Supplementary Material 7: Supplementary Table 6. Details of genes identified within flanking regions of reliably associated QTNs with leaf rust resistance.

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Author's contributions

JKY: Investigation, writing original draft; SS: Investigation, review and editing; HS, AKS, TKS, investigation, SKJ: Investigation, resources, review and editing ,

Jyoti Kumari: resources review & editing, MV, SK, RS, GPS: writing, review & editing; AKS: Conceptualization, resources, writing, review and editing. All authors reviewed the manuscript.

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Data availability
Genotyping data of the GDP accessions used in this study are accessible at https://wheat.pw.usda.gov/GG3/global_durum_genomic_resources.

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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