Original article

Genome wide association mapping through 90K SNP array against leaf rust pathogen in bread wheat genotypes under field conditions

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ABSTRACT

In wheat crop the leaf rust is an infectious disease, threatening wheat production annually. Identification of resistance genes or markers traits associations for effective field resistance could greatly enhance our ability to breed durably resistant varieties. Herein 105 bread wheat genotypes were evaluated against the leaf rust susceptible and resistance during the two seasons 2018–19 and 2019–20 under field conditions using CI, AUDPC and FDS parameters. Based on the performance of genotypes against leaf rust pathogen, 34 genotypes categorised as resistance (R), followed by 30, 32 and 9 genotypes classified as moderately resistance (MR), moderately susceptible (MS) and susceptible (S) genotypes. respectively. We also applied a genome wide association study (GWAS) approach to identify markers trait associations (MTAs) resistance against leaf rust using 90 K SNP Array. Marker–trait association results indicated that total 56 MTAs were significantly associated at $P \leq 10^{-3}$ using Bonferroni adjustment after crossed FDR ≤ 0.05 with studied parameters under field conditions. Among them, total numbers of associated MTAs 17, 23 and 16 were identified for CI, AUDPC and FDS parameters, respectively. Maximum significant MTAs were identified on chromosomes 2A (7), 3B (7), 5A (7) followed by 4A (5), 7B (5), 1A (4) and 7A (4) in the studied genotypes. The locus (*Kukri_c55051_414*) showed pleotropic effects for leaf rust identified parameters on chromosome 5A at position of 68.2 cM under field conditions. This study demonstrated the use of genome-wide association mapping for the identification of potentially new genomic regions associated with studied parameters. Leaf rust resistant genotypes identified in this study proved to be more productive under preliminary field conditions against leaf rust pathogen. Thus, these resistant accessions could be used as parental genotypes in breeding programs for leaf rust resistance.

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1. Introduction

Wheat leaf rust, caused by the fungus *Puccinia triticina* Eriks, is a threat to world wheat production. Reduced yield imposes food security challenges as a result of increasing demands for wheat consumption from the same land or even decreasing area to feed the ever increasing population of the world (Anwaar et al., 2019). Genetic resistance is the most effective, economic, and environmentally safe method to control and reduce losses caused by this disease (Zegeye et al., 2014). Identification of resistance genes

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using molecular markers is an important step toward marker assisted selection and resistance breeding. To date, there have been several leaf rust resistance genes identified, the majority of which confer leaf rust resistance in the seedling stage and are race-specific. However, continuous evolution of new leaf rust races requires constant search for new sources of resistance with novel QTL/genes (Juliana et al., 2018). Earlier, (Pathan and Park 2006) assessed a kind of partial resistance called adult plant resistance (APR) to brown rust in European lines and reported various levels of APR among the investigated wheat lines by means of average coefficient of infection (ACI) by estimating average coefficient of infection (CI) across different locations.

Based on the final disease severity (FDS) and area under disease progress curve (AUDPC), (Macharia and Wanyera 2012) reported infection responses of the wheat cultivars and classified them into four discrete categories as 1 susceptible (S), 2 moderately susceptible (MS), 3 moderately resistant (MR) and 4 resistant (R). Wheat scientists (Taye et al., 2015; Anwaar et al., 2019) reported that area under disease progress curve (AUDPC) and the final disease severity (FDS) were negatively correlated with final grain yield as well as yield contributing components, and very positively correlated with grain yield loss. Thus, it demonstrated a negative relation when there is an increase in these disease parameters there will be a decline in yield related parameters and vice versa.

(Macharia and Wanyera 2012) investigated fifteen wheat cultivars including six advanced lines from CIMMYT germplasm and others were commercially grown cultivars in Kenya and depicted that disease severity and area under disease progress curve were highly positively correlated with yield losses thus, were negatively correlated with grain yield and yield contributing components. (Hasan et al., 2012) assessed grain yield losses due to brown rust disease on the five-local commercial susceptible wheat cultivars under field conditions during 2011–2012 growing seasons and was depicted a relationship between rust severity and yield components which resultantly converted to financial losses. These results endorsed by (El-Shamy et al., 2011) who found a significant relation among percentage losses of 1000 grain weight, grain yield and final rust severity.

(Ochoa and Parlevliet 2007) described that yield losses were strongly correlated with area under disease progress curve in cereal crops. (Singh et al., 2011) reported highly significant ($P < 0.01$) negative correlation of rust severity and AUDPC with grain weight and yield while evaluating 5107 advanced breeding lines in field trials at Mexico in 2001, only 2.4% lines were highly resistant, 28% were moderately resistant and the rest of the lines were moderately susceptible to susceptible. Association mapping (AM) using phenotypic and genotypic data of association panels has become an important approach in identifying molecular markers (QTLs) linked to traits of interest for potential use in marker assisted selection for the fact that it enables to use diverse set of germplasm (landraces, cultivars, elite breeding lines, etc), and provides broader genomic region/allelic coverage with high resolution (Gao et al., 2016; Leonova et al., 2020).

Genome-wide association studies (GWAS) offer complementary approaches to understanding natural variation. GWAS generally combine phenotype and genotype data from 100 or more accessions to identify loci with allele frequency correlations to phenotypic variation or environment (Juliana et al., 2018). This approach can thus incorporate a relatively large portion of natural variation in a species and localize associations to much smaller genomic regions, because the sampled diversity incorporates many more recombination events than traditional recombinant inbred lines and/or doubled haploid populations (Zegeye et al., 2014). Information on the level of resistance of modern wheat varieties and genetic basis of resistance is needed to expand the pool of resistant cultivars available for breeding. GWAS allows us to deter-

mine the presence of both known resistance genes and previously unidentified loci (Leonova et al., 2020). For example, using this approach, a collection of 338 cultivated varieties of spring soft wheat was studied, and 46 QTLs responsible for resistance to leaf rust at seedling and adult plant stages were detected (Gao et al., 2016).

The main feature of this mapping panel is that a large amount of the germplasm possesses resistance to leaf rust and in some cases specific genes providing the resistance are known. It is possible that such a panel will allow us to identify resistance alleles that are normally not detected due to low allele frequency. This study aims to validate known genomic loci effective to leaf rust resistance and to identify novel genes or QTLs that are effective against the leaf rust pathogen in the seedling and (or) adult plant stages. Meanwhile, this study also explores the genetic architecture and phenotypic correlations for seedling and adult plant resistance and discusses ways to implement our research results in plant breeding and genetics efforts.

2. Materials and methods

2.1. Germplasm collection

The total 105 bread wheat genotypes were studied in this experiment. According to the maintaining sources, the germplasm divided into three groups as mentioned in Supplementary Table 1 (Ahmed et al., 2019). In first group the genotypes G-1 to G-20 developed in the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad (PBG-UAF), Pakistan, while second group genotypes G-21 to G-55 were from exotic source and third group genotypes G-56 to G-105 were from indigenous sources.

2.2. Experimental site and sowing conditions

Studied germplasm were sown by hand drill for screening against leaf rust in research area of Department of Plant Breeding and Genetics, The Islamia University of Bahawalpur (29º 24′N latitude, 71º 41′E longitude and 214 m above sea level) Pakistan under randomized complete block design (RCBD) in field with three replications to evaluate the leaf rust susceptible and resistance genotypes during the two seasons 2018–19 and 2019–20. For analysis purpose averaged data based on over years were used. Each genotype was planted in plot size of 1.2 m × 2.5 m and the experimental plots were surrounded by planting three rows of highly susceptible genotype Morocco. Inoculation was done artificially by means of various methods like dusting with talcum powder, rubbing, spraying with distilled water and needle injection methods on Morocco twice in a week at tillering and heading stage for the development of a heavy rust infection pressure (Hussain et al., 2015).

2.3. Data recording of leaf rust under field conditions

Disease severity of leaf rust in percentage and host response were observed by modified Cobb's scale described by (Peterson et al., 1948). Disease severity was recorded four time with 10 days interval when Morocco showed 40–50% rust severity. Rating of the final disease severity (FDS) was recorded when Morocco variety showed 90–100% disease severity. The values of coefficient of infection (CI) were calculated by the equation described by (Pathan and Park 2006). Area under disease progress curve (AUDPC) was estimated for each genotype by using the following equation (Anwaar et al., 2019).

$$AUDPC = d[1/2(y_1 + y_k) + (y_2 + y_3 + - - - - - + y_{k-1})]$$

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where d = days between two consecutive records (time intervals)

$y_1 + y_k$ = Sum of the 1st and last disease scores
$y_2 + y_3 + - - - - - + y_{k-1}$ = Sum of all in between disease scores.

2.4. Statistical analysis

Recorded data were subjected to analysis of variance (ANOVA) (Steel et al., 1997) using the GenStat (v10) software (Payne et al., 2008). The significance level = 0.01 was used for highly significant effects and = 0.05 was used for significant effects. RADAR-graph were developed for mean value using Excel-Stat in which display values relative to a centre point for examined traits under both environments (Ahmed et al., 2020).

2.5. DNA extraction and genotyping

In green-house, wheat seeds were sown in small plastic trays for healthy seedlings. After three weeks, fresh leaves were collected for DNA according to the CIMMYT Molecular Genetics Manual procedure in 96 well-plates. The concentration and quality of isolated DNA was assessed by Nano-drop (ND1000, Thermo Scientific, USA). The DNA of each genotype (70–100 ng/μl) preserved in 96-well plate and prepared for genotyping with high-density illumina 90 K Infinium SNP array (Dreisigacker et al., 2013). After genotyping, the genome-wide positions of SNPs in terms of genetic distance (cM) situated on chromosomes founded on consensus genetic map of bread wheat (Wang et al., 2014). For further analysis SNPs were filtered and excluded the monomorphic SNPs, more than 20% missing SNPs, minor alleles and allelic frequency < 5% for GWAS in the present study.

2.6. Population STRUCTURE and GWAS analysis

Bayesian clustering technique was used to classify the group of genetically similar population via statistical software STRUCTURE v.2.3 (Pritchard et al., 2000). Burn-in iterations of 10^4 cycles, followed by a simulation run of 10^6 cycles and the admixture model selection were used. Web-based analysis "Structure Harvester v0.6.93" was applied to obtain maximum value or peak of "K" for validation to understand the STRUCTURE results which were based on ad-hoc techniques. We selected the K values ranged 1–10 and 6 independent runs to attain reliable effects.

GAPIT (genome association and prediction integrated tool) was also applied with the model selection preference to test the reliability of the results. It was advanced in R package which offer maximum likelihood precision and run in a computationally effective method. GAPIT implements unconventional statistical approaches containing the compressed mixed linear model (CMLM) and CMLM-based genomic prediction and selection. P-values, R^2 and marker effects were extracted from GWAS results (Lipka et al., 2012). The threshold level for significant marker-trait associations (MTA) was 10^{-3} (log10p) or above (Rahimi et al., 2019) after applying the false discovery rate (FDR) < 0.05 correction using Bonferroni adjustment (P = 1/n, n = total number of SNPs) (Benjamini and Hochberg 1995). Mixed linear model (MLM) estimated from newly developed GWAS. To define the spurious associations derived from population structure, covariates from either STRUCTURE or principal components (PCs) were considered as fixed effects. The relationships among individuals were calculated using a kinship matrix and incorporated MLM. Overall 18,500 SNPs from functional iSelect bead chip analyses visually showed polymorphism; to locate them on the published genetic map in the studied genotypes.

3. Results and discussions

3.1. Field evaluation of disease severity parameters against leaf rust

A field evaluation was conducted of 105 wheat varieties over two growing seasons 2018–19 and 2019–20 against leaf rust disease using disease severity Coefficient of infection (CI), Area under disease progress curve (AUDPC) and Final Disease Severity (FDS) parameters. Mean squares results from analysis of variances (ANOVA) showed highly significant ($P \leq 0.001$) effects among investigated wheat genotypes for coefficient of infection under field conditions (Table 1). The mean responses of studied wheat genotypes to leaf rust under field conditions are shown in Fig. 1. In 2014–15 growing season, all investigated wheat genotypes showed the significant values of coefficient of infection. Data analysis and mean comparison indicated that wheat cultivars were considerably different based on the coefficient of infection values. The values 10–20, 21–30, 31–45 and 46–80 were categorized as high, moderate, low and very low levels of resistant, respectively. During this study, an effort was also done to elucidate the association between described parameters. Among the studied germplasm, G-36 and G-27 and G-102 showed highest values of coefficient of infection 77.6, 77.6 and 76.0 followed by G-6 and G-42 having values of 63.6 and 63.0 respectively, thus, these were classified as susceptible wheat genotypes. However, G-28 showed the minimum value of coefficient of infection 12.0 and followed by G-30, G-35, G-38, G-2, G-11, G-23, G-16 and G-48 having values of 14.6, so were ranked as resistant genotypes against leaf rust pathogen (Fig. 1). The described results are in harmony with the results of (Anwaar et al., 2019). (Ali et al., 2009) investigated 20 wheat genotypes and 'Morocco' as susceptible check at NIFA for describing variability for field based partial resistance to rust and lines with coefficient of infection values of 0–20, 21–40 and 41–60 were considered as possessing better, moderate and low levels of partial resistance, respectively. Only Morocco (a susceptible check) was possessing 100 CI value.

Analysis of variances results showed highly significant ($P \leq 0.001$) effects among studied wheat genotypes for area under disease progress curve under field conditions (Table 1). Data analysis and mean comparison indicated that leaf rust infection was well established apparently in all the tested wheat cultivars screened for the disease. The results of the mean values of area under disease progress curve of different wheat varieties to leaf rust is shown in Fig. 1. The genotypes G-102, G-36, G-27 and G-6 were ranked as susceptible to area under disease progress curve

Table 1

Mean Squares of ANOVA for FDS, AUDPC and CI of leaf rust under field conditions based on data averaged over two seasons 2016–17 and 2017–18.

Mean squares				
Source of variation	Df	Coefficient of Infection (CI)	Area under disease progress curve (AUDPC)	Final Disease severity (FDS)
Replications	2	9177.88	2,421,550	6845.00
Genotypes	104	940.66**	256423**	602.66**
Error	208	209.31	16,752	150.10

* = Significant (P < 0.05) ** = Highly Significant (P < 0.01) NS = Non-significant.
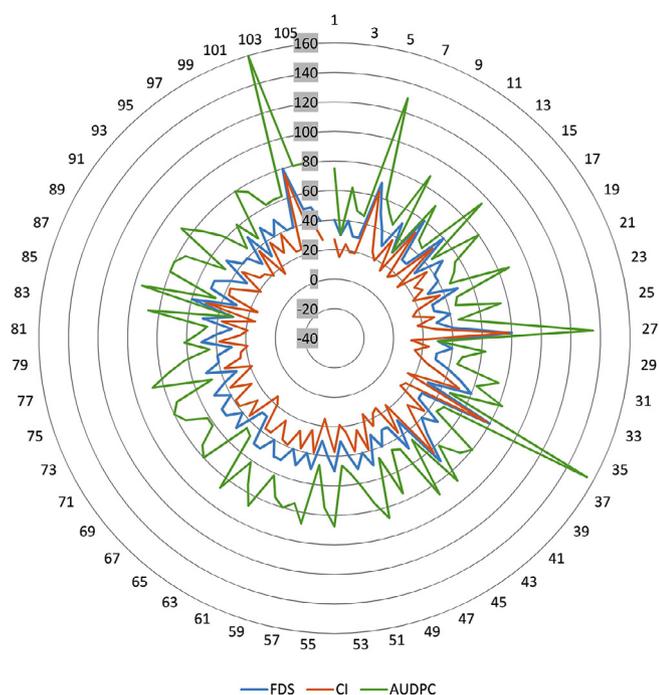
Fig. 1. Performance of 105 wheat genotypes against leaf rust pathogen using Final Disease severity (FDS), Area under disease progress curve (AUDPC) and Coefficient of Infection (CI) parameters based on data averaged over two seasons 2018–19 and 2019–20 under field conditions.

(AUDPC) with the values of 1600, 1550, 1350 and 1300 respectively, whereas G-42, G-11 and G-2 were ranked as resistant having the minimum value 300 of area under disease progress curve. The rest of the cultivars had the range of value from 450 to 850 were classified as moderately resistant (MR) to moderately susceptible (MS) genotypes. Results of current study showed that AUDPC run in a parallel manner to rust severity. It is obvious from the previous findings by Safavi (2015), Taye et al. (2015), Anwaar et al. (2019) that a very strong positive relation found between AUDPC and final rust severity that means severely infected cultivar showed higher AUDPC values. And the second thing was disease progress rates and AUDPC values were positively correlated and highly significant.

Disease damage or lesions covered on host tissues or organ described in percentage is called disease severity. Severity results from the number and size of the lesions. Data analysis and mean comparison indicated that 105 wheat genotypes were significantly different based on the final disease severity (FDS) parameter. ANOVA Results showed highly significant (P ≤ 0.001) effects among investigated wheat genotypes for disease severity under field conditions (Table 1). Mean results of the response of different studied wheat genotypes against leaf rust disease under field conditions are shown in Fig. 1. All the studied wheat genotypes exhibited different disease severity ranged from 30 to 80%. The genotypes of G-102, G-27 and G-36 showed the highest final rust severity of 80% followed by G-6 and G-42 which had the value of 70% rust severity and classified as leaf rust susceptible genotypes. While the minimum final disease severity value was 30 % showed by the genotypes G-30, G-37, G-25 and some other cultivars, thus, were more resistant to the pathogen of the leaf rust as indicated in Fig. 1. Current results are in a harmony with results reported by Macharia and Wanyera (2012), Taye et al. (2015). A study by Hasan et al. (2012) was aimed to estimate of losses of grain yield because of brown rust disease on the five local commercial susceptible wheat cultivars in field conditions during 2011–2012 growing

seasons at Gemmeiza Agriculture Research Station, Egypt. The investigated cultivars exhibited 5–80% disease severity. And was depicted a relationship between rust severity and yield components which resultantly converted to financial losses.

3.2. Population STRUCTURE

Bayesian method execute in statistical software package STRUCTURE used to estimate the genetic structure of 105 bread wheat genotypes. The results from this technique showed that highest (peak) number of K = 4 which demonstrating the germplasm distributed into four sub-population (Fig. 2A). Different types of coloured in Fig. 2B exhibits the distinct group and overall germplasm allocated into four sub-groups. This technique has been applied in wheat breeding scheme by many scientists and were obtained the explanatory outcomes (Wang et al., 2021). In current experiment, existence of different groups clearly showed the genetic variations between 105 bread wheat genotypes and genetically diverse to one another. Fundamentally, this is the indication of genetic divergence between the clusters or groups and resultantly the presence of more genetic diversity in studied germplasm. Several wheat breeders evaluated the genetic diversity (Rahimi et al., 2019; Leonova et al., 2020) using the similar techniques which were applied in current study and some extent they got the similar results. Development of novel bread wheat genotypes should be attaining the significance level of genetic diversity. Presence of more variation in 105 bread wheat genotypes which indicate the maximum genetic diversity, fearlessly, that the studied germplasm introduced from different sources or assumable mechanical mixing.

According to the provided pedigree record there are three groups of 105 bread wheat genotypes as shown in Supplementary Table 1. In first group, genotypes G-1 to G-20 which was developed in PBG-UAF, while in second group the genotypes G21 to G-55 were from exotic source, and in third group, genotypes G-56 to G-105 was from indigenous sources. But according to molecular analysis these genotypes divided into four populations. The maximum genetic distance between groups exhibited indicating genetic similarity within groups and genetic dissimilarity between the groups (Lipka et al., 2012; Zegeye et al., 2014). Particularly, results were useable conferring to the previously known pedigree record and origin of wheat genotypes. Genetic diversity evaluation could be helpful to identify the different genotypes for the advancement and improve the future wheat breeding scheme. The genotypes with different genetic makeup can be selected for desirable combinations to develop complex and significant attributes to obtaining maximum yield. Discrimination of wheat genotypes based on their genetic basis would be useful for effective and early selection of desired genotypes in wheat breeding scheme for developing promising wheat genotypes (Juliana et al., 2018; Leonova et al., 2020).

3.3. Genome-wide markers–traits associations for disease severity parameters

Marker-trait association (MTA) study recognized the connection between particular morphological and genetic variation within a genome, which ultimately perceived locus underpinning related characters at the end. In this study, 18,500 high density SNP markers from the 90 K Illumina iSelect SNP array were evaluated to perceive SNPs associated with disease severity for leaf rust related indices. Before analyses of GWAS and genomic prediction, scientists should validate and maintain genotype quality. The GAPIT provides a series of diagnostic tools to help users perform quality control on genotypes (Lipka et al., 2012). Total 56 significant SNPs were correlated with observed characters at or above

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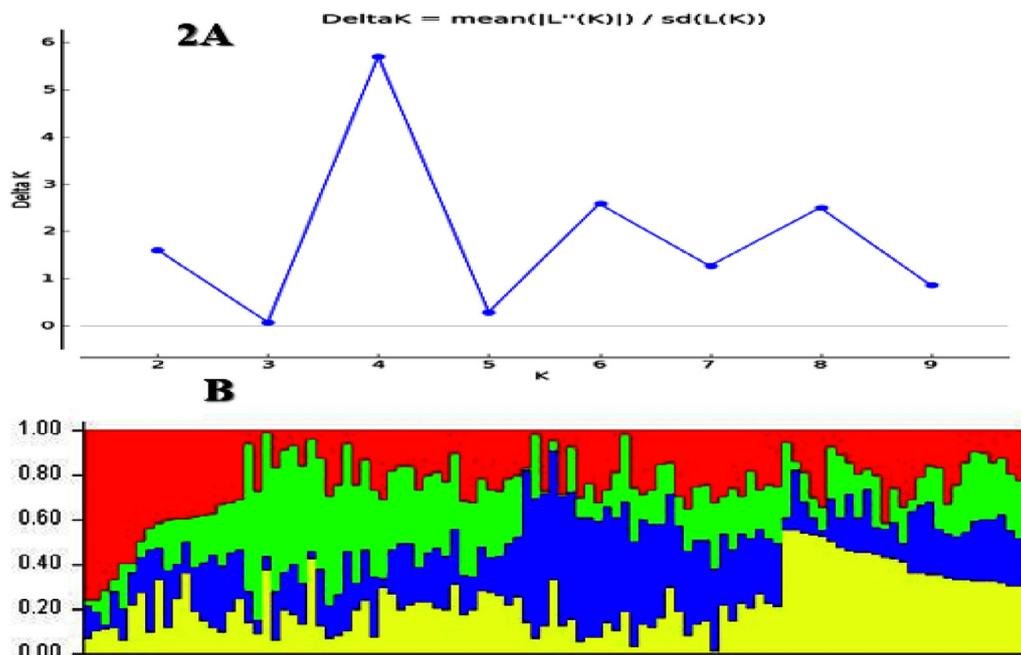
Fig. 2. (A) This result achieved of 105 wheat genotypes using 90 k SNP Array from Structure Harvester analysis. It's based on the second order derivation on the variance of the maximum likelihood estimation of model given a specific K. Delta K shows only the uppermost clustering level and number of subpopulations in main population. (B) Population structure of 105 wheat genotypes based on Bayesian method analysed with 90 k SNP Array detecting 4 groups. The dissimilar colours in this figure demonstrating the different group.
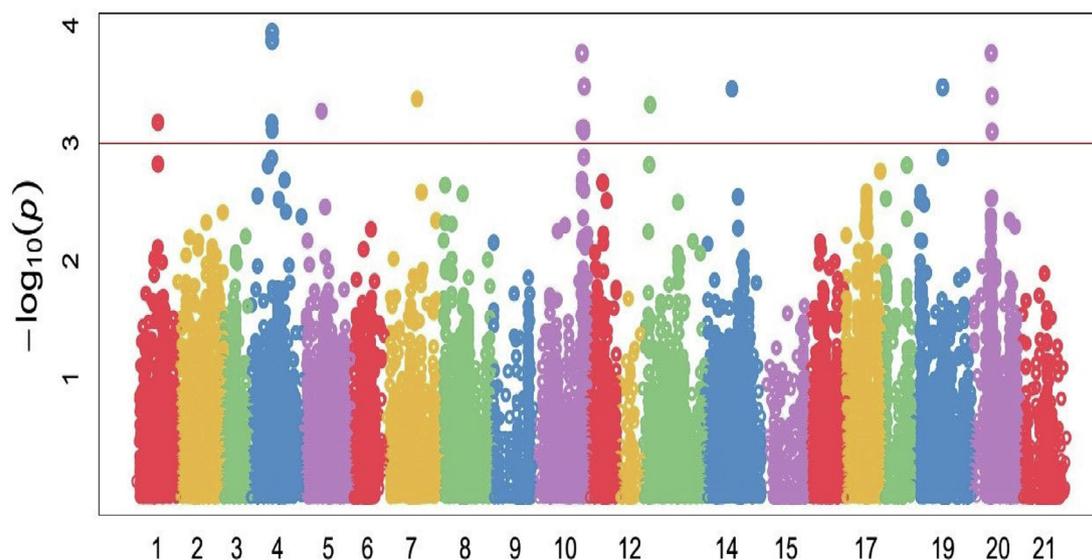
Fig. 3. Manhattan plots showing the location of significant SNPs and −log10(p) associated with Coefficient of Infection (CI) in 105 wheat genotypes against leaf rust pathogen under field conditions. The red horizontal line on Manhattan plot entitles the threshold level at $P \leq 10^{-3}$ of significance for SNPs with specific traits.

−log 10 ($P < 0.0001$) threshold level using MLM (mixed linear model) for studied traits as mentioned in Table 3. The significance level for p values were measured using Bonferroni adjustment at $P \leq 10^{-3}$ after crossed FDR < 0.05 threshold, some scientists previously used this criteria with small population size as in current study (Qaseem et al., 2018; Rahimi et al., 2019). Manhattan plots (Figs. 3–5) presentation the site of significant SNPs at ($P < 0.0001$) −log10(p) which significantly linked with the desired characters under studied conditions. The *red horizontal line* on Manhattan plot entitles the threshold level ($P < 0.0001$) of significance for SNPs with specific traits. Current results were supported

with the findings of (Rahimi et al., 2019; Leonova et al., 2020). In this study, 18,500 high density, polymorphic SNP markers from 90 K Illumina iSelect SNPs array (Wang et al., 2014) were examined to notice SNPs associated with disease severity for leaf rust related associated indices.

A total of 17 significantly marker trait associations were found with coefficient of infection, Including 5 markers located on chromosomes 4A, 4 on chromosome 2A, 3 on 7B and the other on 1A, 2B, 3A, 5A and 7A (Fig. 3). In GWAS analysis, *wsnp_Ex_c5412_9564046* on chromosome 2A at position 261.05 cM and *Kukri_c4709_53* on chromosome 4A were significantly correlated
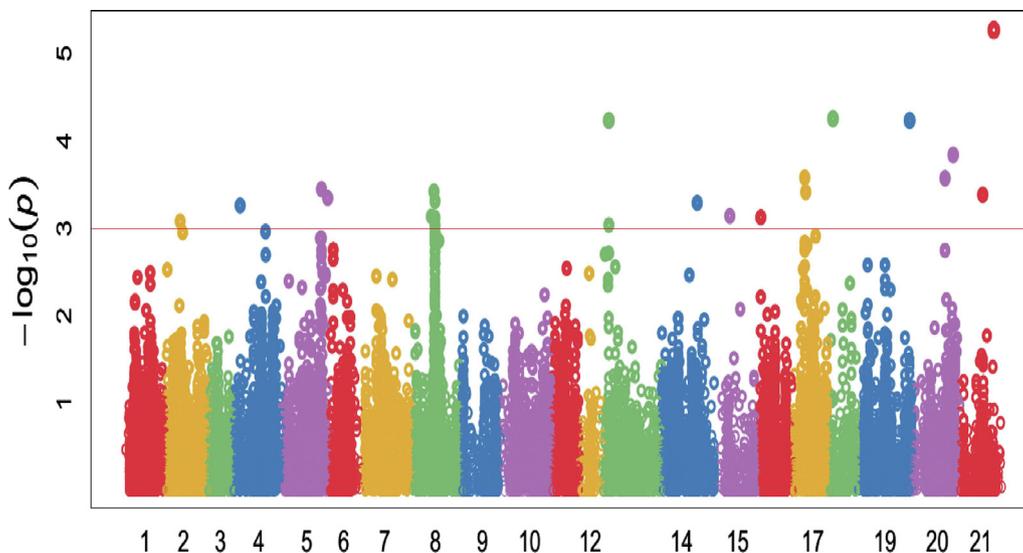
Fig. 4. Manhattan plots showing the location of significant SNPs and −log10(p) associated with Area under disease progress curve (AUDPC) in 105 wheat genotypes against leaf rust pathogen under field conditions. The red horizontal line on Manhattan plot entitles the threshold level at P ≤ 10^{-3} of significance for SNPs with specific traits.
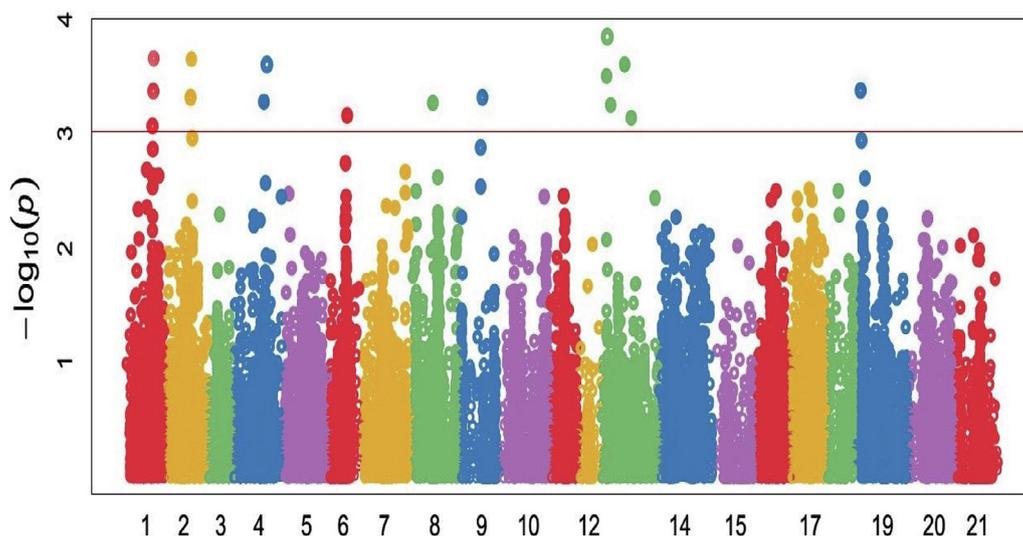
Fig. 5. Manhattan plots showing the location of significant SNPs and −log10(p) associated with Final Disease severity (FDS) in 105 wheat genotypes against leaf rust pathogen under field conditions. The red horizontal line on Manhattan plot entitles the threshold level at P ≤ 10^{-3} of significance for SNPs with specific traits.

with this trait covering 20.49% and 16.60% of the phenotypic variation respectively. Reliable MTAs were detected for disease severity parameters which located on the 5A, 6D, 6A and 6A chromosomes by wheat scientists (Singh et al., 2009). Associations with SNPs of chromosome 5A were also established a link with current study. In addition, association mapping revealed a Genomic region on chromosome 5A, which determines the presence of resistance loci against leaf rust. Based on the location of QTLs, as well as their origin, the locus on 5A found in this study cannot be attributed to any of the known genes (Singh et al., 2009; Maccaferri et al., 2010; Leonova et al., 2020). This suggests the presence of a previously unknown QTL for resistance to leaf rust in the genomes of studied germplasm.

Total 23 significant SNP markers were strongly linked with Area under disease progress curve (AUDPC) including 6 markers located on chromosomes 3B, 2 on each chromosome 2B, 5A, 6B, 7B, 7D and the other on 1B, 6A, 6D and 7A (Fig. 4). These markers explained

11.64% to 23.03% of the phenotypic variation in AUDPC under field conditions. The marker (D_contig29746_525) on chromosome 7D at 436.21 cM explained maximum variation 23.03% while the marker (BS00037898_51) on 3B at 274.8 cM explained (Table 3) minimum variation (11.64%) in this parameter. In the study of (Wang et al., 2021) 32 markers located on seven chromosomes were identified which similar to current study results, as significantly associated (P < 0.00o1) with FDS and AUDPC. The identified resistant accessions and resistance loci will be useful in the ongoing effort to develop new wheat cultivars with strong resistance to leaf rust (Yao et al., 2019; Wang et al., 2021).

Final Disease Severity (%) was highly associated with sixteen QTL regions. Four QTLs associated regions were located on chromosomes 5A, three on 1A, two on each 1B, 2A and 7A, one on each 3B and 3 D (Fig. 5). These MTAs explained 12.97 % to 19.39 % of the variation in FDS (Table 3). The marker (Ku_c10756_1197) explained maximum phenotypic trait variability (19.39%) on chromosome 5A

Table 2
Response of 105 wheat genotypes against leaf rust pathogen under field conditions using studied parameters based on data averaged over two seasons 2016–17 and 2017–18.

Resistant (R) genotypes	Moderately Resistant (MR) genotypes	Moderately Susceptible (MS) genotypes	Susceptible (S) genotypes
Total 34 genotypes	Total 30 genotypes	Total 32 genotypes	Total 9 genotypes
32.39%	28.59%	30.49%	8.60%
G2, G4, G5, G9, G11, G13, G14, G16, G20, G22, G23, G25, G28, G30, G31, G35, G37, G38, G43 G45, G46, G48, G53, G56, G66, G67, G75, G79, G82, G84, G86, G90, G94, G96.	G1, G3, G8, G18, G19, G24, G26, G29, G32, G40, G47, G50, G52, G55, G58, G60, G63, G69, G72, G74, G77, G78, G81, G89, G92, G93, G98, G100, G101, G105.	G7, G10, G17, G21, G33, G39, G41, G44, G49, G51, G54, G57, G59, G61, G62, G64, G65, G68, G70, G71, G73, G76, G80, G83, G87, G88, G91, G95, G97, G99, G103, G104.	G6, G12, G15, G27, G34, G36, G42, G85, G102.

Table 3
Significant marker trait associations MTAs at FDR < 0.05 threshold for leaf rust pathogen under field conditions.

	SNP	Chromosome	Position	P.value	PV
CI	wsnp_Ex_c5412_9564046	4	261.05	0.0001157	20.49
	Ra_c58279_684	4	261.05	0.0001357	20.16
	RAC875_c45591_79	20	246.47	0.0001444	19.96
	GENE-4367_562	10	575.49	0.0001738	19.66
	wsnp_Ex_c27666_36847022	20	213.28	0.0001823	19.36
	BS00109911_51	10	603.44	0.0003316	18.35
	Kukri_c55051_414	13	68.20	0.0003347	18.32
	BS00038787_51	19	369.49	0.0003378	18.31
	wsnp_Ra_c3176_5975986	20	246.93	0.0004007	17.97
	wsnp_Ex_c15475_23757972	7	419.04	0.0004056	17.37
	wsnp_Ex_c5412_9564346	4	257.26	0.0006717	16.95
	Excalibur_c26971_1730	10	598.31	0.0007480	16.74
	Tdurum_contig46583_2203	10	575.49	0.0007507	16.73
	wsnp_RFL_Contig2744_2471775	5	277.23	0.0007613	16.69
	wsnp_Ex_c5412_9564550	4	261.05	0.0007791	16.66
	wsnp_Ku_c10292_17066821	1	261.32	0.0007838	16.62
	Kukri_c4709_53	10	598.31	0.0008009	16.60
AUDPC	D_contig29746_525	21	436.21	0.0000053	23.03
	Kukri_c43200_235	18	58.30	0.0000552	17.69
	Ex_c54098_174	13	78.01	0.0000577	17.59
	Excalibur_c33112_226	19	683.43	0.0000577	17.59
	RAC875_c4876_2096	20	522.37	0.0001431	15.61
	RAC875_c19425_903	17	134.00	0.0002610	14.31
	Excalibur_c58468_162	20	418.37	0.0002655	14.28
	BS00100117_51	5	504.16	0.0003548	13.66
	TA004110-0731	8	262.18	0.0003758	13.54
	RAC875_c31280_122	17	146.01	0.0003820	13.51
	D_GA8KES401D0K1W_269	21	294.97	0.0004101	13.36
	RFL_Contig4549_196	5	583.38	0.0004468	13.18
	BobWhite_c35249_110	8	275.44	0.0004843	13.01
	RAC875_rep_c75915_103	14	461.74	0.0005085	12.91
	Excalibur_c10307_254	4	86.88	0.0005443	12.76
	RFL_Contig2949_500	15	194.19	0.0007172	12.19
	GENE-4918_283	8	228.92	0.0007274	12.16
	GENE-1756_564	16	15.48	0.0007440	12.11
	wsnp_Ex_c7172_12318529	8	275.44	0.0007492	12.10
	Excalibur_c10528_347	2	195.12	0.0008269	11.90
	wsnp_Ex_c5378_9505533	8	274.80	0.0009118	11.70
	RAC875_c28449_88	13	78.13	0.0009120	11.69
	BS00037898_51	8	274.80	0.0009350	11.64
FSD	Ku_c10756_1197	13	690.36	0.0001310	19.39
	Excalibur_c65152_572	2	344.72	0.0001330	19.05
	RAC875_s117925_244	13	77.77	0.0001409	18.49
	Excalibur_c111818_435	13	249.32	0.0001420	18.33
	RAC875_c16333_340	4	427.05	0.0002290	17.93
	BobWhite_rep_c66990_294	8	320.47	0.0002350	17.88
	Kukri_c55051_414	13	68.20	0.0002460	17.66
	RAC875_c33823_279	9	245.89	0.0002770	17.20
	Excalibur_c34115_271	19	103.70	0.0002840	17.15
	BobWhite_c26570_282	4	410.90	0.0008310	16.78
	Tdurum_contig62445_667	19	98.21	0.0008410	16.67
	BS00110877_51	1	333.28	0.0008510	15.38
	BS00037976_51	1	337.41	0.0008510	15.25
	Tdurum_contig30517_310	2	269.73	0.0008520	15.21
	wsnp_Ex_c3831_6965890	1	260.38	0.0008530	15.11
	BobWhite_c15453_678	6	208.94	0.0008840	12.97

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at 690.36 cM while the marker (*BobWhite_c15453_678*) on chromosome 2D at 208.94 cM explained minimum value (12.97 %) of phenotypic variability. MTA for FDS were distributed across 16 chromosomes, including, 11 SNPs at A-genome, 3 at B-genome and 2 at B-genome. Leaf rust resistance genes designated *Lr1* to *Lr60* have been have been characterized in common (Bolton et al., 2008). These resistance genes are widely distributed across all the chromosomes of the wheat genome. In present study, significant SNPs were observed and matched with previously identified location of genes by several scientists. Genes *Lr2a*, *Lr2b* and *Lr2c* were mapped to a locus on chromosome 2D, Chromosomes 6B exhibited the *Lr3a*, *Lr3ka* and *Lr3g* genes against leaf rust (Li et al., 2016) The other genes *Lr17a* and *Lr17b* are at a locus on chromosome 2A and *Lr22a* and *Lr22b* at a locus on chromosome 2D. Genes *Lr14a* and *Lr14b* are extremely tightly linked on chromosome 7B and are considered as alleles for all practical purposes (Bolton et al., 2008; McIntosh et al., 2014).

Based on the disease severity parameters of FDS and AUDPC (Macharia and Wanyera 2012), reported infection responses of the wheat cultivars and classified them into four discrete categories: a) S-susceptible) MS-moderately susceptible c) MR-moderately resistant d) R-resistant which was previously reported by several scientists. Resistant wheat varieties are compromised by the continuously evolving races of rust pathogens, which causes leaf rust of wheat and appears to be the most damaging pathogen that threatens global food security by inducing yield reductions in wheat (Li et al., 2016). Diseased symptoms are prevalent on leaf blades, leaf sheaths and glumes. Pathogenic attack in the early crop stages may result in increased yield losses up to 30% (Anwaar et al., 2019).

In the current study total 105 genotypes were investigated, among them 34 genotypes showed resistant results against leaf rust pathogen. Moderately resistance showed by 30 genotypes against this disease in studied germplasm. Total 32 genotypes exhibited moderately susceptible performance to leaf rust pathogen while 9 genotypes showed susceptible behaviour against this pathogen (Table 2). Leaf rust resistant accessions recognised in this experiment verified to be additional fruitful in preliminary field environments against leaf rust pathogen. Thus, these resistant genotypes could be helpful as parental genotypes in breeding schemes for leaf rust resistance. The locus (*Kukri_c55051_414*) showed pleotropic effects for leaf rust identified parameters on chromosome 5A at position of 68.2 cM under field conditions\. The main objectives of this study were to identify sources of resistance, and to map genomic loci associated with leaf rust resistance using genome wide association study (GWAS) approach. The new identified sources of resistance and MTAs/QTL could be used in wheat breeding programs to improve leaf rust resistance for sustainable food security.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2021.101628.

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