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Full Length Article

Genome Wide Association Analysis for Leaf Rust Resistance in Spring Wheat (*Triticum aestivum* L.) Germplasm

Sher Muhammad[1], Aqeel Ahmad[2], Faisal Saeed Awan[3], Azeem Iqbal Khan[4], Muhammad Qasim[1], Aziz-ur-Rehman[5], Abdul Rehman[6], Muhammad Aslam Javed[7], Irfan Manzoor[1] and Muhammad Sajjad[8]

[1]*Department of Bioinformatics and Biotechnology, GC University Faisalabad, Pakistan*
[2]*Department of Environmental Sciences and Engineering, GC University Faisalabad, Pakistan*
[3]*Centre of Agricultural Biochemistry and Biotechnology (CABB) and Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan*
[4]*Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan*
[5]*Wheat Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad–38950, Pakistan*
[6]*Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan*
[7]*Agricultural Biotechnology Research Institute (ABRI), AARI, Faisalabad, Punjab, Pakistan*
[8]*Department of Environmental Sciences, COMSATS University Islamabad, Vehari Campus, Pakistan*
[*]For correspondence: sher1325@hotmail.com

Abstract

Leaf rust caused by *Puccinia triticina*, is an important disease of wheat in Pakistan and many other wheat growing countries. Seventy six leaf rust resistance genes have been cataloged based on genetic analysis in different mapping populations. A majority of these genes belong to all stage resistance category. Only a small proportion of these genes are currently effective against *P. triticina* pathotypes of Pakistan. A set of 94 wheat genotypes was scored for adult plant leaf rust response variation under field conditions in Faisalabad, Pakistan during the 2011 and 2012 crop seasons and was genotyped by using 203 microsatellite markers covering all 21 wheat chromosomes. Marker-leaf rust associations were determined using single marker tests. The most important genomic regions for leaf rust resistance were IDS, 4AL, 4BL, 5AS, 5BS, 6AL, 6BS, 6DS. Different loci that were not mapped in previous studied were tagged to different chromosomal locations thus the tagged loci may be further studied for identification and designation of new genes to different chromosomes. This will help the wheat breeders to use this information for the development of new resistant cultivars against wheat leaf rust via utilizing marker assisted selection. © 2018 Friends Science Publishers

Keywords: Association mapping; *P. triticina*; SSR markers; *Triticum aestivum* L.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops worldwide (Curtis and Halford, 2014). Leaf rust (LR), caused by *P. triticina* is a constant threat to wheat crop all over the world. Rusts pose the most serious threats to wheat production in Pakistan. Pakistan has experienced major epidemics of leaf rust in 1948, 1954, 1973 and 1978, resulting in 30-50% yield reductions. The leaf rust epidemic in 1978 caused a huge loss to country's GDP of about US$ 86 million (Hussain *et al*., 1980). The use of fungicides to control LR may lead to accumulation of fungicide residues in grains or resurgence of the disease under conducive conditions if the rusts develop resistance to fungicides (Luo, 2009). Therefore, developing cultivars with resistance to LR races is the most effective, economical and environmentally safe approach.

Genetically, resistance to rusts is of two types, viz., race specific/vertical and race non-specific/horizontal resistance. Race specific resistance is controlled by one major gene or simple combinations of few major genes. The cultivars with this type of resistance do not have long life and collapse when a new pathotype emerges with a new virulence factor (Rehman *et al.,* 2013). Conversely, race non-specific resistance which is also termed as adult plant resistance (APR), partial or slow rusting resistance is controlled by several minor genes which typically reduces development of pathogens on adult plants. The cultivars having APR resistance show durable resistance for longer time with almost same level of reaction against different races in different climatic conditions. For example, Lyalpur-73 (a major cultivar of Pakistan in 1970's) with several minor APR genes plus *Lr34* still show very high level of resistance in screening nurseries (Rehman *et al*., 2013).

More than 100 LR genes have been identified and 76 have been catalogued and assigned specific names and symbols (McIntosh *et al*., 2016). Most of these genes are race specific and there resistance can be broken easily by a

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new LR race. Among the 76 cataloged genes, only four genes namely, *Lr34*, *Lr46*, *Lr67* and *Lr67* are APR genes which are being pyramided in modern wheat cultivars (Dyck, 1977; Singh *et al*., 1998; Herrera-Foessel *et al*., 2011, 2012; Li and Peng, 2014). In addition to the four cataloged APR genes, 80 other APR QTLs for LR have been identified on 16 chromosomes in various wheat populations (Li and Peng, 2014). Though minor gene resistance is considered more durable than major gene resistance but it also can be broken by slow evolution in the existing LR races (McDonald and Linde, 2002). To deal with such scenario in future, it is very important to identify more APR genes in local wheat germplasm for avoiding anymore LR epidemics in Pakistan.

Genome wide association analysis using wheat populations of un-related individuals and SSR markers have been widely used to map QTLs for various traits related to yield (Yu *et al*., 2011; Sajjad *et al*., 2014), quality (Reif *et al*., 2011; Shahzad *et al*., 2016), biotic (Zhao *et al*., 2014; Liu *et al*., 2017) and abiotic stress tolerance (Ain *et al*., 2015; Mwadzingen *et al*., 2017). The present study was conducted to identify APR QTLs for leaf rust resistance and their closely linked molecular markers for marker assisted selection (MAS) in wheat breeding. A panel of 94 diverse spring wheat accessions were scored of LR disease intensity for two years and scanned with 203 genome wide SSR markers.

Materials and Methods

Plant Material and Phenotypic Data Recording

Three hundred and twenty five genotypes of bread wheat (*Triticum aestivum* L.) collected from all over Pakistan, CIMMYT, ICARDA and other countries of the world were used for the current study. Seeds of collected germplasm were sown in November in the experimental area of Plant Pathology in the University of Agriculture, Faisalabad (Latitude = 31˚-26' N, Longitude = 73˚-06' E, Altitude = 184.4 m) during the years 2010-11 and 2011-12. Each entry was planted in 1.5 m long row by keeping row to row distance of 30 cm and sowing was done by putting two seeds per hole by maintaining 8 cm plant to plant distance. The experiment was conducted following the augmented design. The whole material was divided in to 5 sets each comprising of 65 entries similarly experimental field was divided in to 5 blocks. A set of 65 test entries and 13 check varieties were randomized in each block. The package of agronomic practices was followed to raise the wheat crop. Leaf rust reaction**,** field response and severity were recorded by the modified Cobb's scale (Peterson *et al*., 1948). Leaf rust data was recorded after seven days interval from mid-February to end March. Leaf rust data were converted into Average Coefficient of Infection (ACI) (Stubbs *et al*., 1986) for further statistical analysis. Of these total 325 wheat genotypes, 94 were selected on the basis of leaf rust reaction, field response and severity. These genotypes represents resistant (R), moderately resistant (MR), moderately resistant to moderately susceptible (MRMS), moderately susceptible (MS), and susceptible (S) against leaf rust of wheat (Muhammad *et al*., 2015). The list of selected genotypes and their origin is given in Table 1.

Genotyping

DNA from fresh leaf tissue of each genotype in the selected set of 94 accessions was extracted following the CTAB method (Doyle and Doyle, 1987). The polymorphic SSR markers were selected from Grain Genes data base (http://wheat.pw.usda.gov) representing gwm (118), wmc (34), sun (13), barc (12), cfd (8), gpw (7), mag (6), gdm (2), cfb (1), CsSr2 (1) and CsLv34 (1) types.

PCR was carried out in a total reaction volume of 10 µL containing 60 ng/µL of DNA, 1× $MgCl_2$ buffer, 0.75× dNTPs, 0.4× 1.25 µM forward primer labelled with M13, 0.4×5 µM Reverse primer, 0.1× 0.50 µM M13-tailed primer labelled with IR Dye 700 or 800 and 0.04 µL× 0.2 U *Taq* polymerase (Bioline). The PCR profile for each SSR was consisted of 10 min at 95°C; followed by 30 cycles with touchdown profile, of 30s at 60˚C and 72˚C for 30s. The annealing temperature was decreased by 1˚C per cycle for the next five cycles followed the first cycle, with a final extension of 5 min at 72°C. 2 µL of the PCR product was separated on 2.5% agarose gel electrophoresis to ensure the presence of specific bands. From the remaining 8 µL product, mixed M700 (1-48 samples) and M800 (49-96 samples) together in a separate plate, each sample 2.5 µL and diluted with the 5 µL of dye (0.5% Fuschin dye+100% formamide liquid + 0.5M EDTA) in each well which yielded 10 µL product. The PCR product was separated and detected by polyacrylamide gel electrophoresis apparatus.

To make polyacrylamide gel solution, 20 mL of KB Plus 6.5% Gel Matrix, 150 µL of APS and 25 µL of TEMED (tetra methyl ethylene diamine) were added and mixed to homogenate before use. The gel solution was drawn in to 60 mL syringe with 14 guage tips and poured to the prepared glass plate. The gel was allowed to polymerize for at least 1 h before use. After polymerization, the comb was removed and the back and front of the plate was carefully cleaned to remove any acrylamide fragments in each well. Following cleaning, the gel apparatus was mounted on the LICOR instrument against the heater plate, with the bottom of the gel sandwich inside the lower buffer tank. The buffer tank was filled with 1× TBE running buffer (10× TBE to 10L of deionized water). After the wells were cleaned using 20cc syringe filled with buffer from the upper tank, pre-running was done for 10 min before the actual electrophoresis started. Following pre-running denatures (95°C for 5 min) samples were loaded and run using $SAGA^{GT}$ software.

Table 1: List of genotypes selected for genome wide association mapping and their origin

S. NO	NAMES OF GENOTYPES	Origin	S.NO	NAMES OF GENOTYPES	Origin
1	FAISALABAD-08 (V-04189)	Pakistan	48	WATAN (V-87094)	Pakistan
2	V-04178 = AARI-10	Pakistan	49	BAU'S' = BAGULA	CIMMYT
3	SKD-1	Pakistan	50	SONOITA=SNI	CIMMYT
4	FAREED-06	Pakistan	51	LAKTA-1	Unknown
5	UFAQ	Pakistan	52	V-04188	CIMMYT
6	SOGHAT-90=PVN	Pakistan	53	NING 8319	China
7	INQILAB 91	Pakistan	54	NEELKANT'S'	CIMMYT
8	ABADGAR-93	Pakistan	55	BACANORA T88=BCN	CIMMYT
9	SASSI	Pakistan	56	V-03007	Pakistan
10	KOHSAR 95	Pakistan	57	MAYA/PVN	CIMMYT
11	ANMOLE-91	Pakistan	58	BULBUL	CIMMYT
12	T.J-83	Pakistan	59	HD2236//SA.42/HARRIER' S= V-97088	Pakistan
13	LASANI-08 (V-03138)	Pakistan	60	WL 711/3/KAL/BB//ALD = V-85054	Pakistan
14	BHITTAI	Pakistan	61	EAGLE	Australia
15	T.D-1	Pakistan	62	WEEBILL-1 = V-3158	CIMMYT
16	MANTHAR	Pakistan	63	BLS/KLT'S'	Pakistan
17	KHIRMAN	Pakistan	64	HARTOG=HTG.(PAVON)	CIMMYT
18	SALEEM 2000	Pakistan	65	LU26/KEA'S'	Pakistan
19	MIRAJ-08	Pakistan	66	V-03094	CIMMYT
20	MARVI-2000	Pakistan	67	KAKATSI	CIMMYT
21	PIRSABAK 2004	Pakistan	68	BOW'S'/SPT'S'	CIMMYT
22	SHAFAQ-06	Pakistan	69	V-04179	Pakistan
23	MEHRAN-89	Pakistan	70	PARULA=PRL	CIMMYT
24	PIRSABAK 2005	Pakistan	71	GAMDOW-6	CIMMYT
25	ZARGOON 79	Pakistan	72	TAM200/TUI	CIMMYT
26	FAISALABAD 85	Pakistan	73	REH/HARE//2*BCN/3/CROC-1/AE.SQ(213)//	CIMMYT
27	PAK 81	Pakistan	74	V-05121	Pakistan
28	ZARLASHTA 99	Pakistan	75	PRL/LU26//TRAP/LU26	Pakistan
29	IQBAL2000	Pakistan	76	V-09221	Pakistan
30	PARWAZ 94	Pakistan	77	V-06068	Pakistan
31	RASKOH 05	Pakistan	78	V-86711TC/SH-88//CROW	Pakistan
32	JAUHAR-78	Pakistan	79	V-08081	Pakistan
33	PASINA 90	Pakistan	80	WAXWING*2/KRITATI = V-7194	CIMMYT
34	SARIAB 92	Pakistan	81	PAK-81/2*V-87094	Pakistan
35	KARAWAN-2	Pakistan	82	NR 388	Pakistan
36	PUNJAB 76	Pakistan	83	V-07007	Pakistan
37	BLUE SILVER = SONALIKA	Pakistan	84	V-03144	Unknown
38	KOHINOOR 83	Pakistan	85	NR 378	Pakistan
39	PUNJAB 96	Pakistan	86	KIRITATI//2*SERI/RAYON	CIMMYT
40	CHENAB 70	Pakistan	87	V-87094/2*ERA/3/PAK-81/2*V-87094/4/SHAFAQ	Pakistan
41	LU 26 (Salt Tolerant)	Pakistan	88	TW 76004	Pakistan
42	SA 42	Pakistan	89	WHEATEAR	CIMMYT
43	CHENAB-2000	Pakistan	90	AS2002/WL711//SHAFAQ	Pakistan
44	LYP 73	Pakistan	91	SEHER-06	Pakistan
45	SHALIMAR 88	Pakistan	92	DOLLARBIRD	Australia
46	FAISALABAD 83	Pakistan	93	TUKURU//BAV92/RAYON*3/3/T.SPELTAP1348449	CIMMYT
47	MEXIPAK 65	Pakistan	94	ZAMINDAR 80	Pakistan

Statistical Analysis

The marker-trait association analysis was performed using the software R. Prior to single marker scan, pattern analysis based on hierarchical clustering and principal coordinate analysis was conducted to investigate the population structure. Classification was achieved using one minus the Czekanowski coefficient (Czekanowski, 1913; Williams, 1976), for calculating the dissimilarity measure and group average UPGMA (Søkal and Michener, 1958; McQuitty, 1967) as the clustering strategy. Principal coordinate analysis was applied to this Czekanowski coefficient. The Czekanowski coefficient is also known variously as the Dice coefficient (Dice, 1945), the Sorensen index (Sorensen, 1948), or the Nei and Li's coefficient (Nei and Li, 1979).

Association analysis was conducted using single-marker tests (Arief, 2010; Hickey *et al*., 2011). For each marker, t test was conducted to determine whether the absence or presence of that marker significantly affected the phenotypic performance of genotypes. The probability (P) of differences between marker class (i.e., absent and present) means was converted into a log score (i.e. -log10P). A log score of three was used as a threshold to declare an association significant. The marker was considered to be positively associated with a trait, if the presence of the marker contributed to better resistance and vice versa for negative association. Since association analysis utilizes gametic phase disequilibrium in the population, this was evaluated using D´ and r2, the two most commonly used measures (Devlin and Risch, 1995). The procedures for QTL detection and digenic interactions analysis between nonallelic QTL were similar with Zhao *et al.* (2014), Qi *et al.* (2016).

Results

Allelic Diversity

Total of 94 wheat genotypes were evaluated using 250 SSR markers selected by their known genetic positions to give a unvarying coverage to the 21 chromosomes in the wheat genomes (A, B and D). Total of 203 markers out of 250 SSRs gave polymorphic bands while rest of them were found monomorphic or did not amplify. Total of 571 alleles were detected. The allele numbers per locus were observed with maximum and minimum values that ranged from 1 to 7 with an average of 2.80 alleles per locus. Maximum number of alleles were detected in B genome as compared to A and D genome.

Linkage Disequilibrium

To estimate inter and intra chromosomal linkage disequilibrium, SSR markers were used to assigned to their map position. The distribution and amount of LD were displayed graphically by plotting intra chromosomal D′ and r^2 values for loci. The average value of r^2 for all pairs was 0.45. The significant decay of LD with the increase of genetic distance was observed. Maccaferri *et al.* (2005) defined four classes of marker pairs: class 1 tight linkage (distance < 10 cM); class 2 moderate linkage (10–20 cM); class 3 loosely linked (20–50 cM); and class 4 independent pairs (>50 cM). Based on D measure of LD, Linkage disequilibrium exhibited between loci distance upto 45 cM. Complete LD exists between loci that were genetically distinct showing superior LD. The overall LD on 21

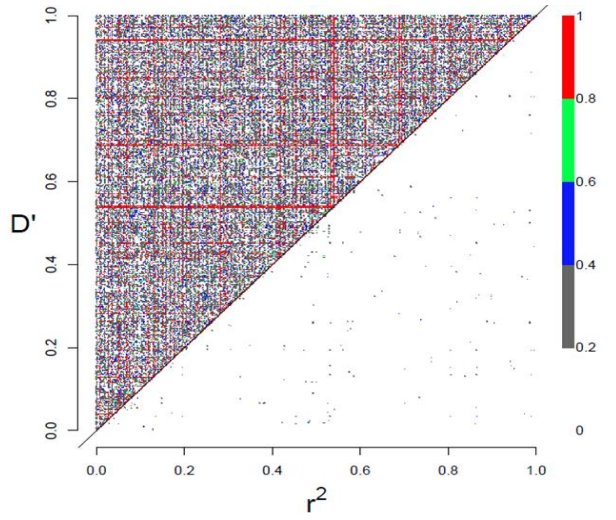

Fig. 1: Graphical representation of the gametic phase disequilibrium coefficients D′ and r^2 matrices among 203 SSR markers covering 21 chromosomes scored in 94 genotypes

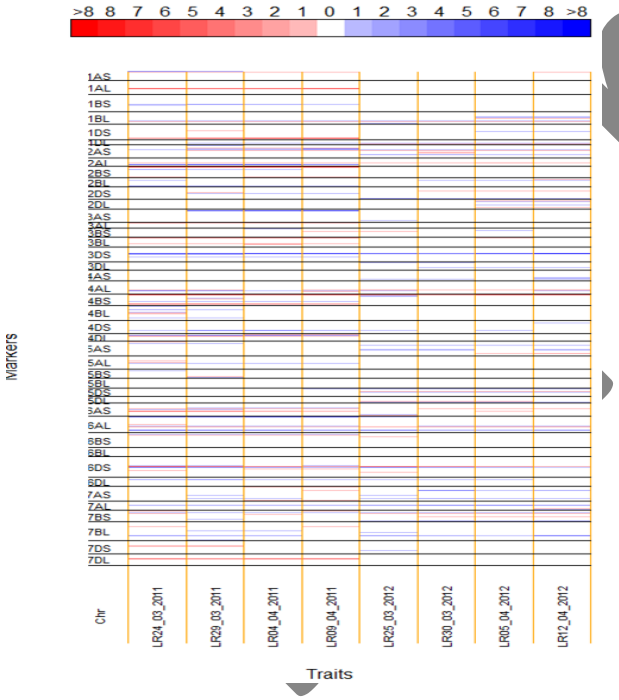

Fig. 2: Marker trait associations (MTAs) for leaf rust of 94 genotypes based on 203 microsatellites markers

Negative associations are showed in red (i.e. the presence of the markers contributed to poorer phenotypic performance or smaller phenotypic value for neutral traits) and positive associations are showed in blue (i.e. the presence of the markers contributed to better phenotypic performance or larger phenotypic value for neutral traits). Increasing colour intensity shows the strength (-log10 probability) of the association (Arief, 2010)

chromosomes is up to the distance of 45 cM. Weaker LD was covered between loci even to that were genetically 20 cM apart (Fig. 1).

Marker Trait Associations

It was observed that several SSR markers were significant on different chromosomes for leaf rust resistance, and many unmapped SSR markers were also showing significant associations as well (Fig. 2). Forty two QTLs were found to be associated with leaf rust resistance on all chromosomes (Table 2 and Fig. 3). The QTLs, *QLr.uaf.1AS* and *QLr.uaf.1AL*, located on chromosomes 1A explained phenotypic variance of 6.8 and 4.2%, respectively. The QTL on 1B, *QLr.uaf.1BL.2*, explained 9.2% phenotypic variation and was linked with genes *Lr33*. The *QLr.uaf.1DS* was spanning around the gene *Lr10* and explained 7.6% phenotypic variation. The *QLr.uaf.2AS* and *QLr.uaf.2BS* contained the genes *Lr17a* and *Lr16*, respectively, explaining phenotypic variation >10%. Each of the *QLr.uaf.2DS*, *QLr.uaf.3BS* and *QLr.uaf.3DS* contained two genes explaining phenotypic variance >11%. The QTL *QLr.uaf.4BS* had three genes, *Lr25*, *Lr49* and *Lr12*, with highest value of phenotypic variance (14.4%). The *QLr.uaf.5BS*, *QLr.uaf.5DS* and *QLr.uaf.5DL* contained the genes *Lr52*, *LrAC* and *Lr*, respectively. The two linked genes *Lr3* and *Lr9* were lying in the *QLr.uaf.6BL*. The *Lr53*, *Lr47*, *Lr72* and *Lr34* were found to be in the regions of *QLr.uaf.6DS*, *QLr.uaf.7AS*, *QLr.uaf.7BS.1* and *QLr.uaf.7DS*, respectively. All these QTLs containing known genes have been detected in previous researches (Table 3). The thirteen QTLs found chromosomes 2AL, 2BL, 2DL, 3BL, 4AS, 5AS, 6A, 6BS, 6DL, 7BL and 7DL containing unknown genes were first time detected in Pakistani germplasm (Table 2).

Discussion

Allelic Diversity

In assaying relatively different wheat genotypes, percentage of polymorphic SSRs may also be improved using capillary electrophoresis. Out of 250 markers used in this study, 203 showed polymorphism on 2.5% high resolution agarose gel electrophoresis and on polyacrylamide gel electrophoresis (PAGE). However in previous study, (Sánchez-Pérez *et al*., 2006) observed no significant difference in results obtained by agarose and PAGE. Gene diversity values are an evidence of sufficient genetic diversity present in the germplasm lines used in this study. Previously, it was found that 6.1 numbers of alleles per locus in a core collection of Chinese wheat accessions (Liu *et al*., 2010) and 108 Chinese wheat accessions showed 5.7 alleles per locus (Yao *et al*., 2009). 60 European wheat accessions showed 4.8 alleles per locus (Stachel *et al*., 2000). Moreover, 4.8 alleles per locus in 95 soft winter cultivars of USA were also reported (Breseghello and Sorrells, 2006). High number of alleles per locus (18.1) were observed in IPK gene bank hexaploid wheat accessions (Huang *et al*., 2002). Allelic diversity found in the present study is very

Table 2: Quantitative trait locus/loci for leaf rust resistance by GWAS in the Pakistani germplasm

Sr.No	QTL[a]	Position (cM[b])	Marker interval	PVE[c](%)	Genes/Loci
1	*QLr.uaf.1AS*	12-27	gwm136-gwm33	6.8	*Lr10*
2	*QLr.uaf.1AL*	60-65	gwm106-cfd22	4.2	Unknown
3	*QLr.uaf.1BL.1*	21	gwm264-wmc619	5.5	Unknown
4	*QLr.uaf.1BL.2*	33-42	wmc419-gpw4002	9.2	*Lr33*
5	*QLr.uaf.1DS*	5-23	gwm354-cfd15	7.6	*Lr10*
6	*QLr.uaf.1DL*	53-59	mag1348-wmc216	4.9	Unknown
7	*QLr.uaf.2AS*	8-13	barc124-gwm497	10.2	*Lr17a*
8	*QLr.uaf.2AL.1*	45-48	gwm66-gwm558	11.9	Unknown
9	***QLr.uaf.2AL.2***	**151-152**	**sun389-sun356**	**7.1**	**Unknown**
10	*QLr.uaf.2BS*	0-5	gwm219-wmc661	10.3	*Lr16*
11	***QLr.uaf.2BL***	**52-79**	**barc183-gwm191**	**6.9**	**Unknown**
12	*QLr.uaf.2DS*	26-43	gwm210-wmc453	12.6	*Lr39, Lr41*
13	***QLr.uaf.2DL***	**91-93**	**gwm539-gwm349**	**3.8**	**Unknown**
14	*QLr.uaf.3AS*	44-47	gwm32-gwm391	5.9	Unknown
15	*QLr.uaf.3BS*	0-6	barc75-gwm533	11.5	*LrSV2, Lr27*
16	***QLr.uaf.3BL***	**68-69**	**gwm107-wmc471**	**4.8**	**Unknown**
17	*QLr.uaf.3DS*	0-3	gwm114-wmc674	11.7	LrSV2, Lr27
18	***QLr.uaf.4AS***	**2-18**	**gwm165-gwm397**	**6.1**	**Unknown**
19	*QLr.uaf.4AL*	79-82	gwm160-wmc776	7.4	Unknown
20	*QLr.uaf.4BS*	11-31	gwm538-gwm368	14.4	*Lr25, Lr49, Lr12*
21	*QLr.uaf.4DS*	27-31	wmc89-gwm608	6.1	Unknown
22	*QLr.uaf.5AS.1*	32-34	gwm205-gwm154	4.5	Unknown
23	***QLr.uaf.5AS.2***	**54-62**	**gwm415-gwm186**	**3.6**	**Unknown**
24	*QLr.uaf.5AL*	73-77	gwm156-gwm617	6.1	Unknown
25	*QLr.uaf.5BS*	32-45	gwm443-sun199	7.1	*Lr52*
26	*QLr.uaf.5DS*	24-26	cfd81-gwm358	5.8	*LrAC*
27	*QLr.uaf.5DL*	108-115	gwm469-gwm465	4.7	*Lr1*
28	*QLr.uaf.6AS.1*	0-2	gwm459-gwm334	3.5	Unknown
29	***QLr.uaf.6AS.2***	**25-29**	**gwm494-wmc182**	**4.1**	**Unknown**
30	***QLr.uaf.6AL.1***	**83-93**	**gwm169-gwm427**	**7.5**	**Unknown**
31	***QLr.uaf.6AL.2***	**148-156**	**wmc254-wmc59**	**3.6**	**Unknown**
32	***QLr.uaf.6BS***	**27-37**	**gwm518-gwm133**	**4.8**	**Unknown**
33	*QLr.uaf.6BL*	48-66	gwm626-wmc621	8.2	*Lr3, Lr9*
34	*QLr.uaf.6DS*	3-25	barc183-gwm469	5.9	*Lr53*
35	***QLr.uaf.6DL***	**70-79**	**wmc748-barc175**	**4.7**	**Unknown**
36	*QLr.uaf.7AS*	4-30	gwm233-gwm60	8.3	*Lr47*
37	*QLr.uaf.7BS.1*	0	Wmc606	5.7	*Lr72*
38	*QLr.uaf.7BS.2*	41-47	gwm537-gwm400	4.6	Unknown
39	*QLr.uaf.7BL.1*	58-77	gwm43-gwm112	5.9	Unknown
40	***QLr.uaf.7BL.2***	**172-174**	**mag1757-mag1811**	**8.4**	**Unknown**
41	*QLr.uaf.7DS*	75-77	cslv34-gwm295	7.4	*Lr34*
42	***QLr.uaf.7DL***	**93-102**	**cfd14-gwm121**	**6.2**	**Unknown**

[a]QTL that overlap in the one-log support confidence intervals were assigned the same symbol. [b]centi morgan. [c]Percentages of phenotypic variance explained by individual QTL. The bold and red coloured QTLs are novel and un-bold and black were also found in previous studies

close to the allelic diversity observed in the Chinese germplasm, European and USA wheat population as discussed above.

Table 3: Markers associated with leaf rust data and their reports in previous studies

Chromosome Name	Markers	Locus/gene	Reference
1AS	gwm136	Unknown	(Vasu *et al.*, 2001)
1AS	gwm33	*Lr10*	(Maccaferri *et al.*, 2010)
1AL	wmc59	Unknown	(Crossa *et al.*, 2007)
1BS	gwm131	Unknown	(Crossa *et al.*, 2007)
1BS	wmc619	Unknown	(Crossa *et al.*, 2007)
1BL	wmc419	*Lr33*	(Maccaferri *et al.*, 2010)
1DS	gdm33	*Lr42*	(Liu *et al.*, 2013)
1DS	cfd15	*Lr10*	(Somers *et al.*, 2004)
1DS	gwm337	Unknown	(Crossa *et al.*, 2007)
1DS	barc149	*Lr60*	(Hiebert *et al.*, 2008)
1DS	wmc432	*Lr42*	(Sun *et al.*, 2010)
1DL	wmc216	Unknown	(Crossa *et al.*, 2007)
2AS	wmc667	*Lr17a*	(Roder *et al.*, 1998; Bremenkamp-Barrett *et al.*, 2008)
2AL	gwm66	Unknown	(Crossa *et al.*, 2007)
2BS	wmc661	*Lr 16*	(McCartney *et al.*, 2005; Crossa *et al.*, 2007)
2BS	barc183	*Lr13, Lr23*	(Maccaferri *et al.*, 2010)
2BS	gwm210	*Lr16*	(McCartney *et al.*, 2005)
2DS	gwm210	*Lr39, Lr41*	(Raupp *et al.*, 2001; Sun *et al.*, 2009)
2DS	gwm102	*Lr15*	(Dholakia *et al.*, 2013)
2DS	barc124	*Lr 41*	(Sun *et al.*, 2009)
3AS	gwm369	Unknown	(Crossa *et al.*, 2007)
3AS	gwm2	Unknown	(Crossa *et al.*, 2007)
3AS	gwm674	Unknown	(Crossa *et al.*, 2007)
3AS	gwm666	Unknown	(Crossa *et al.*, 2007)
3AL	gwm155	Unknown	(Crossa *et al.*, 2007)
3AL	gwm480	Unknown	(Crossa *et al.*, 2007)
3AL	gwm497	Unknown	(Crossa *et al.*, 2007)
3BS	gwm533	*LrSV2*	(Ingala *et al.*, 2012)
3BS	gwm389	*Lr27*	(Crossa *et al.*, 2007)
3BS	gwm383	Unknown	(Crossa *et al.*, 2007)
3BL	wmc334	Unknown	(Crossa *et al.*, 2007)
4AS	gwm160	Unknown	(Crossa *et al.*, 2007)
4BS	gwm538	*Lr25*	(Singh *et al.*, 2012)
4BS	wmc349	*Lr49*	(Bansal *et al.*, 2008)
4BS	gwm251	*Lr12*	(Herrera-Foessel *et al.*, 2011)
4BS	gwm149	*Lr12*	(Singh *et al.*, 2011)
4BL	gwm513	Unknown	(Crossa *et al.*, 2007)
4BL	gwm495	Unknown	(Crossa *et al.*, 2007)
4DS	wmc48	Unknown	(Crossa *et al.*, 2007)
4DL	gwm192	*Lr67*	(Herrera-Foessel *et al.*, 2011)
4DL	gwm165	*Lr67*	(Herrera-Foessel *et al.*, 2011)
5AS	gwm154	Unknown	(Crossa *et al.*, 2007)
5AL	gwm617	Unknown	(Crossa *et al.*, 2007)
5BS	gwm443	*Lr52*	(Hiebert *et al.*, 2005)
5DS	cfd81	*LrAC*	(Riar *et al.*, 2012)
5DL	gwm469	*Lr1*	(Somers *et al.*, 2004)
6AS	gwm334	Unknown	(Crossa *et al.*, 2007)
6BL	wmc621	*Lr3, Lr9*	(Maccaferri *et al.*, 2010)
6BL	barc178	Unknown	(Crossa *et al.*, 2007)
6DS	cfd1	*Lr53*	(Dadkhodaie *et al.*, 2011)
7AS	gwm60	*Lr47*	(Vanzetti *et al.*, 2006)
7BS	wmc606	*Lr72*	(Herrera-Foessel *et al.*, 2014)
7BL	gwm232	Unknown	(Crossa *et al.*, 2007)
7DS	cslv34	*Lr34*	(Lagudah *et al.*, 2006; Singh *et al.*, 2007)

Linkage Disequilibrium

The prevalence of LD in the targeted genomic region is pre-requisite for association mapping. A review of previous reports on LD extent indicates that LD extent varies with varying wheat populations (Chao *et al*. 2007; Sajjad *et al.*, 2012). examined genome wide LD among 43 US cultivars using 242 SSRs distributed throughout the genome. For this germplasm genome wide LD decayed to 0.2 within a distance of 10 cM and 20 cM. This LD extent is much higher compared to that reported previously (Breseghello and Sorrells, 2006). They observed LD on chromosome 3B, extended up to 0.5 cM in 44 varieties and in 240 RILs; it was extended up to 30 cM. This study favours the usefulness of germplasm over bi-parental populations in association mapping. LD extent up to ~ 80 cM (r^2 = 0.015) on chromosome 3A in 94 spring wheat genotypes including mostly Pakistani landraces, cultivars and elite lines surveyed with 23 polymorphic SSRs was observed (Sajjad *et al.*, 2014). LD by r^2 at $P < 0.001$ by using 660 DArT markers were also estimated (Mulki *et al.*, 2013). On a genome-wide level, almost 25% of all pairs of loci were in significant LD. These reports on LD structure in hexaploid wheat reveal that LD structure varies with populations, genomic regions and type of marker. The changes in LD along chromosomes are indicative of areas of genome that are under selection pressure were suggested (Somers *et al.*, 2007).

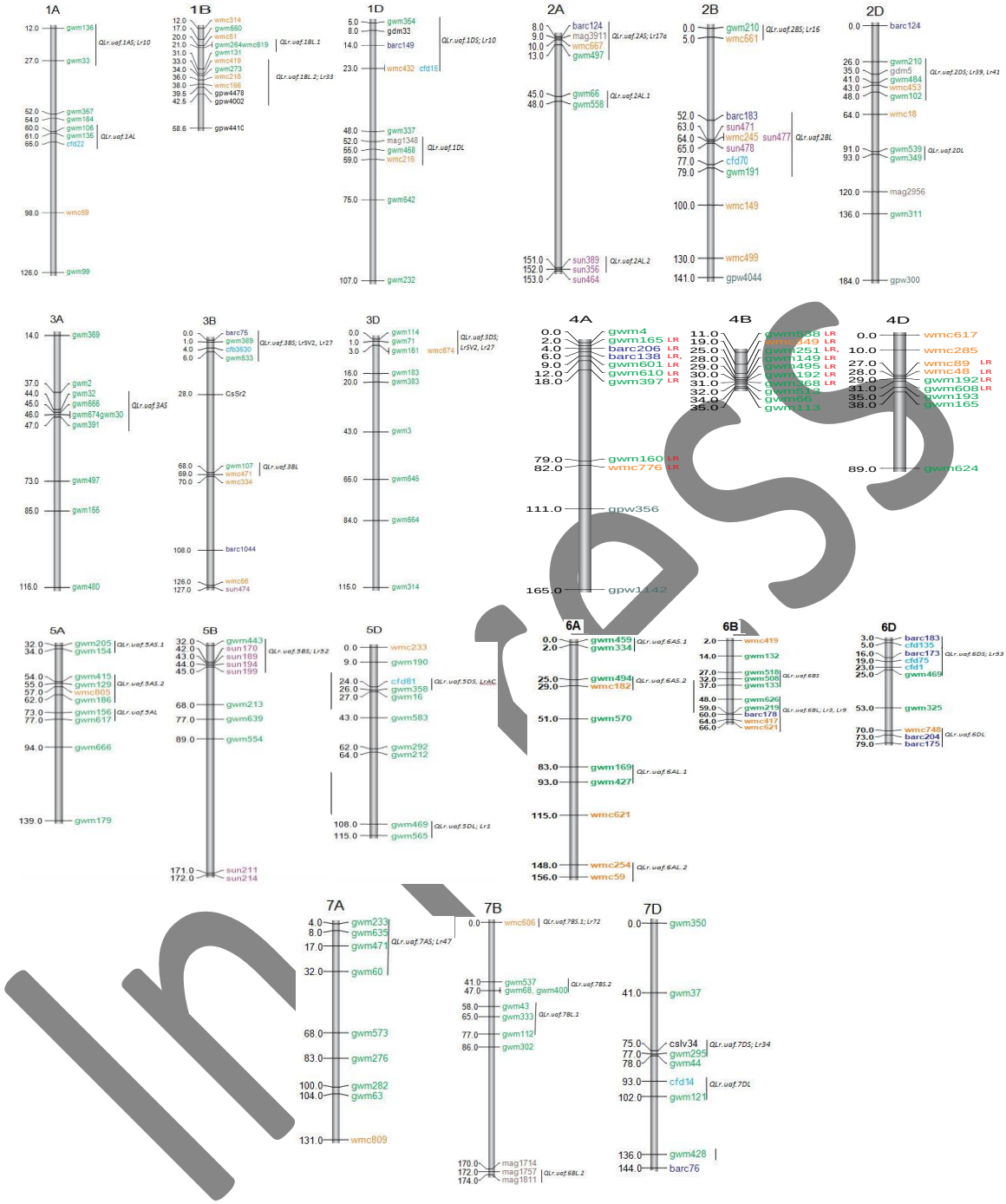

Fig. 3: A microsatellite consensus map of all 21 chromosomes of wheat showing QTLs for LR resistance detected in the study

Marker-trait Associations

Seven major QTLs with percentage of Phenotypic Variance Estimates (PVE) value of > 10% were identified in this study. The QLr.uaf.2AS mapped in the marker interval barc124-gwm497. This QTL contained a known genes *Lr17a* and have been identified in winter and spring wheat germplasm (Roder *et al*., 1998; Bremenkamp-Barrett *et al*., 2008). The *QLr.uaf.2AL*.1 mapped between markers gwm66 and gwm558 where three known LR QTLs, QLr.cimmyt-2AL (Rosewarne *et al*., 2012), QLr.sfr-2AL (Schnurbusch *et al*., 2004) and QLr.ubo-2A (Maccaferri *et al*., 2008), were reported.

The *QLr.uaf.2BS* mapped in marker interval gwm219-wmc661 was located on chromosome 2BS with six known Lr genes, *Lr13*, *Lr16*, *LrZH22*, *Lr23*, *Lr35*, and *Lr48* (Wang *et al*., 2016; Zhang *et al*., 2017). The *QLr.uaf.2DS* mapped between gwm210-wmc453 had two known genes *Lr39* and *Lr41* (Raupp *et al*., 2001; Sun *et al*., 2009). The *QLr.uaf.3BS* mapped between barc75 and gwm533 had two known genes *LrSV2* and *Lr27* and identified in previous studies (Crossa *et al*., 2007; Ingala *et al*., 2012). The *QLr.uaf.4BS* mapped in gwm538-gwm368 was rich in Lr genes viz. *Lr12* (Singh and Bowden, 2011), *Lr25* (Singh *et al*., 2012) and *Lr49* (Bansal *et al*., 2008). The thirteen novel QTLs identified kin this study have unknown genes need to be verified in future studies.

Conclusion

Seven out of 42 QTLs identified were major QTLs with PVE value of > 10. Many known *Lr* genes were located in these major QTLs. Thirteen tentative QTLs with unknown genes were also found and need to be verified in future studies. The major QTLs validated in Pakistani germplasm could be used through marker assisted backcrossing scheme to develop cultivars with improved *Lr* resistance in elite wheat backgrounds.

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