The Journal of Animal & Plant Sciences, 31(2): 2021, Page: 459-467
ISSN (print): 1018-7081; ISSN (online): 2309-8694

IDENTIFICATION OF MULTIPLE LEAF RUST RESISTANCE GENES IN SELECTED GERMPLASM OF PAKISTANI BREAD WHEAT USING MOLECULAR MARKERS

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ABSTRACT

Leaf rust, caused by *Puccinia triticina* f. sp. tritici, is the most prevalent disease of wheat in Pakistan. Host resistance is the most effective and economical method of controlling the disease. In the present study, 254 Pakistani wheat germplasm were screened for the presence or absence of 17 leaf rust resistance (*Lr*) genes. *Lr* 09, 10, 20, 24, 25, 27, 28, 29, 35, 37, 39, 46, 47, 50, 51, 58 and *Lr* 61 were detected either as pyramided triplet and doublet genes, or as a single gene harbored varieties/germplasm. Triple and double gene pyramids were detected in 9 and 50 varieties/germplasm, respectively, while single *Lr* genes were detected in 29 varieties/germplasm. Genotype 10731, having the *Lr*28/*Lr35* combination, was the leading one with highest grain yield of 20.62 g/plant. Other leading germplasm were 10754, 10767 and 10758, having combinations of *Lr28/Lr35*, *Lr28/Lr29* and *Lr25/Lr27*, and grain yield of 18.84, 16.63 and 15.65, respectively. Ata Habib, with triplet pyramid of *Lr*27/*Lr*50/*Lr*51, was the leading cultivar with 12g/plant grain yield. Identification of *Lr* genes in Pakistani wheat germplasm will help accelerate the current programs of breeding for resistance to leaf rust in the varieties/germplasm of interest.

Key words: Leaf rust, *Puccinia triticina* , germplasm, *Lr* genes, PCR, Wheat, MAS.

https://doi.org/10.36899/JAPS.2021.2.0235 *Published online October 03,2020*

INTRODUCTION

Wheat is among the highest cultivated cereals due to its broad-spectrum adaptation, uses and production, and is grown by nearly half of the world population as a principal food (Inamullah *et al.* 2006; Paux *et al*. 2008). Because of its huge impact on human life, the crop has also been termed as miracle crop. Wheat has a huge impact on global civilization and provides 20% of food calories and proteins, and is cultivated over 220 million hectares of land worldwide, producing 670 million tons of grains annually (Shiferaw *et al*. 2013). Furthermore, the global demand for wheat is increasing with rising human population. As to any other crop, wheat also faces the problems of both abiotic and biotic stresses. Biotic stresses include threats from different rust pathogens, which severely affect wheat productivity. For example, the stem rust alone was estimated to cause a loss of US$ 1-2 billion in Asia (Jin and Singh, 2006). The epidemic caused by stripe rust was severe in late sowing varieties in the Khyber Pakhtunkhwa province of Pakistan. Even the early sowing varieties which escaped the epidemics inflicted a loss of approximately US$ 100 million in the province. One of the economically important rust diseases is the leaf rust of wheat, caused by fungal pathogen *Puccinia triticina*. The disease greatly affects the wheat production, to the extent, that the loss goes upto 50 % and beyond in case of severe incidences (Singh and Hughes, 2006). In addition to cultural practices like cropping intensity and monocropping, the nature of the disease resistance genes distributed in the population of a crop variety also affects the intensity of rust occurrence. Uniformity of disease resistant genes in a cultivar, for example, increases the occurrence of rust. The scenario provides an apt environment for the pathogen to rapidly evolve, arising into a new rust pathotype, and turning a previously resistance host gene into susceptible one (Marasas *et al.* 2004).

Developing host resistance is effective, economically feasible, and environment friendly approach to contain wheat rust (Vanzetti *et al.* 2011). Utilization of new sources of resistance from collected germplasm and transfer of resistance gene(s) in the adapted wheat cultivars is an effective strategy toward this end (Singh *et al.* 2011). Resistance is, essentially, achieved by the products of *R* genes in the plant and an associated avirulent (*avr*) gene of the pathogen. The Avr-R interaction elicits a cascade of pathogen race-specific responses in the host, resulting into the establishment of dominant resistance (Dangl and Jones, 2001). Identification of leaf rust (*Lr)* candidate *R* genes in wheat cultivars and germplasm as source of resistance and their introgression into a cultivar is the best approach and a stepping stone for a successful breeding program. The strategy helps avoid the prevalence of cultivars which are genetically uniform and homozygous for susceptibility genes (Messmer *et al.* 2000 and Mebrate *et al.* 2008).

DNA markers are effective tools to detect genes of interest in the host germplasm and are widely being used in the breeding for resistance against wheat rust, an alternative to the traditional approach of gene postulation, including gene interactions and expression at different growth stages of the plant. Advancements in the *Lr* genes mapping and development of markers for many *Lr* genes have paved the way for markers assisted selection (MAS) to characterize individual genotypes in the advanced breeding programs (Helguera *et al.* 2000, 2003, 2005; Gupta *et al.* 2006; Lagudah *et al.* 2006). Recently, a QTL genetic marker was co-localized for yellow rust on chromosome 6BL in wheat (Zeng *et al.* 2019). Similarly, the leaf and yellow rust genes have been fine mapped to 5D chromosome of *Aegilops peregrine*, a wild relative of common wheat (Narang *et al.* 2019). MAS is therefore, a helpful tool to identify cultivars harboring alleles for the traits of interest at specific loci, and develop varieties of improved yield and high rust resistance in wheat. Furthermore, MAS not only corroborates the authenticity of conventional breeding and its associated QTL analysis but also shortens the time required for a variety development. Detection of *Lr* gene(s) present in selected Pakistani germplasm of bread wheat using molecular DNA markers is reported in the present study. It is an effort to categorize the selected germplasm into different batches based on the presence/absence of the corresponding *Lr* gene(s).

MATERIALS AND METHODS

Plant material: Common wheat varieties and germplasm consisting of 254 genotypes, and acquired from Plant Genetic Resources Institute (PGRI) Islamabad and Cereal Crop Research Institute (CCRI) Nowshera, were grown in the field at Hazara University Mansehra. The selected genotypes were characterized for various yield contributing traits and leaf rust resistance genes *Lr* 09, 10, 20, 24, 25, 27, 28, 29, 35, 37, 39, 46, 47, 50, 51, 58 and *Lr* 61 by employing molecular markers viz: STS, SSR, CAPS and SCAR etc.

DNA extraction: Genomic DNA was isolated from young leaves using the protocol of Brunel (1992).

Molecular markers-based identification of leaf rust genes: The basic information regarding all the 17-leaf rust resistant genes using different molecular markers, primers sequences, annealing temperature and expected product size along with the original references is given in Table 1.

Polymerase chain reaction for 37 cycles was carried out in a total volume of 25 µl having 50 ng of template DNA using Applied Biosystems 2720 Thermal Cycler. Other ingredients of PCR included 10xTaq buffer (Fermantos), 5 U Taq DNA polymerase enzyme (Fermantos), 10mM of each deoxynucleotides (AGTC), 10µM of each primer and 2.5 mM of $MgCl_2$. The PCR program consisted of 94◦C for 1 min as denaturation temperature followed by 50-64◦C for 1 min as annealing temperature and 72◦C for 1min as extension temperature and a final extension round of 72◦C for 10 min. Amplification products were visualized under UV light using gel doc system.

Data analysis: Statistical analysis was performed through R-packages for the presence of different *Lr* genes as triplet, doublet or singlet, in the varieties/germlasm in the present study.

Table 1. Primers associated with 17 molecular marker *Lr* genes and the relevant parameters.

S. No	Leaf rust gene	Marker used	Olegonucleotide sequences	Annealing temp(°C)	Required band size	Reference
1	Lr 09	STS	TCCTTTTATTCCGCACGCCGG CCACACTACCCCAAAGAGACG	58	1100 bp	Schachermayr *et al.* 1994
2	Lr 10	STS	GTGTAATGCATGCAGGTTCC AGGTGTGAGTGAGTTATGTT	55	310 bp	Schachermayr *et al.* 1997
3	Lr 20	STS	GCGGTGACTACACAGCGATGAAGCAATGAAA GCGGTGACTAGTCCAGTTGGTTGATGGAAT	52	542 bp	Neu *and* Keller, 2002
4	Lr 24	STS	TCTAGTCTGTACATGGGGGC TGGCACATGAACTCCATACG	60	310 bp	Schachermayr *et al.* 1995
5	Lr 25	SCAR	CCACCCAGAGTATACCAGAG CCACCCAGAGCTCATAGAA	58	1800 bp	Procunier *et al.* 1995
6	Lr 27	EST	GGGTGCACATCCATTGACTTT TTCTCTCAAGAGCGGGTGCT	60	393 kb	Mago *et al.* 2011
7	Lr 28	STS	CCCGGCATAAGTCTATGGTT CAATGAATGAGATACGTGAA	50	378 bp	Naik *et al.* 1998
8	Lr 29	SCAR	GTGACCTCAGGCAATGCACAGT GTGACCTCAGAACCGATGTCCATC	64	900 bp	Procunier *et al.* 1995
9	Lr 35	STS	AGAGAGAGTAGAAGAGCTGC	51	900bp	Gold *et al.* 1999

			AGAGAGAGAGCATCCACC			
10	Lr37	CAPS	GGTCGCCCGGGCTTGCACCT TGCAGCTACAGCAGTATGTACACAAAA	64	285 bp	Helguera *et al.* 2003
11	Lr 39	SSR	CCTGCTCTGCCCTAGATACG ATGTGAATGTGATGCATGGCA	56	160 bp	Raupp *et al.* 2001
12	Lr46	SSR	AGGGAAAAGACATCTTTTTTTTC CGACCGACTTCGGGTTC	50	110 bp	Rosewarne *et al.* 2006
13	Lr47	CAPS	GCTGATGACCCTGACCGGT TCTTCATGCCCGGTCGGGT	55	487 bp	Helguera *et al.* 2000
14	Lr50	SSR	gtcagataacgccgtccaat ctacgtgcaccaccattttg	56	139 bp	Brown-Guedira *et al.* 2003
15	Lr51	CAPS	gcatcaacaagatattcgttatgacc tggcggctcagaaaactggacc	60	783 bp	Helguera *et al.* 2005
16	Lr 58	SSR	TTCTGCAACATTTTGTCCCA CGTATGATCCTAACGAGGGC	60	261bp	Kuraparthy *et al.* 2007
17	Lr 61	SSR	cAAATTTggccAccATTTTAcA cggTTcAATccTTggATTTAcA	55	160 bp	Herrera-Foessel *et al.* 2005

Table 2. Germplasm studied in the present study.

S. No	Group	Number of genes studied	Number of germplasm	Germplasm
1	Group I	5	52	11882, 10818, 10821, 10819, 10879, 10878, 10814, 10875, 10730, 10815, 10816, 10813, 10877, 10738, 10817, 10771, 10803, 10726, 10772, 10727, 10718, 10732, 10801, 11876, 10808, 10809, 11863, 10717, 10735, 10810, 10759, 10725, 10755, 10733, 10719, 10780, 10740, 10743, Lasani08, Faisalabad08, Uqab2000, gomal, suleman, NARC 2009, Sahar 2006, Dera 98, Atta-Habib, KT 2000, Kaghan-93, Pak- 81, PS 85, Tatara.
2	Group II	4	52	10805, 10799, 10798, 10758, 10802, 10748, 10804, 10786, 10742, 10797, 10807, 10751, 10800, 10787, 10789, 10792, 10760, 10794, 10791, 10724, 10793, 10776, 10761, 10715, 10795, 10784, 10769, 10782, 10766, 10765, 10778, 10796, 10768, 10746, 10783, 10790, 10762, 10785, Lasani-2008, FSD 2008, Auqab-2000, Gomal, Suleman-96, NARC- 2009, Sahar-2006, Dera 98, Atta - habib, KT 2000, Kaghan-93, Pak 81, PS-85, Tatara.
3	Group III	2	48	Punjab96, Mumal2002, Zamindar80, Iqbal2000, SH2003, Anmol91, LU26, Chenab96, Faisalabad83, Zarghoon79, C228, Shahkar95, Punjab88, Nuri70, Punjab81, C591, Sutlag86, C250, Blue silver, RWP94, Sariab92, Wafaq2008, Anza+2NS, *Lr*51YR, AUP5000, WL711, SA75, SA42, Marwat01, Barani83, Potohar93, Kohinoor83, Potohar70, Pak81, Pirsabak85, C273, Tandojam83, Dirk, Bahalwapur79, Lasani08, Sussi, Khyber79, FPD08, Sandal, Kiran, Wardak85, Meraj08, C518
4	Group IV	3	51	11868, 10854, 10853, 10850, 10849, 10825, 10845, 10847, 10862, 10860, 10861, 10866, 10809, 10873, 10842, 10841, 10833, 10843, 10848, 10832, 10829, 10852, 10834, 11831, 10835, 10830, 11865, 10826, 10822, 10881, 10828, 10824, 10874, 10827, 10864, 10820, 10867,Shafaq2006, PS2004, Siran2010, Noshera96, Khyber87, Wafaq, Hashim, PS08, Zam, Saleem2000, PS05, Janbaz, Haider2000, ARE10
5	Group V	3	51	11880, 10750, 10777, 10781, 10752, 10775, 10774, 10744, 11872, 10753, 10770, 10736, 10812, 10779, 10741, 11870, 10763, 10757, 10739, 11871, 10731, 10773, 10764, 10745, 10806, 10716, 10767, 10749, 11869, 10754, 10722, 10728, 10720, 10721, 10756, 10787, 10788, Shafaq2006, PS2004, Siran2010, Noshera96, Khyber87, Wafaq, Hashim, PS08, Zam, Saleem2000, PS05, Janbaz, Haider2000, ARE10
	Total	17	254	

RESULTS

Uncovering the genetic background of commercially important wheat cultivars is important for deployment of the disease resistance genes and development of molecular markers. In the present study, 254 genotypes and varieties of Pakistani wheat germplasm were screened for the presence of 17 *Lr* genes via PCR using the primers specific to the respective *Lr* genes (Tables 1, 2).

The germplasm/varieties in the current study were grouped into three categories relative to the number of *Lr* genes present in them. The germplasm/varieties either harbored triple or double *Lr* genes pyramided or single *Lr* gene present in the corresponding category. Based on literature, a wheat plant produces approximately 250 grains on average, while 1000 wheat grains weigh approximately 40 grams (Afzal and Iqbal, 2015). By this account, 10 g/plant grain weight, as an arbitrary cut off value, was set for selection of a cultivar/germplasm with *Lr* gene marker (Afzal and Iqbal, 2015).

A total of 9 varieties/germplasm contained a set of triple genes pyramided in them. Ata Habib, with triplet combination of *Lr*27/*Lr*50/*Lr*51 was the leading cultivar with 12g/plant grain yield. Other cultivars with triplet *Lr* genes were Kaghan 93 and Faisalabad 08, having the *Lr* combinations of *Lr*27/*Lr37/Lr47* and *Lr47/Lr50/Lr51*, and grain yield of 11.53g/plant and 10g/plant, respectively. The germplasm 10879 had the combination of *Lr37/Lr47/Lr50* and grain yield of 11.25 g/plant (Table 3).

Fifty varieties/ germplasm were naturally pyramided with double *Lr* genes. Germplasm 10731, having the *Lr*28/*Lr35* combination, was the leading germplasm with highest grain yield of 20.62 g/plant. Other leading germplasm included 10754, 10767 and 10758, having combinations of *Lr28/Lr35*, *Lr28/Lr29* and *Lr25/Lr27*, and grain yield of 18.84, 16.63 and 15.65, respectively (Table 3).

There were eleven *Lr* genes present as a singlet in 29 varieties/germplasm, out of which 12 varieties/germplasm harbored *Lr61*. Further, 10822 and 11881 were among the highest yielding varieties/germplasm which harbored *Lr61* with 19.53 g/plant and 16.14 g/plant grain yield, respectively (Table 3).

Table 3. PCR-based detection of *Lr* gene markers as triplet, doublet and singlet, and grain yield/plant in the selected bread wheat varieties/germplasm.

***Lr* Genes**	**Germplasam**	**Grain yield/plant (grams)**
Lr20, Lr25, Lr27	10802	6.918
Lr28, Lr29, Lr35	11870	9.83
Lr 28, Lr35, Lr39	11871	7.35
Lr37, Lr47, Lr50	**10879**	**11.25**
Lr37, Lr46, Lr51	10814	9.24
Lr25, Lr27, Lr46	Pak-81	9.81
Lr27, Lr50, Lr51	**Atta Habib**	**11.98**
Lr27, Lr37, Lr47	**Kaghan93**	**11.53**
Lr47, Lr50, Lr51	**Faisalabad08**	**10.09**
Lr20, Lr27	10748	6.457
Lr24, Lr27	10798	8.86
Lr25, Lr27	**10805, 10758, 10742, 10807**	**13.54, 15.65, 14.02, 12.81**
Lr28, Lr35	**10774**, 10753, 10736, **10812, 10731, 10716, 10754, 10720**	**11.66**, 8.49, 9.71, **10.29**, **20.62**, **10.22**, **18.84**, **13.06**
Lr29, Lr35	**10744**	**12.14**
Lr37, Lr51	10821, 10759, 10725	4.63, 16.36, 11.24
Lr37, Lr47	10755, Gomal	8.04, 7.04
Lr28, Lr29	**10767**	**16.63**
Lr37, Lr46	10819	6.01
Lr37, Lr50	10743	9.75
Lr46, Lr50	10730	6.83
Lr46, Lr51	10875, 10732,	9.30, 10.32
Lr47, Lr51	11882	7.08
Lr47, Lr50	10771	6.74
Lr50, Lr51	10877	9.57
Lr25, Lr47	**Suliman96**	**12.75**
Lr27, Lr47	KT-2000	7.37
Lr28, Lr29	Zam	9.77
Lr9, Lr10	Punjab96, Mumal2002, Zamindar80, Iqbal2000, SH2003, Anmol94, LU26, Chenab96, Faisalabad83, Zarghoon79, C228, Shahkar95, Nuri70, Punjab81, Sutlaj86, Blue Silver, RWP94, Pirsabak85	4.83, 5.03, 4.78, 5.36, 6.66, 5.43, 7.26, 5.63, 6.83, 6.26, 5.13, 6.46, 6.16, 5.40, 4.83, 9.16, 5.93, 6.36
Lr25	**10800**, **10776**, 10769, 10765	**12.12**, **10.71**, 8.15, 8.85
Lr27	**10799, 10786,** 10785	**11.57, 12.25,** 8.56
Lr28	10750, **10777**, 10770, 10757, 10739, 10749, **11869, 10756,** 10787, **PS05**	7.46, **14.42**, 9.71, 6.21, 9.61 6.23, **10.67**, **12.61,** 8.68, **10.0**

Lr29	10741, **10728,** Saleem-2000	9.20, **12.6,** 9.6
Lr35	10781, **10752,** 11872, 10773, 10745, **10806, 10721**	9.77, **12.50,** 8.50, 5.78, 4.66, **10.72, 16.76**
Lr37	10818, 11876, 10780, 10740, Lasani08	7.50, **10.0,** 4.90, 6.22, 6.30
Lr46	10816, 10718, **Uqab2000**	4.74, 7.92, **11.90**
Lr50	**10878, 10815,**	**10.15, 13.15**
Lr51	10813, 10803, 10772, **10809, 10717**, 10810	5.51, 7.26, 6.54, 8.81, **10.70**, **11.52**
Lr58	**10874, 10827, 10820**	**11.90, 15.48, 11.28**
Lr61	11868, 10854, **11860**, 11809, **10829**, **0852**, **10834,** 10831**, 11865, 10822, 11881, 11874**	7.35, 9.32, **11.22**, 7.81, **10.1**, **13.12** **12.72**, 9.40, **11.64, 19.53, 16.14, 11.90**
Lr9	Anza+2NS, AUP5000, WL711, SA75, SA42, Marvat01, Dirk, Lasani08, Sandal, Kiran	4.6, 5.23, 5.33, 6.26, 5.63 5.40, 8.33, 6.30, 4.83, 4.66
Lr10	C-228, C-591, C-250, Sariab92, Barani-83, Kohinoor83, Potohar70, C-273	5.13, 6.10, 6.6, 6.76, 3.73 5.06, 5.2, 6.63

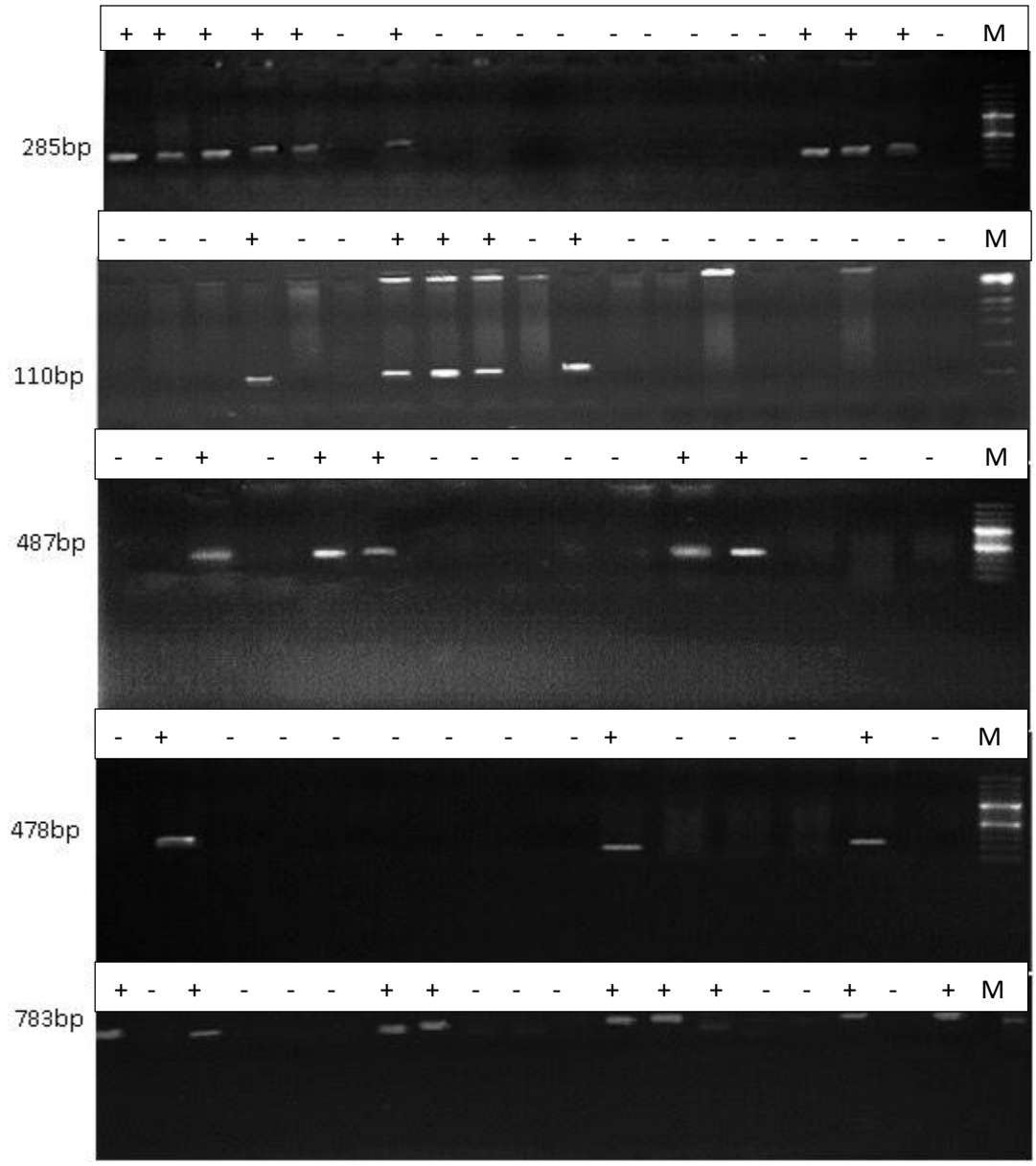

Fig. 1. Selected gel picture of some genes studied M= 100bp marker (A) Amplification profile of Lr37. (B) Amplification profile of Lr46 (C) Amplification profile of Lr 47. (D) Amplification profile of Lr 50 (E) Amplification profile of Lr 51.

DISCUSSION

To survive and sustain, pathogens respond positively to the selection pressure of the host and environment with fast genetic changes. This results into the emergence of new races of pathogens. In context of this ever-evolving nature of the pathogens, a single gene-derived resistance is neither broad nor durable, and thus, any time soon, can be defeated by the emergence of the new virulent races of the pathogen. In USA, for example, *Lr9* gene conferred resistance to soft red winter wheat in the 1970s against leaf rust only for few years. In another example, the races of leaf rust which could defeat *Lr41* and *Lr50* genes were identified in USA even before the cultivars harboring these genes could be released (Kolmer *et al.,* 2007a). Similarly, races having virulence to *Lr19*, *Lr26*, *Lr41* and *Lr51* have been identified in Argentina in cultivars that originally were resistant to leaf rust (Vanzetti *et al.,* 2011). Therefore, pyramiding more than one effective resistance genes in a single background can enhance the spectrum and durability of the rust resistance. Hard red spring wheat cultivars having *Lr16* and *Lr24* were highly resistant to leaf rust (Oelke and Kolmer 2004). The development of wheat germplasm with high level of resistance will, therefore, depend on the ability to select genotypes that have combinations of effective resistance genes such as *Lr16*, *Lr23* and *Lr34* genes as suggested by Kolmer *et al.,* (2007a). In Argentina, however, resistant local cultivars were detected for the presence of *Lr16* gene but not *Lr34* and *Lr23* genes. The results propose the possibility that *Lr16*, in combination with some unknown *Lr* gene(s) other than *Lr*23 and *Lr*34, gives a high level of resistance relative to different geo-ecological environments (Vanzetti *et al.,* 2011).

Gene pyramiding of *Lr* genes is, therefore, an effective strategy to develop broad and durable resistance in wheat cultivars against leaf rust. MAS has been well understood and applied to follow the genes of interest in germplasm and varieties. It is an effective way for identification and pyramiding of disease resistance genes into a single variety. In breeding for leaf rust resistance in wheat, PCR-based markers are already available for around 30 resistance genes and alleles (Samsampour *et al.* 2010). Some of the markers appropriate for seedling resistance include *Lr*9 (Schachermayr *et al.* 1994; Gupta *et al.* 2005), *Lr*16 (McCartney *et al.* 2005), *Lr*19 (Prins *et al.* 2001; Gupta *et al.* 2006), *Lr*41 (Sun *et al.* 2009), *Lr*51 (Helguera *et al.* 2005), *Lr Lr*53 (Dadkhodaie *et al.* 2010), *Lr*56 (Marais *et al.* 2010a), *Lr*57 (Kuraparthy *et al.* 2009), *Lr*59 (Marais *et al.* 2008), *Lr*62 (Marais *et al.* 2009), *Lr*63 (Kolmer *et al.* 2010), and *Lr*66 (Marais *et al.* 2010b). The markers associated with adult plant resistance genes are *Lr*12 (Singh and Bowden, 2010), *Lr*22a (Hiebert *et al.* 2007), SV2 (Ingala *et al.* 2007), *Lr*46 (Mateos-Hernandez *et al.* 2006), *Lr*48 (Singh *et al.* 2011) and *Lr*67 (Herrera-Foessel *et al,* 2011) are available along with ones used in the current study.

Substantial efforts have been made and are being put together to develop genomic resources for different plant species of economic importance, wheat being one of them (Schreiber *et al.* 2012). Innovations in mapping and sequencing tools have increased the number of effective *Lr* genes showing partial resistance (slow rusting) at seedling or adult plant stages. This has broadened the pool of available *Lr* genes and provided a flexibility to deploy a combination of *Lr* genes for broad and durable resistance against the rapidly evolving strains of rust pathogens. Multiple minor or major, adult or seedling plants-resistance genes need to be combined for the optimal level of durable and broad-spectrum resistance. Through this strategy, the resistance response of the plants to rust can be exploited in two ways i.e basal resistance mediated by minor genes and race-specific resistance mediated by major genes. A rigorous assessment of host fitness of the combined genes deployment in terms of agronomic characteristics including phenotype and yield will be needed to either acquire or discard this new modulated strategy. The presence of *Lr* genes, as triplet, doublet or singlet, in different number of varieties/germplasms in the current study showed the dynamic nature of the immunity of these varieties/germplasms. Based on MAS, the genotypes positive for *Lr* genes in the present study can be valuable resources for future breeding for rust resistance and high grain yield programs.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest: None to declare.

Research involving Human Participants and/or Animals: None to declare.

Informed consent: None to declare.

Conclusions: In the present study, 254 Pakistani wheat germplasm were screened for the presence/absence of 17 leaf rust resistance (*Lr*) genes. Triple and double *Lr* genes pyramids were detected in 9 and 50 varieties/germplasm, respectively, while single *Lr* genes were detected in 29 varieties/germplasm. Germplasm 10731, having the *Lr*28/*Lr35* combination, was the leading germplasm with highest grain yield of 20.62 g/plant. Other leading germplasm included 10754, 10767 and 10758, having combinations of *Lr28/Lr35*, *Lr28/Lr29* and *Lr25/Lr27*, and grain yield of 18.84, 16.63 and 15.65, respectively.

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