The Journal of Animal & Plant Sciences, 31(3): 2021, Page: 877-887
ISSN (print): 1018-7081; ISSN (online): 2309-8694

MICROSATELLITE MARKERS BASED ASSESSMENT OF RUST RESISTANCE GENES IN PAKISTANI BREAD WHEAT GENOTYPES

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ABSTRACT

Development of rust resistant wheat cultivars to meet ever-increasing wheat production targets for national food security in climate change scenario is currently at high priority in Pakistan. Focusing on this, here we report the screening of 40 wheat genotypes including advanced breeding lines and commercial varieties through PCR based DNA markers to detect genes conferring resistance against different rust races. In this study 22 SSR/STS markers were used which included nine leaf rust (Lr19, Lr22a, Lr27, Lr28, Lr34, Lr35, Lr37, Lr46 and Lr67), eight stripe rust (Yr5, Yr15, Yr17, Yr18, Yr26, Yr29, Yr30 and Yr46) and seven stem rust resistance genes (Sr2, Sr22, Sr24, Sr25, Sr26, Sr38 and Sr39). The results revealed that allelic frequency for rust resistance genes (Lr, Yr and Sr) was maximum (70.4%) in three advanced wheat lines namely V-9452, V-11153 and NNG-3. However, minimum frequency (51.9%) was found in 25 genotypes. Three advanced lines NNG-3, V-9432 and V-11153 had maximum number of rust resistance genes among all the reported wheat genotypes which indicated narrow genetic base of Pakistani wheat cultivars for rust resistance. Therefore, it is suggested that newly discovered rust resistance genes especially Lr and Yr should be used along with existing genes for effective and durable rust resistance in new wheat varieties.

Key words: Gene, heat map, DNA markers, PCR, SSR.

https://doi.org/10.36899/JAPS.2021.3.0285 *Published online November 11, 2020*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is widely grown and consumed food crop worldwide. It gives about 20% of calories consumed by the world population. Its importance is increasing with increasing global population (Tadesse *et al*., 2013; Akhtar *et al*., 2018). It is also an important cereal crop of Pakistan due to its consumption as staple food of more than 220 million populations. In Pakistan, bread wheat is cultivated on 8740 thousand hectares having production of 25.195 million tons (Government of Pakistan, 2019). Although Pakistan has attained self-sufficiency in wheat production but per hectare yield is still lower than other wheat producing countries (FAO, 2018). Therefore, advanced technologies need to be adopted to save wheat crop from biotic and abiotic stresses and to enhance wheat production to fulfill the food requirements of large population.

It is observed that in Pakistan rusts reduce wheat yield nearly 10% every year in later growth stages. But in early growth stages, yield losses may have reached up to 20% (Ejaz *et al*., 2012; Singh *et al*., 2016). Wheat rusts caused by *Puccinia spp.* are among the major production constraints worldwide (Qamar *et al.,* 2014; Bhardwaj *et al.,* 2019). Leaf rust (Lr) attack on wheat occurs more frequently than stripe rust (Yr) or stem rust (Sr). Pakistan suffered from leaf rust epidemics in 1972-73 when the disease severity level was 30-50% while in 1976-78, disease severity increased up to 50-80% (Khan, 1997). Similarly, in 1995, 2005 and 2019 stripe rust and leaf rust epidemics have caused significant yield losses (Qamar *et al*., 2014 and Gessese, 2019). The emergence of new races of stem rust (Sr) has brought a major challenge of world wheat production (Gessese, 2019) The prime objective of wheat breeders is to develop new wheat varieties with optimum levels of disease resistance (McCallum *et al*., 2016). The wheat germplasm has a large set of resistance genes and more than 50 wheat rust resistant genes for each of the three rust species have been reported by different wheat researchers (Pretorius *et al*., 2012; Pal *et al*., 2015; Gessese, 2019).

Previously, conventional breeding techniques were used for development of new rust resistant wheat genotypes through gene recombination but recently, marker assisted selection, by far, the most efficient and broadly used tool for the monitoring of *Pst* pathotypes and threats of Ug99-complex races (Bhardwaj *et al.,* 2019; Gessese, 2019). Microsatellites (SSR/STS) as reported in the literature are one of the most suitable molecular markers for the detection of rust resistance genes in hexaploid wheat (Singh *et al*., 2016). At present, approximately 2,800 SSRs have been reported and genetically mapped in wheat genome to investigate wheat rust resistant genes.

This study was designed to identify rust resistant genes in candidate wheat varieties and existing commercial cultivars by using DNA based markers tightly linked to rust resistance genes in bread wheat.

MATERIALS AND METHODS

Healthy seeds of 40 wheat genotypes (30 advanced lines and 10 commercial varieties) were sown in the experimental area of Agricultural Biotechnology Research Institute (ABRI), Ayub Agriculture Research Institute, Faisalabad, Pakistan in Alpha-Lattice design with two replications (Table 1). At tillering stage, 2-3 fresh leaves of selected plants from each genotype were collected and stored at -20^{o}C. A modified CTAB method was used to extract DNA of studied wheat genotypes (Rahman *et al*., 2002).

The quality of extracted DNA was analyzed by gel electrophoresis using 0.8 % agarose gel. The concentration of total genomic DNA of 40 wheat lines/varieties was determined by an automated NanoDrop-1000 3.3.1 apparatus. Quantified DNA was diluted to the working concentration of about 30 ng/µl.

A total of 22 Simple Sequence Repeats (SSR) and Simple Tendom Sequence (STS) markers were used in the experiment to identify genes that conferred resistance to various rust races in wheat genotypes (Table 2) along with 20 gene differentials to confirm the presence of resistant gene of interest in studied wheat genotypes. DNA amplification was done in a thermo-cycler machine using volume of 20 µl per sample having 2 µl 10X *Taq* buffer, 2 µl $MgCl_2$ (25mM), 1 µl each of dATP, dCTP, dTTP and dGTP (2.5 mM), 1.5 µl primer (30 ng/ µl), 2 µl of genomic DNA (30 ng/µl), 0.2 µl (5unit/µl) *Taq* DNA Polymerase and 8.3 µl ddH_2O.

Amplification reactions were programmed for 40 cycles. Amplification products were electrophoresed in 3g/l agarose gel. DNA ladder of 1000bp with known molecular weight bands was loaded along with PCR amplified DNA products on gel to determine the size/molecular weight of the polymorphic DNA fragments. Then amplified products were photographed using Syngene Gel Documentation System. Polyacrylamide Gel Electrophoresis (PAGE) was also performed for those samples which had small DNA fragments to study them precisely. Presence of rust resistant gene with adequate band size was scored as positive (+) while susceptible gene was scored as negative (-) as shown in Tables 6,7 & 8.

Estimation of allelic frequency (%) and allelic diversity were determined by R. and Power Maker V.0.3 software package. The polygenetic analysis with reference to rust resistant genes was determined by using R-software version 3.0.2. All amplified loci based on SSR/STS banding pattern were recorded as present (1) and absent (0) in the 40 genotypes. The bivariate 1-0 was used to construct heat map dendrogram by using cluster method between group linkage and genetic distance was determined through Squared Euclidean Distance.

RESULTS

A total of 22 molecular markers (SSR/STS) were used in this study to determine the presence/absence of rust resistance genes (Lr,Yr & Sr) in 40 wheat genotypes (Table 2). Xgwm533, Xwmc44, Xgwm11, Lr28, csLV34, Sr26#43, BF145935, Xcfd23, Xcfd71, Xbarc352, cfa2123, SCS253 and Xgwm296 provided reproducible results whereas csSr2, Sr39#22r, Sr39F_2, STS7/8, STS9/10, BARC71, Xgwm437, Ventriup LN2 and SCS265 failed to amplify fragments for Sr24, Lr37 and Yr5 due to some experimental error. Gene differentials (20) were also used to confirm band size of rust resistant genes in studied wheat genotypes. Regarding leaf rust (Lr) resistance genes, nine genes were investigated. Among these, five leaf rust (Lr) resistance genes Lr.19. Lr.22a, Lr.28, Lr.35 and Lr.67 genes were identified in all studied wheat genotypes while Lr.24 and Lr.37 were not found. Whereas, Lr.34 and Lr.46 genes were present in 10-B9346 and NR-310 wheat genotypes, respectively (Table 3). Regarding eight studied stripe rust resistance conferring genes, only Yr.15, Yr.26 Yr.30 and Yr.46 were observed in all 40 wheat genotypes while Yr.5 and Yr.17 were absent in all genotypes. Stripe rust resistance genes Yr.18 and Yr. 29 were also identified in limited number of wheat genotypes i.e. 10B9346 and 10B2003 genotypes, respectively (Table 4). Stem rust resistance genes were examined in current wheat genotypes by using seven Sr. markers and it was observed that only Sr.2, Sr.22, Sr.25 and Sr.39 were present in all 40 wheat genotypes to confer stem rust resistance while Sr. 24, Sr.26 and Sr. 38 were lacking in all genotypes (Table 5). Phenotypic evaluation of wheat genotypes for leaf, stripe and stem rusts is presented in Table 9.

Allelic frequency of rust resistance genes (Lr, Yr and Sr) was observed in studied wheat genotypes and found maximum frequency (70.4%) in 3 advance lines namely V-9452, V-11153 and NNG-3. However minimum frequency (51.9%) was estimated in twenty-five genotypes in which 10 were commercial varieties and other 15 were advanced wheat lines (Fig 1).

The genetic association among advanced wheat breeding lines for rust resistance genes was determined by developing heat map based on the SSR/STS banding patterns. Heat map indicated two groups; Group-A contained three clusters indicating wheat genotypes along Y-axis, whereas, Group-B indicated clusters of rust resistance genes (along with their primers) on X-axis (Fig 2). Red colour in map indicated presence of rust resistance genes in respective genotypes. However, yellow colour indicated absence of required genes. Cluster-A comprised of two leaves. Leaf-1 contained two genes Sr25 and Lr19 while leaf-2 had 11 genes (Lr19, Lr22a, Lr28, Lr67, Yr49, Sr2, Yr30, Sr39, Sr22 and Yr15), which indicated the existence of rust resistance genes in 40 wheat genotypes. Cluster-B discriminated five rust resistance genes (Lr34, Lr46, Yr18, Yr29 and Sr57) in red and yellow patches and cluster-C indicated five genes (Yr5, Sr24, Lr37, Yr17 and Sr38) in yellow patch for absence of resistance genes of rust. In group-B the cluster A' comprised of two leaves. Leaf 1 contains three genotypes (NNG-3, V-9452 and V-11153) with maximum allelic frequency of rust resistance genes (70.4%), whereas leaf 2 contains eight genotypes (AARI-11, Ufaq-2002, TW86014, V-10355, 09B9110, V-11161, NR-310 and 10B2003) with 63% allelic frequency. Cluster B' comprised of two leaves 4 and 5. Leaf 4 contained the largest number of 24 genotypes with allelic frequency of 51.9%. However, leaf 5 contains three genotypes (NR-378, V-11156 and TW96018) showing 59.3% allelic frequency. Cluster C' comprised of Leaf 3 contains only one genotype (Seher-06) with 59.3% allelic frequency.

All this characterization of leaf rust, stripe rust and stem rust resistant genes represented by + and - signs in wheat genotypes showed in tables 6,7 and 8, respectively.

Sr.25 is one of the stem rust resistance gene which exhibits strong linkage with Lr.19 in wheat for adult plant resistance. In this study, the primer BF145935 amplified 198 bp and 180bp DNA fragments of Sr.25 gene in all the studied wheat genotypes which conferred stem rust resistance at two fragment lengths (Fig 3). DNA fragment length of 330 bp linked with primer Lr28 indicated the presence of leaf rust resistance gene Lr.28 in all studied wheat genotypes (Fig 4). The primer Xgwm11 amplified 213bp DNA fragment length for Yr. 15 gene in most of the studied wheat genotypes which indicated the occurrence of stripe rust resistance gene. The banding pattern on agarose gel only for 10 wheat genotypes. The results of remaining gels are not reported here (Fig 5).

Table 1. Name of wheat varieties and advanced breeding lines as supplementary material.

S. No.	Name of wheat genotypes	Source	S. No.	Name of wheat genotypes	Source
1.	NR-399	NARC, Islamabad	21.	V-10031	WRI Faisalabad
2.	V-9452	UAF Faisalabad	22.	Shafaq-06	WRI Faisalabad
3.	V-11153	WRI Faisalabad	23.	SH-2002	WRI Faisalabad
4.	V-11154	WRI Faisalabad	24.	V-10104	WRI Faisalabad
5.	NNG-3	NIBGE Faisalabad	25.	TW96018	AZRI Bhakkar
6.	V-11156	WRI Faisalabad	26.	V-10110	WRI Faisalabad
7.	NR-310	NARC, Faisalabad	27.	V-11166	WRI Faisalabad
8.	V-11160	WRI Faisalabad	28.	NR-400	NARC, Islamabad
9.	V-10B2003	RARI Bahwalpur	29.	V-TW86014	AZRI Bhakkar
10.	V-11161	WRI Faisalabad	30.	NR-399	NARC, Islamabad
11.	V-10-B9346	RARI Bahwalpur	31.	NR-378	NARC, Islamabad
12.	V-11164	WRI Faisalabad	32.	Pb-11	WRI Faisalabad
13.	V-09B9110	RARI Bahwalpur	33.	Galaxy-13	WRI Faisalabad
14.	V-10355	WRI Faisalabad	34.	Ufaq-2002	WRI Faisalabad
15.	V-09-BT043	ABRI Faisalabad	35.	AARI-11	WRI Faisalabad
16.	V-10217	WRI Faisalabad	36.	Sehar-06	WRI Faisalabad
17.	V-10-BT002	ABRI Faisalabad	37.	Inq-91	WRI Faisalabad
18.	V-09B9172	RARI Bahwalpur	38.	Millat-11	WRI Faisalabad
19.	V-10025	WRI Faisalabad	39.	Fsd-08	WRI Faisalabad
20.	V-10193	WRI Faisalabad	40.	Lasani-08	WRI Faisalabad

Table 2. List of SSR/STS markers demonstrating in the experiment.

Sr. #	Primer Name	Linked gene	Location	Size (bp)	Reference
1	Xgwm 296	Lr22a	2DS	121/131	Hiebert *et al*., 2007
2	Xwmc 44	Lr46/Yr29	1BL	242	Suenaga *et al*. (2001)
3	Xgwm 437	Lr19	7DL	130	(http://maswheat.ucdavis.edu)
4	X-barc 352	Lr34/Yr18	4D.7DS	250	(http://maswheat.ucdavis.edu)
5	Xgwm-11	Yr15/Yr26	1B,1BS	213	(http://maswheat.ucdavis.edu)
6	Xgwm-533	Sr2	3B	120	Spielmeyer *et al*., (2003)
7	Cfa2123	Sr22	7A	245/260	Hiebert *et al*., 2010
8	csSr2	Sr2/Yr30	3B	378	(http://maswheat.ucdavis.edu)
9	Lr28	Lr28	4AL	330	(http://www.graingenes.com)

10	BF145935	Sr25/Lr19	7D.7A	180/198	Mago *et al*., 2011
11	STS(7/8)	Yr5	1B	500	Campbell *et al*., 2009
12	Sr26#43	Sr26	6AS.6AL	206	Liu *et al*., 2010
13	csLv34	Lr34/Yr18	7DS	150	(http://maswheat.ucdavis.edu)
14	Sr39#22r	Sr39/Lr35	2B	487	Mago *et al*., 2011
15	Xcfd71	Lr67/Yr26	4DL	214	Hiebert *et al*., 2010
16	Xcfd23	Lr67	4DL	211	Hiebert *et al*., 2010
17	BARC71	Sr24/Lr24	3DL	85/103	(http://maswheat.ucdavis.edu)
18	STS-9/10	Yr5/Yr17	2BL	439	(http://maswheat.ucdavis.edu)
19	SCS253	Lr19	7D.7A	736	(http://maswheat.ucdavis.edu)
20	Ventriup LN2	Lr37/Sr38	2AS	259	(http://maswheat.ucdavis.edu)
21	SCS265	Lr19	7D.7Ag	512	(http://maswheat.ucdavis.edu)
22	$Sr39F_2$	Sr39	2BS	900	(http://maswheat.ucdavis.edu)

Table 3. Wheat genotypes showing presence/absence of leaf rust (Lr) resistance of genes.

Sr. #	Primers	Wheat genotypes	Lr genes
1	SCS265	All genotypes were positive for this marker.	(Lr19) +ve
2	Xgwm 296	All genotypes were positive for this marker.	(Lr22a) +ve
3	BARC71	All genotypes were negative for this marker.	(Lr24) -ve
4	Lr28	All genotypes were positive for this marker.	(Lr28)+ve
5	Xbarc-352Lr34	9452, 11153, NNG-3, NR-310, 10B2003, 11161, 09B9110, 10355, TW86014, Ufaq-2002, AARI-11 were positive for this marker.	(Lr34) +ve
6	Sr39#22r	All genotypes were positive for this marker.	(Lr35) +ve
7	VentriupLN2	All genotypes were negative for this marker.	(Lr37) -ve
8	Xwmc-44	9452, 11153, NNG-3, NR-378, 11156, TW96018, Sehar-06 were positive for this marker.	(Lr46) +ve
9	Xcfd 23	All genotypes were positive for this marker.	(Lr67)+ve

+ = Presence & - = Absence of leaf rust resistance genes

Table 4. Wheat genotypes showing presence/absence of stripe rust (Yr) resistance genes.

Sr. #	Primers	Wheat genotypes	Yr genes
1	STS(7-8)	All genotypes were negative for this marker.	(Yr5) –ve
2	Xgwm-11	All genotypes were positive for this marker.	(Yr15) +ve
3	STS9/10	All genotypes were negative for this marker.	(Yr17) –ve
4	csLv34	9452, 11153, NNG-3, NR-310, 10B2003, 10355, 11161, TW86014, Ufaq-2002, AARI-11, 09B9110 were positive for this marker.	(Yr18) +ve
5	Xgwm-11	All genotypes were positive for this marker.	(Yr26) + ve
6	Xwmc-44	9452, 11153, NNG-3, NR-310, 11156, , 10355, TW96018, NR-378, Sehar-06	(Yr29) +ve
7	Xgwm-533	All genotypes were positive for this marker.	(Yr30) +ve
8	Xcfd71	All genotypes were positive for this marker.	(Yr46) +ve

+ = Presence & - = Absence of stripe rust resistance genes

Table 5. Wheat genotypes showing presence/absence of stem rust (Sr) resistance genes.

Sr. #	Primers	Wheat genotypes	Sr genes
1	Xgwm-533	All genotypes were positive for this marker.	(Sr2) +ve
2	cfa2123	All genotypes were positive for this marker.	(Sr22) +ve
3	Sr24	All genotypes were negative for this marker.	(Sr24) –ve
4	BF145935	All genotypes were positive for this marker.	(Sr25) +ve
5	Sr26#43	All genotypes were negative for this marker.	(Sr26) –ve
6	Ventriup/LN2	All genotypes were negative for this marker.	(Sr38) –ve
7	Sr39#22r	All genotypes were positive for this marker.	(Sr39) +ve

+ = Presence & - = Absence of stem rust resistance genes

Table 6. Characterization of Leaf Rust Resistant Genes (Lr) in Wheat Genotypes.

Sr. #	Genotypes	Lr19	Lr22a	Lr24	Lr28	Lr34	Lr35	Lr37	Lr46	Lr67
1.	NR-399	+	+	-	+	-	+	-	-	+
2.	V-9452	+	+	-	+	+	+	-	+	+
3.	V-11153	+	+	-	+	+	+	-	+	+
4.	V-11154	+	+	-	+	-	+	-	-	+
5.	NNG-3	+	+	-	+	+	+	-	+	+
6.	V-11156	+	+	-	+	-	+	-	+	+
7.	NR-310	+	+	-	+	+	+	-	-	+
8.	V-11160	+	+	-	+	-	+	-	-	+
9.	10B2003	+	+	-	+	+	+	-	-	+
10.	V-11161	+	+	-	+	+	+	-	-	+
11.	10-B9346	+	+	-	+	-	+	-	-	+
12.	V-11164	+	+	-	+	-	+	-	-	+
13.	09B9110	+	+	-	+	+	+	-	-	+
14.	V-10355	+	+	-	+	+	+	-	-	+
15.	09-BT043	+	+	-	+	-	+	-	-	+
16.	V-10217	+	+	-	+	-	+	-	-	+
17.	10-BT002	+	+	-	+	-	+	-	-	+
18.	09B9172	+	+	-	+	-	+	-	-	+
19.	V-10025	+	+	-	+	-	+	-	-	+
20.	V-10193	+	+	-	+	-	+	-	-	+
21.	V-10031	+	+	-	+	-	+	-	-	+
22.	Shafaq-06	+	+	-	+	-	+	-	-	+
23.	SH-2002	+	+	-	+	-	+	-	-	+
24.	V-10104	+	+	-	+	-	+	-	-	+
25.	TW96018	+	+	-	+	-	+	-	+	+
26.	V-10110	+	+	-	+	-	+	-	-	+
27.	V-11166	+	+	-	+	-	+	-	-	+
28.	NR-400	+	+	-	+	-	+	-	-	+
29.	TW86014	+	+	-	+	+	+	-	-	+
30.	KANZO	+	+	-	+	-	+	-	-	+
31.	NR-378	+	+	-	+	-	+	-	+	+
32.	Pb-11	+	+	-	+	-	+	-	-	+
33.	V-07096	+	+	-	+	-	+	-	-	+
34.	Ufaq-2002	+	+	-	+	+	+	-	-	+
35.	AARI-11	+	+	-	+	+	+	-	-	+
36.	Seher-06	+	+	-	+	-	+	-	+	+
37.	Inq-91	+	+	-	+	-	+	-	-	+
38.	Millat-11	+	+	-	+	-	+	-	-	+
39.	Fsd-08	+	+	-	+	-	+	-	-	+
40.	Lasani-08	+	+	-	+	-	+	-	-	+

+ = Presence of required gene-, - = Absence of required gene

Table 7. Characterization of Stripe Rust Resistant Genes (Yr) in Wheat Genotypes.

Sr. #	Genotypes	Yr5	Yr15	Yr17	Yr18	Yr26	Yr29	Yr30	Yr46
1.	NR-399	-	+	-	-	+	-	+	+
2.	V-9452	-	+	-	+	+	+	+	+
3.	V-11153	-	+	-	+	+	+	+	+
4.	V-11154	-	+	-	-	+	-	+	+
5.	NNG-3	-	+	-	+	+	+	+	+
6.	V-11156	-	+	-	-	+	+	+	+
7.	NR-310	-	+	-	+	+	-	+	+
8.	V-11160	-	+	-	-	+	-	+	+

9.	10B2003	-	+	-	+	+	-	+	+
10.	V-11161	-	+	-	+	+	-	+	+
11.	10-B9346	-	+	-	-	+	-	+	+
12.	V-11164	-	+	-	-	+	-	+	+
13.	09B9110	-	+	-	+	+	-	+	+
14.	V-10355	-	+	-	+	+	-	+	+
15.	09-BT043	-	+	-	-	+	-	+	+
16.	V-10217	-	+	-	-	+	-	+	+
17.	10-BT002	-	+	-	-	+	-	+	+
18.	09B9172	-	+	-	-	+	-	+	+
19.	V-10025	-	+	-	-	+	-	+	+
20.	V-10193	-	+	-	-	+	-	+	+
21.	V-10031	-	+	-	-	+	-	+	+
22.	Shafaq-06	-	+	-	-	+	-	+	+
23.	SH-2002	-	+	-	-	+	-	+	+
24.	V-10104	-	+	-	-	+	-	+	+
25.	TW96018	-	+	-	-	+	+	+	+
26.	V-10110	-	+	-	-	+	-	+	+
27.	V-11166	-	+	-	-	+	-	+	+
28.	NR-400	-	+	-	-	+	-	+	+
29.	TW86014	-	+	-	+	+	-	+	+
30.	KANZO	-	+	-	-	+	-	+	+
31.	NR-378	-	+	-	-	+	+	+	+
32.	Pb-11	-	+	-	-	+	-	+	+
33.	V-07096	-	+	-	-	+	-	+	+
34.	Ufaq-2002	-	+	-	+	+	-	+	+
35.	AARI-11		+	-	+	+	-	+	+
36.	Seher-06	-	+	-	-	+	+	+	+
37.	Inq-91	-	+	-	-	+	-	+	+
38.	Millat-11	-	+	-	-	+	-	+	+
39.	Fsd-08	-	+	-	-	+	-	+	+
40.	Lasani-08	-	+	-	-	+	-	+	+

*+ = *Presence of required gene, - = Absence of required gene*

Table 8. Characterization of Stem Rust Resistant Genes (Sr) in Wheat Genotypes.

Sr. #	Genotypes	Sr2	Sr22	Sr24	Sr25	Sr26	Sr38	Sr39
1.	NR-399	+	+	-	+	-	-	+
2.	V-9452	+	+	-	+	-	-	+
3.	V-11153	+	+	-	+	-	-	+
4.	V-11154	+	+	-	+	-	-	+
5.	NNG-3	+	+	-	+	-	-	+
6.	V-11156	+	+	-	+	-	-	+
7.	NR-310	+	+	-	+	-	-	+
8.	V-11160	+	+	-	+	-	-	+
9.	10B2003	+	+	-	+	-	-	+
10.	V-11161	+	+	-	+	-	-	+
11.	10-B9346	+	+	-	-	-	-	+
12.	V-11164	+	+	-	+	-	-	+
13.	09B9110	+	+	-	+	-	-	+
14.	V-10355	+	+	-	+	-	-	+
15.	09-BT043	+	+	-	+	-	-	+
16.	V-10217	+	+	-	+	-	-	+
17.	10-BT002	+	+	-	+	-	-	+
18.	09B9172	+	+	-	+	-	-	+
19.	V-10025	+	+	-	+	-	-	+

20.	V-10193	+	+	-	+	-	-	+
21.	V-10031	+	+	-	+	-	-	+
22.	Shafaq-06	+	+	-	+	-	-	+
23.	SH-2002	+	+	-	+	-	-	+
24.	V-10104	+	+	-	+	-	-	+
25.	TW96018	+	+	-	+	-	-	+
26.	V-10110	+	+	-	+	-	-	+
27.	V-11166	+	+	-	+	-	-	+
28.	NR-400	+	+	-	+	-	-	+
29.	TW86014	+	+	-	+	-	-	+
30.	KANZO	+	+	-	+	-	-	+
31.	NR-378	+	+	-	+	-	-	+
32.	Pb-11	+	+	-	+	-	-	+
33.	V-07096	+	+	-	+	-	-	+
34.	Ufaq-2002	+	+	-	+	-	-	+
35.	AARI-11	+	+	-	+	-	-	+
36.	Seher-06	+	+	-	+	-	-	+
37.	Inq-91	+	+	-	+	-	-	+
38.	Millat-11	+	+	-	+	-	-	+
39.	Fsd-08	+	+	-	+	-	-	+
40.	Lasani-08	+	+	-	+	-	-	+

+ = Presence of required gene, - = Absence of required gene

Table 9. Phenotypic expression of wheat genotypes for rust (Lr, Yr and Sr) resistance.

Sr #	Genotype	Leaf Rust (Lr) Resistance Genes	Stripe Rust (Yr) Resistance Genes	Stem Rust (Sr) Resistance Genes
1	NR-399	MR	MR	MR
2	V-9452	R	R	R
3	V-11153	R	R	R
4	11154	MR	MR	MR
5	NNG-3	R	R	R
6	V-11156	MR	MR	MR
7	NR-310	MS	MS	MR
8	V-11160	MS	MS	MS
9	10B2003	MS	MS	MR
10	V-11161	MS	MS	MR
11	10-B9346	MS	MR	MR
12	V-11164	S	MS	MS
13	09B9110	MS	MS	MR
14	V-10355	MS	MS	MR
15	09-BT043	MS	MS	MR
16	V-10217	MS	MS	MR
17	10-BT002	MS	MS	MR
18	09B9172	S	MS	MR
19	V-10025	MS	MS	MR
20	V-10193	MS	MS	MR
21	V-10031	MS	MR	MS
22	Shafaq-06	MS	MS	MR
23	SH-2002	MS	MS	MR
24	V-10104	MS	MS	MR
25	TW96018	MS	MS	MR
26	V-10110	MS	MS	MR
27	V-11166	MS	MS	MR
28	NR-400	MS	MS	MR
29	TW86014	MS	MS	MR

30	KANZO	MS	MS	MR
31	NR-378	MS	MS	MR
32	Pb-11	MS	MS	MR
33	V07096	MS	MS	MR
34	Ufaq-02	MS	MS	MR
35	AARI-11	MS	MS	MR
36	Seher-06	MS	MS	MR
37	Inq-91	MS	MR	MR
38	Millet-11	MS	MS	MR
39	Fsd-08	MS	MS	MR
40	Lasani-08	MS	MS	MR

Note: R: resistant, MR: moderately resistant, MS: moderately susceptible and S: susceptible.

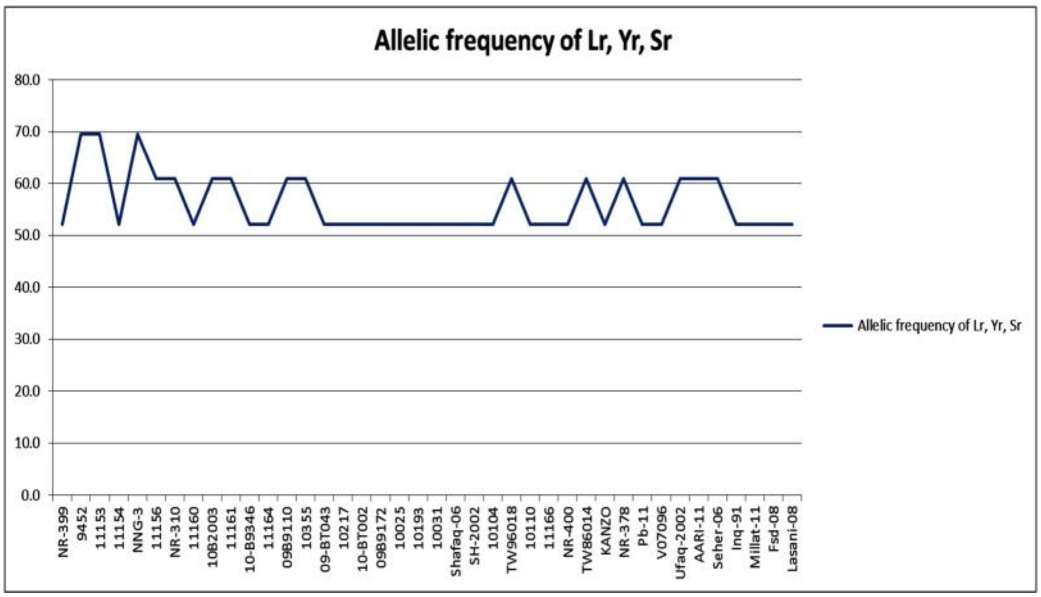

Figure 1. Allelic frequency of rust resistance genes in studied bread wheat genotypes.

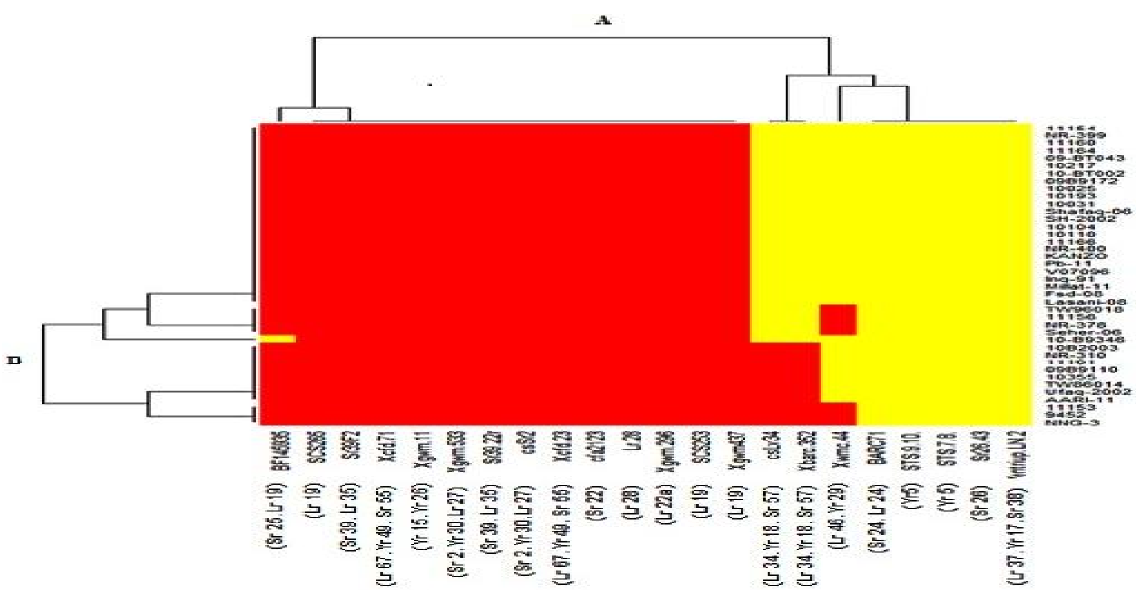

Figure 2. Heat map dandrogram showing genotype clusters along Y-axis and gene clusters (with name of primer) along X-axis. Red color in map indicated presence of rust resistance genes in respective genotypes. However, yellow color indicated absence of required genes.

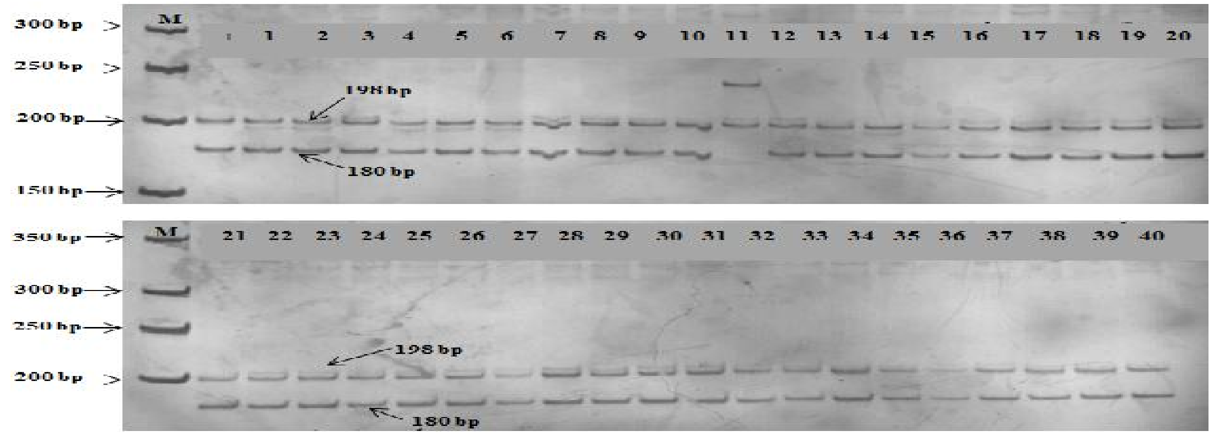

Figure 3. Wheat genotypes showing resistance against stem rust (Sr.25/Lr.19) by primer BF145935.

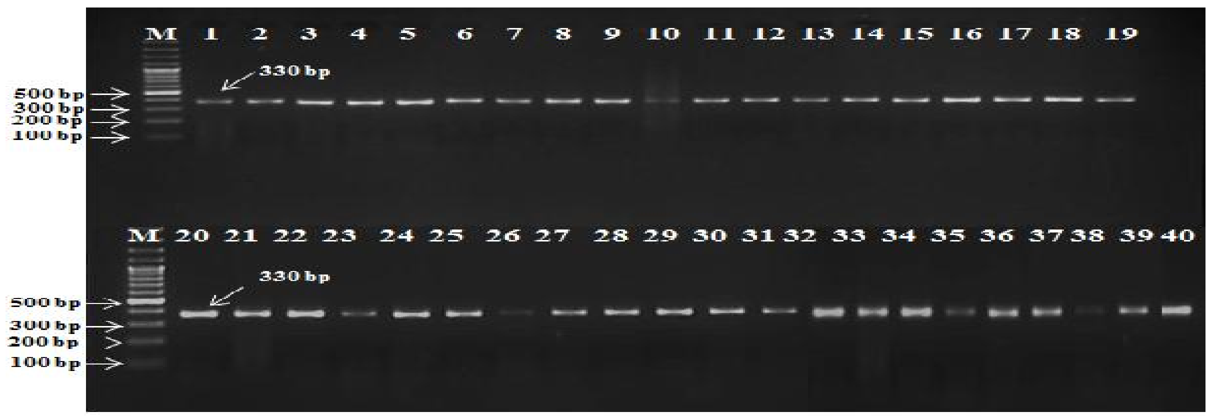

Figure 4. Wheat genotypes showing resistance against leaf rust (Lr.28) by primer Lr28.

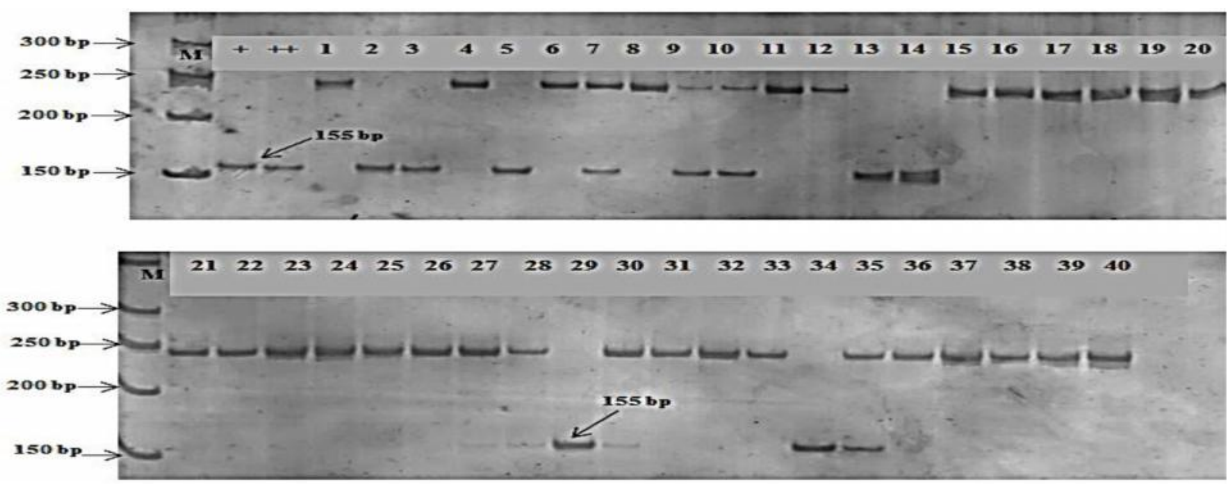

Figure 5. Wheat genotypes showing resistance against stripe rust (Yr. 18) by primer csLv34

DISCUSSION

The proposed study was aimed to screen advanced lines/commercial varieties of bread wheat developed by various wheat breeding institutes of Pakistan using molecular markers tightly linked with the genes conferring resistance to different rust races. Plant breeders broadly categorized rust resistance genes into seedling as well as adult plant resistance (APR) genes. The APR genes reveals durable, slow rusting, non-specific and horizontal resistance which are effective against rust epidemics (Jeffrey *et al*., 2014). Basnet *et al*., 2013 reported that at seedling stage rust resistance genes reveal the phenotypes of major effect and with varying infection types whereas most of the APR genes are limited in effect with varying disease severity levels. In this study various durable, slow rusting/non-specific rust resistance gene (Lr34/Yr18, Lr46/Yr29, Sr2/Yr30, Lr67/Yr49) have been observed. Based on the results, it was observed that only five out of nine reported Lr genes (Lr.19. Lr.22a, Lr.28, Lr.35 and Lr.67) were identified in all present wheat genotypes, while Lr.34 and Lr.46 genes were detected in 11 and 7 wheat genotypes respectively, which conferred leaf rust resistance in studied wheat genotypes. This indicated that a satisfactory number of leaf rust genes were not present in present Pakistani wheat genotypes and it was strongly suggested to broaden the wheat genetic back ground to cope with future rust epidemics (Qamar *et al*., 2014; McCallum *et al*., 2016, Bhardwaj *et al.,* 2019; Gessese, 2019). Though in some cases, the results were not as per expectation as Lr. 24 did not show any band on agarose gel from the amplified DNA of studied wheat genotypes but previously these genes were observed in some bread wheat genotypes (Ejaz *et al*., 2012). This may be due to either improper amplification of PCR products by the primers or absence of these genes in studied wheat genotypes.

A total of eight stripe rust (Yr) resistance genes were observed and found four Yr resistance genes (Yr.15, Yr.26, Yr.30 & Yr.46) in all studied wheat genotypes except Yr.5 and Yr.17 which did not show the bands on agarose gel. This might be the failure of STS primers to amplify the PCR products. Yr.18 and Yr.29 were also observed in different wheat genotypes. This indicated that Pakistani wheat genotypes have enough resistance against stripe rust pathogens. Our results get support from the results of Ejaz *et al*., (2012); Yu *et al*., (2012); Parveen *et al*., (2014); Wang *et al*., (2015); Bhardwaj *et al.,* 2019).

Stem rust (Sr) is an important disease of wheat throughout the world and resistance against Sr in newly developed wheat genotypes is very important to combat foreseen threat of Ug99 epidemics in Pakistan. A total of seven stem rust resistance genes (Sr2, Sr22, Sr24, Sr25, Sr26, Sr38 and Sr39) were studied in 40 wheat genotypes using already reported primers which amplified the DNA fragments in wheat. Among these four stem rust genes (Sr2, Sr22, Sr25 and Sr39) were found in all studied wheat genotypes while the remaining did not show bands on agarose gel. It reveals that although Pakistani wheat genotypes have enough resistance against stem rust and did not identified in Pakistan yet but chances are there to spread in Pakistan through Iran and currently Ug99 resistant wheat genotypes may become susceptible to this virulent. It is an unremitting straggle and should be continued in future to save wheat from rusts. Furthermore, rust resistant genes (Lr, Yr, Sr) as discovered world widely should be introduced in our wheat breeding program to add in new breeding lines through hybridization. Our results get support from the conclusions of Parveen *et al*., (2014); Kosgey *et al*., (2015); Bhardwaj *et al.,* (2019); Gessese, (2019). However, Ejaz *et al*., (2012) found contrary results and reported insufficient stem resistance in Pakistani wheat genotypes. This might be due to study of different stem rust genes in different wheat genotypes.

Conclusion: The results of the study revealed that Pakistani wheat genotypes have enough resistance against stem rust (Sr) pathogens yet but satisfactory number of genes are not available for leaf rust (Lr) and stripe rust (Yr). Therefore, it is strongly proposed that local wheat germplasm should be strengthen through introduction of rusts resistant exotic genotypes developed by internationally to cope the future rust epidemics. Furthermore, in current climate change scenario, it is the need of hour to add in maximum discovered rusts resistance genes in newly developing candidate wheat lines to ensure food security in Pakistan.

Acknowledgements: The research project was conducted by the research fund provided by University of Sargodha under the program “Research and Development”.

REFERENCES

Akhtar, N., A. Waseem, T. Mehmood, S. Bano, A. Raza and Ahsan Aziz (2018). Gene action appraisal for seed yield and related traits in bread wheat. The J. Anim. & Plant Sci**,** 28: 1457-1465.

Basnet, B.R., P.R. Singh, S.A. Herrera-Foessel, A.M.H. Ibrahim, J. Huerta-Espino,V. Calvo-Salazar and J.C. Rudd. (2013). Genetic analysis of adult plant resistance to yellow rust and leaf rust in common spring wheat quaiu 3. Pl. Dis. 97: 728-736.

Bhardwaj S. C. G.P. Singh., O, P. Gangwar., P, Prasad and S. Kumar. (2019). Status of Wheat Rust Research and Progress in Rust Management-Indian Context. Agron., 9:1-14.

Ejaz, M., M. Iqbal, A. Shahzad, A.I. Atiq-ur-Rehman and G. M. Ali. (2012). Genetic variation for markers linked to stem rust resistance genes in Pakistani wheat varieties. Crop Sci. 52: 2038-2648.

FAO. (2018). FAO statistical year book. Food and Agriculture organization (FAO) of the United Nations, Rome, Italy. Available at http://faostat3.fao.org/browse/Q/QC/E.

Gessese, M. K. (2019) Description of Wheat Rusts and Their Virulence Variations Determined through Annual Pathotype Surveys and Controlled Multi-Pathotype Tests. Advan. Agri., 2019: 1-7.

Government of Pakistan. (2019). Federal Bureau of Statistics. Economic Survey of Pakistan.

Jeffrey, G. E., S. L. Evans, S. Wolfgang and N.D. Peter. (2014). The past, present and future of breeding rust resistant wheat. Fron. Pl. Sci. 5: 641-651.

Khan, M.A. (1997). Evaluation of Multiple Regression Models based on Epidemiological Factors to predict Leaf Rust on Wheat. Pakistan J. Agric. Sci. 34: 1-7.

Kosgey, Z., J.O. Owuoche, M.A. Okiror and P.N. Njau. (2015). Inheritance of stem rust (*Puccinia graminis* Pers. F. Sp. Triticiericks and E. Hen) resistance in bread wheat (*Triticum aestivum* L.) lines to TTKST race. Int. J. Agron. Agric. Res.7: 1-13.

McCallum, B.D., C.W. Hiebert, S. Cloutier, D. Bakkeren, S.B. Rosa, Humphreys DG, Marais GF, McCartney CA, Panwar V, Rampitsch C, Saville BJ, Wang X (2016). A review of wheat leaf rust research and the development of resistant cultivars in Canada. Canadian J Pl Path 38: 1-18.

Pal, D., S.C. Bhardwaj, D. Sharma, S. Kumari, M.V. Patial and P. Sharma. (2015). Assessment of Genetic Diversity and Validating Rust Resistance Gene Sources Using Molecular Markers in Wheat (*Triticum aestivum* L.). SABRAO J. Br. Genet. 47: 89-98.

Parveen, Z., M. Iqbal, S. Rahman, M. Younis, M. Nawaz, S.H. Raza and M.Z. Iqbal. (2014). Rust resistance evaluation of advanced wheat (*Triticum aestivum* L.) genotypes using PCR-based DNA markers. Pakistan J. Bot. 46: 251-257.

Pretorius, Z.A., Y. Jin, C.M. Bender, L. Herselman, and R. Prins. (2012). Seedling resistance to stem rust race *Ug99* and marker analysis for *Sr2*, *Sr24* and *Sr31* in South African wheat cultivars and lines. Euphy. 186:15-23.

Qamar, M., S.D. Ahmad, M.S. Rabbani, Z.K. Shinwari and M. Iqbal. (2014). Determination of rust resistance genes in Pakistani bread wheats. Pakistan J. Bot. 46: 613-617.

Rahman, M., D. Hussain and Y. Zafar. (2002). Estimation of genetic divergence among elite cotton cultivars genotypes by DNA fingerprinting technology. Crop Sci. 42:2137-2144.

Singh, R.P.; Singh, P.K.; Rutkoski, J.; Hodson, D.P.; Xinyao He; Jorgensen, L.N.; Hovmoller, M.S. and Huerta-Espino (2016) Disease impact on wheat yield potential and prospects of genetic control. J. Annual Review of Phytopathology 54: 303-322.

Tadesse, W., S. Tawkaz, M.N. Inagaki, E. Picard and M. Baum. (2013). Methods and Applications of Doubled Haploid Technology in Wheat Breeding. ICARDA, Aleppo, Syria, pp 03.

Wang, H., F. Qin, Q. Liu, L. Ruan, R. Wang, Z. Ma, X. Li, P. Cheng and H. Wang. (2015). Identification and disease index inversion of wheat stripe rust and wheat leaf rust based on hyperspectral data at canopy level. J Spect ID 651810, 10 pages.

Yu, L.X., A. Morgounov, R. Wanyera, M. Keser, S.K.Singh and M. Sorrells. (2012). Identification of Ug99 stem rust resistance loci in winter wheat germplasm using genome-wide association analysis. Theo. Appl. Genet. 125: 749-758.