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Identification of stripe rust (*Puccinia striiformis*) resistant genes among Pakistani spring wheat by using molecular markers

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Abstract

The present study was designed to identify the stripe rust resistant genes in Pakistani wheat by using molecular markers. First of all, one hundred and twenty five wheat varieties/lines (will be further named as genotypes) were screened under glass house and field conditions against a dominant Pakistani stripe rust race 574232. Sixty eight wheat genotypes exhibited low infection type and only two genotypes showed high infection type while all other genotypes were moderately resistant to moderately susceptible under both situations. Twelve pairs of stripe rust linked microsatellite and sequence tag site markers were used to identify *Yr* genes in wheat genotypes. Stripe rust resistant gene *Yr* 5 was found in 36.8% and 62.4% wheat genotypes through S19M97 and S23M41 markers, respectively. The marker Iag95 identified *Yr* 9 in 27.2% wheat genotypes and marker Psp 3000 identified *Yr* 10 in 87.2% genotypes. *Yr* 17 was found in 34.4% genotypes through Venturip-LN2. *Yr* 18 was found in 24.8% and 11.2% genotypes by marker CsLV 34 and Cssfr 5, respectively. *Yr* 26 linked Xwmc 419 and Xgwm 11 showed amplification in 98.4% and 82.4% genotypes, respectively while *Yr* 29 linked Xwmc 367 showed amplification in 91.2% wheat genotypes. This study will help breeders for gene pyramiding in high yielding wheat genotypes to get rid of stripe rust.

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Introduction

Bread wheat is the leading staple food grain crop of Pakistan and considered as the main source of proteins, carbohydrates, vitamins and minerals. Multiple pathogens affect wheat yield, among them stripe rust (*Puccinia striiformis* f. sp. *tritici*) is the most important and widely distributed. Previously, it was assumed as a disease of cold climate (Singh *et al.,* 2004; Hovmoller *et al.,* 2010; Shah *et al.,* 2010; Ali *et al.,* 2014) but recently its epidemics have been recorded in the warmer climate also that may indicate the adaptations of its races from cooler to hot climate (Chen *et al.,* 2014). For many years it was assumed that stripe rust pathogen reproduces asexually but recently Jin *et al.* (2010) identified its sexual reproduction on *Berberis chinenensis*. Now multiple *Berberis* species have been documented as alternate hosts of stripe rust pathogen (Zhao *et al.*, 2013). The Himalayan region in the subcontinent have multiple *Berberis* species which may contribute in the sexual recombination of *P. striiformis* that develop new and aggressive races (Ali *et al.,* 2014). Till to date thirteen wheat rusts epidemics have been reported in Pakistan (Afzal *et al.,* 2008; Ibrahim *et al.,* 2015). Epidemic on the Inqilab-91 (*Yr* 27) due to stripe rust during 2003-04 season was the alarming situation as 80% of the wheat area was just planted with a single variety.

There are two different approaches to combat wheat rusts; application of fungicides and development of resistant wheat varieties however, fungicides are expensive and non-environment friendly (Singh *et al.,* 2005; Asad *et al.,* 2012). Therefore, development of resistant varieties is environment friendly and relatively durable approach (Iqbal *et al.,* 2016). To accomplish this approach breeding is the best strategy which depends on accurate gene postulation of wheat varieties. Conventional methods of gene postulation/identification by using different races are stage and environment dependent. Moreover, to preserve these races and to maintain their purity takes much time. Advancement in molecular genetics have provided the opportunity to the researcher to incorporate different economically important traits after identifying them in different food and fiber crops (Begum *et al.,* 2014).

Different molecular marker systems are available (Semagn *et al.,* 2006) for tagging genes controlling economically important traits.

Presently, 70 yellow rust resistance genes have been designated and many of them have been characterized into commercial wheat varieties (Mclntosh *et al*, 2008; Qamar *et al.,* 2014). Among them only *Yr* 18, *Yr* 29 *Yr* 30 and *Yr* 46 are non-race specific and have association with other traits (Suenaga *et al.,* 2003; William *et al.,* 2003). Different DNA markers for identifying the stripe rust resistant genes are available now a days. Juan *et al.* (2008) detected stripe rust resistance gene *Yr26* through SSR marker. Tabassum *et al.* (2010) postulated the *Yr* 5, *Yr*8, *Yr* 9, *Yr* 15 and *Yr* 18 in hundred Pakistani wheats by using SSR, STS and RGAP markers. Begum *et al.* (2014) used the twenty one SSRs and STSs marker to identify *Yr* 5, *Yr* 9, *Yr* 10, *Yr* 17, *Yr* 18, *Yr* 26 and *Yr* 29 in wheat genotypes. Qamar *et al.* (2014) screened 52 Pakistani wheat genotypes to identify the APR *Yr* 18/Lr34 gene complex by using STS marker csLV 34. Similarly, Shah *et al.* (2014) characterized slow rusting gene *Yr* 18/Lr34 in 50 Pakistani genotypes using STS marker csLV 34. Yuan *et al.* (2012) screened 659 Chinese wheat genotypes with gene specific marker and found that genotypes have *Yr10* and *Yr* 18 genes. Similarly, Zeng *et al.* (2014) worked on four hundred ninety four wheat genotypes to identify yellow rusts genes. This study was designed to extend such efforts by identifying genes controlling stripe rust resistance in 125 Pakistani wheat genotypes. Present study will provide the information about the presence of these genes in Pakistani wheats and their response under field condition which will help breeders in gene pyramiding.

Materials and methods

Glasshouse screening of wheat genotypes

To evaluate the wheat germplasm at seedling, eight to ten seeds of 125 wheat genotypes were planted in plastic trays having equal mixture of soil and peat moss. Glass house facility of Crop Disease Research and Protection Centre Murree was used. After 12 days of sowing, seedlings were inoculated with a most dominant stripe rust race 574232 having avirulence/virulence formula: *Yr5, 10, 15, 41, 43, 46, Tr1, Tye/Yr1, 6,7, 8,9,17,42,44,45*, by following similar method of Sohail *et al.* (2015).

After drying in sunlight for two hours, seedlings were shifted in the growth chamber for 48 hours with 100% relative humidity and the temperature maintained in between 5-9°C. After two days, seedlings were shifted to glass house at 12-18°C. After 15 days of inoculation, infection types (IT) were recorded by using 0-9 scale as described by McNeal *et al.* (1971).

Screening of wheat genotypes in field

Field evaluation was carried out at National Agricultural Research Centre, Islamabad and Cereal Crop Research Institute Nowshera during the cropping season 2012-13 and 2013-14 by following the sowing method of Sohail *et al.* (2015). All the plants were inoculated with a dominant Pakistani stripe rust race 574232 in end of January to first week of March (Sohail *et al.,* 2015). Field was irrigated four times during crop season and simple water spray was also conducted for creating the continuous humidity. Stripe rust data was recorded when the susceptible wheat variety Morocco developed upto 60-70 percent rust severity. Observations and severity of stripe rust was recorded according to Loegering (1959).

The severity was recorded as per cent of rust infection on the plants according to the modified Cob scale (Peterson *et al.,* 1948).

Molecular analysis

Total genomic DNA was extracted from fresh leaf tissues of wheat genotypes following Doyle and Doyle (1987). Quality of DNA was checked at 1% agarose gel. Polymerase chain reaction (PCR) was prepared in a volume of 20uL. Twelve SSR and STS markers were applied to identify the stripe rust resistant genes for the presence/absence. PCR amplification was performed in an Applied Biosystems Thermal Cycler (Veriti 96 well) at 94°C for 4 minutes followed by 35 cycles each consisting of one denaturation step for 40 sec at 94°C, an annealing step 72°C for 40 sec and a step of extension at 72°C for 1min. In the last and final step of extension was performed at 72°C for ten min. The fragments of amplification were separated on 1.5% agarose gel (Pre stained with ethidium bromide) and were visualized in Gel documentation System (UV1Pro Platinum Uvitec, Cambridge UK). Bands proved to be associated with various stripe rust resistant genes were scored based on size (Table 1).

Table 1. DNA markers along with linkage, product size and PCR profile used to postulate stripe rust resistance genes in wheat lines.

Marker	Linkage	Product (bp)*	PCR Profile	Primer Sequence	Distance	Reference
S19M93-100	Yr5	P=100	94°C: 4min 1 cycle 94°C: 40s, 62°C: 40s, 72°C: 1min 35 cycles 72°C 7min 1 cycle	TAATTGGGACCGAGAGACG TTCTTGCAGCTCCAAAACCT	Diagnostic	Smith *et al.* (2007)
S23M41-275	Yr5	P=275	94°C : 4min 1 cycle 94°C: 40s, 58°C: 40s, 72°C: 1min 35 cycles 72°C: 7min 1 cycle	TCAACGGAACCTCCAATTTC AGGTAGGTGTTCCAGCTTGC	0.7 cM	Smith *et al.* (2007)
iag95	Yr9	P=1100	94°C : 4min 1 cycle 94°C: 40s, 55°C: 40s, 72°C: 1min 35 cycles 72°C: 7min 1 cycle	CTCTGTGGATAGTTACTTGATCGA CCTAGAACATGCATGGCTGTTACA	Diagnostic	Mango *et al.* (2007)
psp3000	Yr10	P= 260 or 286 A= 240	94°C : 4min 1 cycle 94°C: 40s, 55°C: 40s, 72°C: 1min 35 cycles 72°C: 7min 1 cycle	GCAGACCTGTGTCATTGGTC GATATAGTGGCCAGCAGGATAC	2.7/3.5%	Bariana *et al.* (2007)
VENTRIUP-LN2	Yr17	P= 252	94°C : 4min 1 cycle 94°C: 40s, 65°C: 40s, 72°C: 1min 35 cycles 72°C: 7min 1 cycle	AGGGGCTACTGACCAAGGCT TGCAGCTACAGCAGTATGTACACAAAA	Diagnostic	Helguera *et al.* (2007)
csLV34	Yr18	P= 150 A= 229	94°C : 4min 1 cycle 94°C: 40s, 58°C: 40s, 72°C: 1min 35 cycles 72°C: 7min 1 cycle	GTTGGTTAAGACTGGTGGTGATGG TGCTTGCTATTGCTGCTGAATAGT	Diagnostic	Lagudah *et al.* (2007)
cssfr-5	Yr18	P= 751 A= 523	94°C : 4min 1 cycle 94°C: 40s, 58°C: 40s, 72°C: 1min 35 cycles 72°C: 7min 1 cycle	TTGATGAAACCAGTTTTTTTTCTA TATGCCATTTAACATAATCATGAA	Diagnostic	Lagudah *et al.* (2007)
CYS-5	Yr26	P=348	94°C : 4min 1 cycle 94°C: 40s, 55°C: 40s, 72°C: 1min 35 cycles 72°C: 7min 1 cycle	TGGGGAAGACGACGAGGTGT GCATTGGAACAAGGTGAAAACC	0.5 cM	Wen *et al.* (2007)
Xgwm419	Yr26	P=141	94°C : 4min 1 cycle 94°C: 40s, 55°C: 40s, 72°C: 1min	GTTTCGGATAAAACCGGAGTGC ACTACTTGTGGGTTATCACCAGCC	3 cM	Smith *et al.* (2007)

Marker	Linkage	Product (bp)*	PCR Profile	Primer Sequence	Distance	Reference
			35 cycles 72°C: 7min 1 cycle			
Xwmc367	Yr29	P=154	94°C : 4min 1 cycle 94°C: 40s, 55°C: 40s, 72°C: 1min 35 cycles 72°C: 7min 1 cycle	CTGACGTTGATGGGCCACTATT GTGGTGGAAGAGGAAGGAGAGG	Diagnostic	Smith *et al.* (2007)
Xgwm140	Yr29	P=185	94°C : 4min 1 cycle 94°C: 40s, 55°C: 40s, 72°C: 1min 35 cycles 72°C: 7min 1 cycle	ATGGAGATATTTGGCCTACAAC CTTGACTTCAAGGCGTGAC	0.3e	Smith *et al.* (2007)
Xgwm11	Yr15/Yr26	P=215	94°C : 4min 1 cycle 94°C: 40s, 55°C: 40s, 72°C: 1min 35 cycles 72°C: 7min 1 cycle	GGATAGTCAGACAATTCTTGTG GTGAATTGTGTCTTGTATGCTTCC	1.9 cM	Smith *et al.* (2007)

Results

Glasshouse and field screening of wheat genotypes

At seedling screening, sixty eight genotypes showed low infection (IT=1-3) while 19 genotypes showed infection type of 3-4 or 4 and were considered as resistant. Thirty six genotypes showed intermediate infection type of 45, 5 and 6 and only two genotypes V-0BT014 and V-0BT005 had high infection types of 89 and 9 (Table 2). At adult plant stage during 2012-13 wheat season, 20 wheat genotypes were found immune, 6 resistant (R), 2 resistant to moderately resistant (RMR), 4 moderately resistant (MR), 31 moderately resistant to moderately susceptible (M), 1 moderately susceptible (MS), 26 moderately susceptible to susceptible (MSS) and 35 susceptible (S). Similarly, during 2013-14 wheat season, 16 wheat genotypes were found immune, 2 resistant, 8 resistant to moderately resistant, 2 moderately resistant, 32 moderately resistant to moderately susceptible, 2 moderately susceptible, 27 moderately susceptible to susceptible and 36 susceptible (Table 2).

Table 2. Infection types, Final diseases severity and Allelic variation of markers linked with stripe rust resistance genes in Pakistan spring wheat.

S. No	Varieties/ Lines	nfectio type	slamab d	owshe a	19M97 (YR5)	23M4 (Yr5)	iag95 (Yr9)	sp300 (Yr10)	entriu -LN2 (Yr17)	CslV34 (Yr18)	cssfr5 (Yr18)	wmc41 (Yr26)	gwm11 (Yr26)	mc-36 (Yr29)
1	AARI-2011	1	10MSS	20MSS	-	-	-	+	-	-	-	+	+	+
2	Punjab-2011	2	10MSS	20MSS	-	-	-	+	-	-	m	+	+	-
3	Millat-2011	2	5M	10M	+	+	-	+	-	-	m	+	+	+
4	Bahawalpur-97	2	5S	20MSS	-	-	-	-	-	-	-	+	+	+
5	Bahawalpur-2000	2	5M	10M	-	-	-	-	-	m	-	+	+	-
6	AAS-2011	4	5M	5M	-	-	-	+	-	m	-	+	+	+
7	V-076346 (Gold-16)	2	5M	5RMR	+	+	-	+	-	m	m	+	+	+
8	DN-84	2	5M	10M	-	-	-	+	-	+	m	+	+	+
9	WG-08030	2	5S	10MSS	-	-	-	-	-	-	m	+	+	+
10	WG-08033	2	5M	10MSS	+	+	-	-	-	+	m	+	+	+
11	V-08068	2	10M	5M	-	+	-	-	-	+	m	+	+	+
12	V-076422	45	5M	10M	-	-	-	+	-	+	m	+	+	+
13	V-9407	3	10MSS	10MSS	-	-	-	+	-	-	m	+	+	+
14	V-088132	6	20S	20S	+	+	-	+	-	-	m	+	+	+
15	V-08173	2	5M	10MSS	-	-	-	+	-	-	m	+	+	+
16	WRIS-12	3	5MR	10MSS	+	+	-	-	-	-	m	+	+	+
17	V-05BT014	89	30S	30S	+	+	-	+	-	-	m	+	+	+
18	NR-397	45	10S	5MSS	-	-	-	+	-	-	m	+	+	+
19	V-06BT005	9	40S	40S	+	+	-	+	-	-	m	+	+	+
20	V-08203 (Ujala-14)	4	0	0	-	-	-	+	-	-	m	+	+	+
21	NR-378	45	10M	5M	-	+	-	+	-	-	m	+	+	+
22	TW-76004	2	40MSS	60MSS	-	+	-	+	-	-	m	+	+	+
23	V-08082	45	30M	20M	+	+	-	+	-	-	-	+	+	+
24	V-7/2011	3	20M	40M	-	-	-	-	+	-	-	+	+	-
25	NRL-0707 (NIFA-Insaf)	1	10MSS	5M	+	+	-	-	-	-	-	+	+	+
26	Aup-1052	5	5M	10M	-	-	-	+	-	-	-	+	+	+
27	06FJS3013	5	0	0	-	-	-	+	+	-	m	+	+	+
28	6C002 (Ehsan-16)	3	5MSS	5M	-	-	-	+	-	-	m	+	+	+
29	AUR-0809	4	10M	10M	-	-	-	+	-	-	-	+	+	+
30	NR-399 (Boroloug-16)	3	0	0	-	-	-	+	-	-	-	+	+	+
31	NR-400	4	0	0	-	-	-	+	-	-	-	+	+	+
32	AUP-1059	4	10MSS	10M	-	-	-	+	-	+	-	+	+	+

S. No	Varieties/ Lines	nfectio type	lamab d	owshe a	19M97 (YR5)	23M4 (Yr5)	iag95 (Yr9)	sp300 (Yr10)	entriu -LN2 (Yr17)	3slV34 (Yr18)	cssfr5 (Yr18)	mc41 (Yr26)	gwm1 (Yr26)	mc-36 Ytr29)
33	9C037	3	0	0	-	-	-	+	+	-	-	+	+	+
34	RCA-1	4	10MR	40MSS	-	-	-	+	-	+	-	+	+	+
35	RCA-2	4	5S	10MS	-	-	-	+	-	+	-	+	+	+
36	NR-401	23	5MS	5M	-	-	-	+	-	-	-	+	+	+
37	NR-403	23	0	0	-	+	-	+	-	-	+	+	+	+
38	NR-409	56	5M	5M	-	+	+	+	+	m	+	+	+	+
39	NR-410	34	10M	5MSS	-	-	-	+	-	m	m	+	+	-
40	NR-411	3	10MSS	10MSS	+	-	-	+	-	-	m	+	+	+
41	NR-413	5	10MSS	10MSS	+	+	-	+	-	-	m	+	+	+
42	NR-414	45	5M	10M	+	-	-	+	-	-	m	+	+	+
43	NR-415	1	5MSS	5M	+	+	-	+	-	-	m	+	+	+
44	NR-416	5	5S	5MSS	+	+	+	+	-	m	m	+	+	+
45	NR-417	5	5S	5S	+	+	-	+	-	-	m	+	+	+
46	NR-418	45	10S	10S	+	+	-	+	-	+	m	+	+	+
47	V-09031	45	20M	20M	+	+	-	+	-	-	m	+	+	+
48	V-09082	2	10MSS	20MSS	+	+	-	+	-	-	m	+	+	+
49	V-09087	12	10S	60S	+	+	-	+	-	-	m	+	+	+
50	V-09006	23	10S	20S	+	-	+	+	-	-	m	+	+	+
51	V-09091	3	10S	20S	+	+	-	+	-	-	m	+	+	+
52	V-10296	3	20S	10S	-	+	-	+	-	+	m	+	+	+
53	V-09136	4	5MSS	10MSS	-	+	+	+	-	-	-	+	+	+
54	V-10309	23	20MSS	10M	+	-	-	-	-	-	m	+	+	+
55	V-10317	23	10M	20M	-	-	-	+	-	-	m	+	+	+
56	V-08118	2	20S	30S	-	+	-	+	-	-	-	+	+	+
57	V-08314	12	10S	20S	-	-	-	+	-	-	-	+	+	+
58	V-08212	45	30S	30MSS	+	+	+	+	-	-	-	+	+	+
59	V-08171	3	0	0	-	+	+	+	+	-	-	+	-	+
60	V-10306	1	5MSS	10MSS	+	-	+	+	-	-	m	+	+	+
61	V-05223	34	10MSS	20MSS	+	-	+	+	-	-	-	+	+	-
62	V-09137	2	5MSS	10MSS	-	-	+	+	-	-	-	+	+	+
63	V-08314	45	10M	5M	-	-	-	+	-	-	-	+	+	+
64	V-10110	2	20S	10S	-	+	+	+	-	+	-	+	+	+
65	V-08204	3	10R	10RMR	-	-	-	+	-	-	-	+	+	+
66	V-082082	45	30S	20S	+	+	+	-	-	-	-	+	+	+
67	V-07096 (Galaxy-13)	2	10MSS	20MSS	-	+	+	+	-	-	-	+	+	+
68	V-10378	2	0	0	+	+	+	+	-	-	-	+	+	+
69	Dharabi-2011	23	10R	10RMR	-	+	-	-	-	-	-	+	+	+
70	DH-31	23	10M	20M	-	+	+	+	-	-	m	+	+	+
71	DN-108	45	5R	5RMR	+	+	-	+	-	+	+	+	+	+
72	DN-109	45	0	10M	+	+	-	-	-	+	-	+	+	+
73	DN-110	4	0	10M	-	-	-	+	-	+	+	-	+	-
74	09FJ04	2	5MSS	10MSS	-	-	+	+	-	+	+	+	-	+
75	09FJ28	12	0	0	-	-	-	+	-	-	m	+	+	+
76	09FJ24	2	5R	5MSS	-	+	-	+	-	-	m	+	+	+
77	09FJ33	1	0	0	+	+	-	+	+	+	-	+	-	-
78	05FJ17	23	5M	5M	-	+	+	+	-	-	m	+	-	-
79	NR-419 (Zincol-16)	4	10M	10M	-	-	-	+	-	+	m	+	-	+
80	NR-420	3	5R	10RMR	+	+	-	+	+	+	m	+	-	+
81	NR-421	4	5MR	10RMR	-	-	-	+	+	-	m	+	-	+
82	SA- 4	4	5R	5R	-	+	+	+	+	+	m	+	-	+
83	SA -7	4	10MSS	10MSS	-	+	-	+	+	-	m	+	-	+
84	SA -11	3	5S	10MSS	-	+	-	+	-	-	m	+	-	+
85	SA -15	3	0	0	-	+	-	+	+	-	m	+	-	+
86	SA -16	3	0	0	-	+	+	+	+	+	m	-	-	+
87	SA -17	45	5MR	5MR	-	+	-	-	-	-	m	+	-	+
88	SA -19	3	10S	15S	-	+	+	+	-	+	m	+	+	+
89	SA -22	4	10M	20M	-	+	-	+	-	-	-	+	+	+
90	SA -34	4	30S	30S	+	+	-	+	+	-	m	+	+	+
91	SA -37	45	5M	15M	-	+	-	+	+	-	-	+	+	+
92	SA -41	3	10M	10MSS	+	-	-	+	+	+	-	+	+	+
93	Atta Habib	2	10M	10M	-	-	+	+	+	-	+	+	+	+
94	Siren	2	5M	5M	-	+	-	+	+	+	m	+	+	+
95	05FJS3074	56	0	0	-	+	-	+	+	-	+	+	-	+
96	09FJ40	5	0	10RMR	-	-	+	+	+	-	-	+	-	+
97	V-076356	3	0	10R	-	+	+	+	-	+	-	+	+	+
98	V-088200	5	30M	10M	+	+	+	+	-	+	+	+	+	+
99	V-099115	3	10MSS	10MSS	+	+	-	+	+	+	m	+	-	+
100	V-079309	3	20S	20MSS	-	-	+	+	-	-	m	+	-	-
101	V-099157	3	50S	50S	+	+	+	-	-	-	m	+	-	+
102	V-9308	4	10MSS	10MSS	+	-	-	-	-	+	m	+	+	+
103	V-076317	5	10S	10MS	-	+	-	+	+	-	+	+	+	+
104	Satluj-86	5	30S	50S	-	-	-	+	+	-	m	+	+	-
105	V-076377	3	20S	10S	+	+	+	+	+	-	-	+	+	+
106	V-093660	56	30S	30S	+	+	-	+	+	+	+	+	-	+
107	V-099160	3	30S	10S	-	+	-	+	+	-	-	+	+	+

S. No	Varieties/ Lines	nfectio type	lamab d	owshe a	19M9 (YR5)	23M4 (Yr5)	iag95 (Yr9)	sp300 (Yr10)	entriu -LN2 (Yr17)	sLV34 (Yr18)	cssfr5 (Yr18)	mc41 (Yr26)	gwm1 (Yr26)	mc-36 Ytr29)
108	V-099174	5	40S	30S	+	+	-	+	+	-	-	+	+	+
109	V-099108	3	20S	40S	-	+	-	-	+	+	+	+	-	+
110	V-099110	3	10M	10MSS	+	+	-	+	+	-	-	+	+	+
111	V-099127	5	10MSS	5MSS	-	+	-	+	+	-	-	+	+	+
112	V-099147	56	10MSS	30MSS	-	+	+	+	+	+	+	+	+	+
113	V-099172 (Jauhar-16)	5	0	0	+	+	+	+	+	+	+	+	+	+
114	V-099199	3	30S	50S	-	+	-	+	+	-	-	+	+	+
115	V-099114	3	10RMR	5RMR	+	+	+	+	+	-	-	+	-	+
116	V-11C025	5	20S	10S	+	+	-	+	+	-	-	+	+	+
117	V-11C026	3	5M	5MSS	+	+	+	+	+	-	-	+	+	+
118	V-10C029	34	5RMR	5S	-	+	-	+	+	+	+	+	+	+
119	V-10C033	5	20S	10S	-	+	+	+	+	-	-	+	+	+
120	V-11C018	5	20S	5S	-	+	-	+	+	-	-	+	+	+
121	V-11C019	3	0	0	-	+	+	+	+	-	-	+	+	-
122	V-11C020	5	0	0	+	+	-	+	+	-	-	+	-	+
123	V-11C021	3	20MSS	10MSS	-	+	+	+	+	-	-	+	+	+
124	V-11C022	3	10MSS	5MSS	-	+	-	+	+	+	+	+	+	+
125	V-11C023	45	5MSS	TMS	-	+	+	+	+	-	+	+	-	+
Frequency					46	78	34	109	43	31	14	123	103	114

Identification of Yr genes through molecular markers

We used two AFLP derived Sequence Tagged Site markers S19M93 and S23M41 to identify *Yr5*. Marker S19M93 amplified 100 bp product in forty six wheat genotypes and positive control 'Avocet *Yr5*' while 79 genotypes and negative control missed the product. Similarly, S23M41 marker identified *Yr5* in 78 wheat genotypes and positive control by amplifying product of 275bp which was absent in thirty seven wheat genotypes and negative control (Fig. 1). Sequence Tagged Site marker *iag95* of *Yr9* amplified a product of 1100 bp in 34 genotypes and positive control *'Avocet Yr9'* while 91 wheat genotypes and negative control missed the band suggesting the absence (Fig. 2).

A co-dominant marker *psp3000* o f *Yr10* amplified 260bp or 286bp bands in 109 genotypes and positive control 'Avocet Yr10' whereas 15 wheat genotypes along with negative control did not amplify the desirable band (Fig. 3). *Yr17* linked *VENTRIUP* and *LN2* markers amplified 259bp fragment in 43 wheat genotypes and positive control that confirmed the presence of this gene while remaining 82 wheat genotypes along with negative control 'Avocet S' did not amplify the desirable 259bp band suggesting the absence of the *Yr17* gene (Fig. 4).

The presence of yellow rust resistant gene *Yr18* was assayed by using STS marker csLV34. This marker has an insertion of 79 bp within interonic sequence of sulphate transporter like gene (Lagudah *et al.,* 2006). There were only thirty two wheat genotypes and positive control produced a band of 150bp which indicate the presence of this non-race specific resistant gene. Eighty seven wheat genotypes and negative control amplified a product of 229 bp indicating the absence of *Yr18* gene while Bahawalpur-2000, Aas-2011, V-076346, NR-409, NR-410 and NR-416 did not amplify any fragment. Another gene specific marker *cssfr-5* amplified two fragments of 523bp and 751bp for identification of *Yr18*. Among 125, only 14 genotypes along with positive control produced 751bp fragment confirming the presence of *Yr18* gene. Among these 68 lines missed to produce any band and rest of the 43 lines and negative control produced a product of 523bp genotypes indicating the absence of this gene (Fig. 5).

Microsatellite marker Xwmc419 was selected to identify the yellow rust resistant gene *Yr26* which amplified 141 bp fragment in 123 genotypes along with positive control indicating the presence of *Yr26*. There were only two genotypes DN-110 and SA-16 did not produce the fragment along with negative control confirming the absence of this gene. Another Microsatellite Marker Xgwm11 was also used to identify the *Yr26*. This marker amplified 215 bp fragment in 103 wheat genotypes and the positive control. Twenty two wheat genotypes and negative control did not amplify the desirable fragment confirming the absence of this gene (Fig. 6).

Microsatellite marker Xwmc367 was used to determine the presence of *Yr29* gene. This marker amplified a 154 bp fragment in 114 wheat genotypes along with positive control. There were only 11 wheat genotypes and negative control did not amplify 154 bp bands indicating the absence of this gene (Fig. 7).

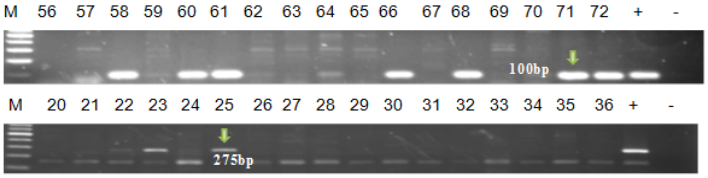

Figure 1. PCR amplified product of a marker S23M41 for detecting *Yr5* gene.

Figure 2. PCR amplified product of a marker iag95 for detecting *Yr9* gene.

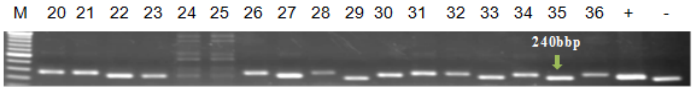

Figure 3. PCR amplified product of a marker psp3000 for detecting *Yr10* gene.

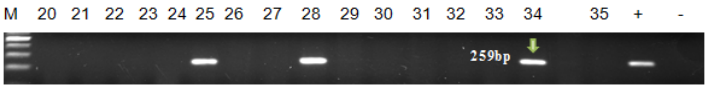

Figure 4. PCR amplified product of a marker Venturip/LN2 for detecting *Yr17* gene.

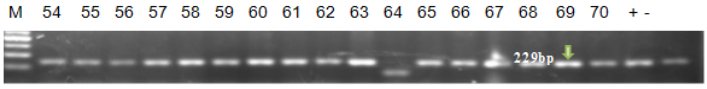

Figure 5. PCR amplified product of a marker ccLV34 for detecting *Yr18* gene.

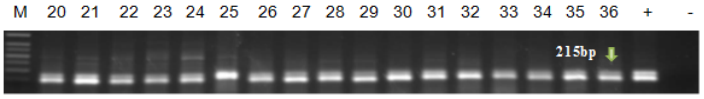

Figure 6. PCR amplified product of a marker Xgwm419 for detecting gene *Yr26*.

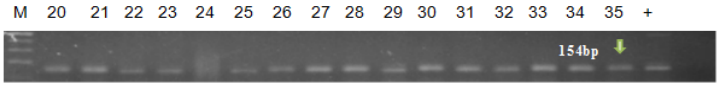

Figure 7. PCR amplified product of a marker Xgwm367 for detecting gene *Yr26*.

Table 3. Pedigree of the Pakistani wheat used for the study.

S. No	Lines	Parentage
1	AARI-2011	SH-88/90A204//MH-97
2	Punjab-2011	AMSEL/ATTILA//INQ-91/PEW'S'
3	Millat-2011	CHENAB2000/INQ-91
4	Bahawalpur-97	MTI 'S'
5	Bahawalpur-2000	AU/UP301//GLL/Sx/3/PEW 'S'/4/MAI 'S'/MAYA 'S'//PEW'S'
6	AAS-2011	KHP/D31708//CM74A370/3/CIAN079/4/RL6043/*4NAC
7	V-076346(Gold-16)	PR-32/INQ-91
8	DN-84	BJY/COC//PRL/BOW/3/URES/JUN/KAUZ
9	WG-08030	NG8201/KAUZ/3/TAN/PEW//SARA/4TAN/PEW//...
10	WG-08033	WBLL*2/2/CHAPIO
11	V-08068	SKAUZ*2/PRL/CM65531//INQ-91
12	V-076422	FERT2/KURUKU//FERT2
13	V-9407	RAWAL87/8073
14	V-088132	V-2068/2237
15	V-08173	ATTILA/3*BCN//BAV92/3/PASTOR
16	WRIS-12	SITE/MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC
17	V-05BT014	BR-83/UFAQ-02//FSD.-83/3/HORK
18	NR-397	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN
19	V-06BT005	FSD.-85/UFAQ-02/2/SH-02/3/CROW'S
20	V-08203(Ujala-14)	KIRITATI/4/2*//WEAVER/TSL//WEAVER/3/WEAVER
21	NR-378	WHEAR//INQILAB-91*2/TUKURU
22	TW-76004	CMH-77A917/PKV1600//RL6010/3/93T308
23	V-08082	V-87094/2*PAK-81//SHAFAQ-06
24	V-7/2011	PF74354//LD/ALD/4/2*DR12*2/3/JUP//PAR214*6
25	NRL-0707(NIFA-Insaf)	TATARA/INQALAB
26	Aup-1052	IBG/AUP/DT1052

S. No	Lines	Parentage
27	06FJS3013	PASTOR//MILAN/KAUZ
28	6C002 (Ehsan-16)	SOKOLLl
29	AUR-0809	SUNSU/CHIBIA
30	NR-399(Boroloug-16)	SOKOLL/3/PASTOR//HXL7573/2*BAU
31	NR-400	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1
32	AUP-1059	IBG/AUP/DT1059
33	9C037	Pastor/Milan//Milan/SHA7
34	RCA-1	Kukuna/3/Pavon76/2*Rohtas90/2/Weebli
35	RCA-2	Pak-81/Bakhtawar/2/Baviacora92/3/MH97
36	NR-401	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/ HUITES /5/PVN
37	NR-403	KBIRD//INQALAB 91*2/TUKURU
38	NR-409	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7
39	NR-410	SOKOLL/3/PASTOR//HXL7573/2*BAU
40	NR-411	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN
41	NR-413	PASTOR//HXL7573/2*BAU/3/SOKOLL/WBLL1
42	NR-414	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN
43	NR-415	NS732/HER//ARRIHANE/3/PGO/SERI//BAU
44	NR-416	WBLL1*2/BRAMBLING
45	NR-417	WBLL1*2/KIRITATI
46	NR-418	KAMB1*2/BRAMBLING
47	V-09031	HP.1744//LU26/HD2179
48	V-09082	INQ.91/FRET.2
49	V-09087	V-87094/2*INQ.91/3/SH88/PAK81/MH97
50	V-09006	INQ.91/3/87094/PB.96//MH.97
51	V-09091	CHENAB-70/5/TE173/4/LEE/KVZ/3/CC/PON/CHA/6/V.00183
52	V-10296	SH-53 V87094/2*ERA//PAK81/2*V-87094/3/SHAFAQ
53	V-09136	TEHLIM/WAXWING//ATILLA2*/PASTOR
54	V-10309	SOONOT-10
55	V-10317	CHRZ//BOW/CROW/3/WBLLI/4/CROC_1/AE.SQUARROSA(213)//PGO
56	V-08118	MILAN/587230//BABAX
57	V-08314	KIRITATI//2*SERI/RAYON
58	V-08212	KIRITATI//2*SERI/RAYON
59	V-08171	MILAN/587230//BABAX
60	V-10306	PFAU/SERI1B//AMAD/3/WAXWING
61	V-05223	SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR
62	V-09137	TEHLIM//2*ATTILA2*/PASTOR
63	V-08314	PFAU/SERI.1B//AMAD/3/WAXWING
64	V-10110	KAUZ/CMH77A-308//BAU/3/INQ-91
65	V-08204	KIRITATI/4/2*//WEAVER/TSL//WEAVER/3/WEAVER
66	V-082082	V-87094/2*PAK81//SHAFAQ-06
67	V-07096(Galaxy-13)	PB96/87094/MH97
68	V-10378	PBW343*/KUKUNA*2//KITE
69	Dharabi-2011	HXL7573/2*BAU//PASTOR
70	DH-31	GA-2002/CHAKWAL-50
71	DN-108	Fakhre-E-Hind x Yecora
72	DN-109	BABAGA
73	DN-110	BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED
74	09FJ04	Barani83/Bhakkar-2002
75	09FJ28	Pak81/01FJ14//LYP73/94-r-30
76	09FJ24	BWL-95/PIRSABAK-91
77	09FJ33	Pak81/01FJ14//LYP73/94-r-30
78	05FJ17	PAK81/MARGALLA99
79	NR-419 (Zincol-16)	OASIS/SKAUZ//4*BCN/3/2*PASTOR/4/T.SPELTA I348449/5/ BAV92/3/ OASIS /SKAUZ//4*BCN/4/PASTOR/6/WBLL1*2/CHAPIO
80	NR-420	CROC_1/AE.SQUARROSA (210)//PBW343*2/KUKUNA/3/PBW343*2/KUKUNA
81	NR-421	CROC_1/AE.SQUARROSA (210)//INQALAB 91*2 / KUKUNA/3/ PBW343*2/KUKUNA
82	SA- 4	WHEAR/VIVITSI//WHEAR
83	SA -7	WHEAR//2*PRL/2*PASTOR
84	SA -11	WHEAR/SOKOLL
85	SA -15	C80.1/3*BATAVIA//2*WBLL1/3/PBW343*2/KUKUNA/4/WBLL1*2/ TUKURU
86	SA -16	WBLL1*2/KIRITATI
87	SA -17	WBLL1*2/KIRITATI
88	SA -19	BABAX/LR42//BABAX*2/3/VIVITSI
89	SA -22	CBRD/KAUZ//ZHENGYOU 6
90	SA -34	BERKUT//PBW343*2/KUKUNA
91	SA -37	C80.1/3*BATAVIA//2*WBLL1/3/C80.1/3*QT4522//2*PASTOR
92	SA -41	WAXWING*2/VARIS
93	Atta Habib	Inqilab91*2/Tukuru
94	Siren	PB343*2/Kukun
95	05FJS3074	MILAN/KAUZ//BABAX
96	09FJ40	Unknown
97	V-076356	Unknown
98	V-088200	Seher-06/INQILAB-91
99	V-099115	IBWSN1126/0663009
100	V-079309	CAL/NH/H567-71/3/SERI/4/------
101	V-099157	V.3009/SHAHKAR-95
102	V-9308	MILAN/AMSES//MESTO
103	V-076317	KAUZ'S'/SHUMA-15

S. No	Lines	Parentage
104	Satluj-86	CMT/YR//MON
105	V-076377	CNDO/R-143//ENTE/MEXI-2/3/---------
106	V-093660	BB/KAL//2460
107	V-099160	V.3009/SH-2002
108	V-099174	BABAX/LR-42//BABAX-*2/3/VIVITISI
109	V-099108	IBWSN1101/032862
110	V-099110	MAIRAJ-08/IBWSN1153
111	V-099127	Unknown
112	V-099147	KAUZ/NAC//SERI/RAYON
113	V-099172 (Jauhar-16)	KAUZ/PASTOR//V.3009
114	V-099199	PRL/2*PASTOR/PB-343*2/KUKUNA
115	V-099114	IBWSN1126/0663009
116	11C025	DURRA-3
117	V-11C026	REYNA-24
118	V-10C029	DARIA-5
119	V-10C033	TC870344/GUI//TEMPORALERAM87/GRA/3/2*WVLLI
120	V-11C018	NS-732/HER/3/PRL/SARA/TSI/VEE#5/4/FRET2
121	V-11C019	FILIN/2*PASTOR//PRL/2*PASTOR
122	V-11C020	HSB 1313/2*WBLLI
123	V-11C021	SOKOLL/EXCALIBUR
124	V-11C022	SOKOLL//SUNCO/2*PASTOR
125	V-11C023	SOKOLL/EXCALIBUR

Discussion

Stripe rust is the most important disease of wheat and spreading from cool to hot climate of the country. Genetic resistance is considered the most economical meth for development of resistant varieties but unfortunately resistance is overcome in a very short period of time by evolving new races in nature. Primary gene pool of wheat is considered the best way to identify the new resistant sources and durable rust resistant genes for developing the new varieties. (Mujeeb-Kazi *et al.*, 2013). The primary gene pool have wild and early domesticated relatives of wheat, landraces, old cultivars and breeding lines. The present study postulated stripe rust resistant genes by using molecular markers (SSR and STS) with evaluation of wheat genotypes at seedling and adult plant stage against dominant race 574232.

Seedling screening and filed evaluation along with markers data provide the complete information of the lines and their trend in future. Only two lines (Entry no. 17 and 18) showed high infection types at seedling and adult plant stages at both tested locations. Low infection types at seedling stage indicate the presence of major genes. The present data showed low infection in many wheat genotypes with increased severity under field conditions against prevailing races of stripe rust. Both the locations are considered hot spots for rust development as the conditions are ideals due to high rain falls and temperature in the months January to March. The data revealed that most of the lines showed similar pattern at both the tested locations.

Data indicating that the rust severity is increasing from 1st to 2nd year. The screening data under field conditions revealed that the rust severity is increasing. As the most of the lines are selected from different nurseries have similar genetic back ground in their blood.

Yellow rust seedling resistance gene *Yr5* is present on the long arm of chromosome of 2B and first time reported by Macer (1966). This translocation was introgressed from spelt wheat to bread wheat (Law, 1976). Two STS markers S19M93 and S23M41 were applied to identify this *Yr5* gene as reported by Smith *et al.* (2007). Thirty eight wheat genotypes were found positive for yellow rust resistance gene *Yr5* with both the markers. Marker S19M93 has been considered a codominant marker which makes it more reliable as compared to S23M41 and is mapped 0.7 cM away from *Yr5* (Smith *et al.,* 2007). Begum *et al.* (2014) also utilized both the markers to identify this gene among 100 wheat cultivars. Both the markers produced different fragments hence the genotypes also behave in the same manner. The marker S19M93 is preferred over S23M41 as it is considered closely linked with *Yr5* (Begum *et al.,* 2014). Till to date no virulence was observed on this gene in Pakistan (Bux *et al.,* 2011) and considered as the resistant gene. Although a single isolate from Nowshera was suggested by Ali *et al.* (2014) having virulence on this gene but trap nurseries data of last five years indicating no virulence for this gene alone or present in combination.

However, both the markers are equally diagnostic and considered reliable for screening the yellow rust resistant gene.

Marker iag95 considered the diagnostic marker for the detection of 1BL.1RS translocation that carries pleiotropic gene *Yr9/Lr26/Sr31/pm8* (Mago *et al.,* 2002). At one time this was the most dominant gene in Pakistani wheat cultivars due to the reason that this translocation linked with high yield. The virulence for this gene in Pakistan has already been described by many researchers (Bux *et al.,* 2012; Ali *et al.,* 2014). Virulence for *Yr9* first appeared in East African countries and then shifted to North Africa, West Asia and South Asia in the late 1980s in the form of huge loss Ethiopia, Turkey, Iran, Afghanistan and Pakistan (Singh *et al.,* 2004).Virulence for *Yr*9 was first time noted in Pakistan during wheat season of 1994-95. This gene was present in many cultivars of the 80s and 90s of Pakistan like Pak-81 and Pirsbaq-85 and there was a considerable loss in KPK (Sumera *et al.,* 2010). The virulence for this gene appears early than any other gene every year in Pakistan (Personal communication). Ejaz *et al.* (2012) and Pretorius *et al.* (2012) used marker Iag95 for the detection of stem rust resistance gene *Sr31* in Pakistani and African wheat. Sohail *et al.* (2015) used this marker iag95 to identify the leaf rusts resistant genes *Lr26*. The reliability of iag95 has been proven by different studies and their results.

Bahri *et al.* (2011) used the French stripe rust races and found this gene in some of the Pakistani wheat. This marker is excellent for diagnostic but the problem appears when applied on the segregating population having hetrozygosity due to its dominant nature (Begum *et al.,* 2014).

Stripe rust resistance gene *Yr10* is located on chromosome 1BS of hexaploid wheat and is 5.0±2.2 cM distal to locus Gli-1B coding gliadin proteins (Payne *et al.*, 1986) and 2.0±0.3 cM apart from Rg1 locus for brown glume color, a phenotypic marker for *Yr10* (Metzeger and Silbaugh, 1970). *Yr10* is a race-specific and dominant gene and was originally reported from a line of Turkish origin (Wang *et al.*, 2002) and can be used to detect *Yr10* at maturity stage.

Presently *Yr10* was identified by the microsatellite marker Xpsp 3000 which is 1.2cM distal to gene (Wang *et al.*, 2002). The alternate allele of *Yr10* was reported in wheat germplasm derived from *Triticum vavilovii* (Bariana *et al.*, 2002). This marker Xpsp 3000 was prepared for a small F_2 population (Wang *et al.*, 2002) but has been applied successfully in other Australian germplasm (Bariana *et al.*, 2002). The co-dominant nature of the marker which makes it an ideal marker from segregating populations at early growth stage that should be used for marker assisted selection for gene pyramiding in breeding program. Presently this gene did not show virulence against the prevailing races in Pakistan (Bux *et al.,* 2012; Ali *et al.,* 2014).

Yellow rust resistant gene *Yr17* located on chromosome 2NS/2AS (Bariana and Mclntosh, 1993) and this pleotropic gene (*Lr37/Yr17/Sr38*) was first introgressed from *Triticum ventricosum* into the winter bread wheat 'VPM1' (Helguera *et al.*, 2003). Qamar *et al.* (2008) identified this gene by diversified Australian pathotypes and was confirmed by using primers VENTRIUP and LN2. Marker was successfully applied to identify the *Yr17* and *Sr38* on different Pakistani wheat genotypes (Begum *et al.,* 2014; Ejaz *et al.*, 2012). Virulent rust races of *Yr17* and *Lr37* are present in different countries of the world (Robert *et al.*, 1999). Still it is assumed that the cluster provide a good level of protection against rusts when used in different combination (Helguera *et al.,* 2003). Virulence for this gene already observed in Pakistan from last many years (unpublished). In future we should be very careful to use this gene in breeding program.

Yellow rust resistance gene *Yr18* located on chromosome 7DS and is completely linked with *Lr34, Sr57, Pm38, Ltn1, Sb1* and Bdv1 (McIntosh, 1992; Singh, 1992; Spielmeyer *et al.*, 2005; Shah *et al.,* 2014). Non-race specific genes considered durable against all *Pst* races and provide adequate level of resistance (Singh *et al.,* 2005). These genes when alone are not effective but provide resistance when used in combination with three to four minor genes (Singh *et al.,* 2005; Fayyaz *et al.,* 2008).

RFLP based co-dominant STS marker csLV34 produces 150 bp fragment for resistant genotypes (Lagudah *et al.*, 2006). In our study csLv34 produced good results. This marker was widely tag this gene in different Pakistani wheat genotypes (Tabassum *et al.,* 2010; Qamer *et al.,* 2014; Begum *et al.,* 2014; Shah *et al.,* 2014). Co-dominant nature of the marker makes it suitable for early segregating generations. The phenotypic marker leaf tip necrosis also provides the chance for selection of this gene. This character is not always reliable as some of the genotypes having this adult plant resistance gene were evolved without leaf tip necrosis. Virulence for this gene is present in Pakistan and severity range was 20 MSS to 70 MSS at different locations (Unpublished data). The frequency of *Yr18* gene is low in our genotypes and it should be increased in wheat varieties to broaden resistance.

Stripe rust resistance gene *Yr26* was translocated from *Haynaldia villosa* and mapped on chromosome 1BS based on linkage marker loci Xgwm11, Xgwm18 and Xgwm 419 (Ma *et al.*, 2001). This marker Xgwm11 is mapped 1.9cM distal away to *Yr26*. Markers Xwmc419 and CYS-5 were considered diagnostic and widely used for detection. *Yr26* has same chromosomal position with *Yr24* and *YrCH42* and all are mapped on chromosome 1B. Many other race specific resistant genes are previously reported on both arms of chromosome but maximum on large arm. Virulence for *Yr26* is common among many countries of the world. The best way to utilize this gene is to use with combination of others stripe rust resistant genes that will ultimately broaden the genetic base against stripe rust races (Begum *et al.,* 2014).

Yr29 gene was the most dominant by marker Xwmc367. *Yr29* is also a non-race specific gene and genetically linked with *Lr46* (Singh *et al.,* 2004). This is the second designated slow rusting and was first introduced in Pavon 76 (Singh *et al.,* 1998; William *et al.,* 2003). Virulence for this gene is already present in Pakistan. Under field conditions. This gene is not effective against the prevailing races of yellow rust (Unpublished trap nursery data). As race non-specific resistance genes are considered ineffective when present alone and in combination they provide good resistance. Hence, these genes should be used in combination (Fayyaz *et al.,* 2008).

Some of the genotypes from this selection now attain the status of released varieties and covering the larger area. V-07096 now gained the status of dominant wheat variety of Pakistan as Galaxy-13 but became susceptible just after release. V07096 (Galaxy-13) was found positive for three yellow rust genes. The rust severity of the Galaxy-13 is indicating that the disease under field condition is increasing. The pedigree of Galaxy-13 suggesting that genotype has the blood of three parents i.e. Punjab-96, V-87094 and MH-97 which are highly susceptible to stripe and leaf rusts under field condition. This variety also has *Yr9* and may be this gene is dominant and the virulence for *Yr9/Lr26* is very common in the country.

V-08203 (Ujala-14) was found immune under field condition and was considered best for rice wheat belt of Pakistan. Similarly, four other varieties NR-399 (Borloug-16), NRL-0707 (NIFA-Insaf), V-076346 (Gold-16) and 6C002 (Ehsan-16) were also released during 2015-16 wheat season. Under field condition these varieties performed good and provided a good level of resistance against stripe rust. Similarly, some of the genotypes are varieties e.g. AARI-2011, Punjab-11, Millat-11, Atta Habib, Dharabi-11 and Siren -11 performing good under field condition and have the combination of genes. Genotypes 11C023, V-099172 (Jauhar-16), NR-409 and SA-4 were found positive for all the markers and were found resistant under field condition.

Many stripe rust epidemics have been reported in Pakistan that has significantly reduced yield. The breakdown of resistant genes *Yr9* and *Yr27* in Pak-81 and Inqilab-91 was alarming situation of Pakistan. Exploitation of yellow rust resistance genes from adapted wheat and their incorporation and confirmation by marker assisted selection provide an effective way for developing resistant varieties. This will ultimately provide the protection from future epidemics. The present study was an attempt to know the status of various stripe rust resistance genes among Pakistani advanced wheat genotypes and to select genotypes to be used as parents in pyramiding of rust resistance genes. Based on marker results, we identified several wheat genotypes with 3 or 5 *Yr* genes in combination.

Wheat breeders can use these genotypes as parents in wheat breeding programs for developing wheat varieties with desirable combinations of stripe rust resistance genes. The frequency of durable adult plant stripe rust resistance gene *Yr18* was low but maximum with *Yr29*. Therefore, genotypes possessing non-race specific genes should be used as donor parents in future hybridization programs to increase the frequency and hence broaden the genetic base of future wheat varieties against stripe rust.

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