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MOLECULAR GENETIC VARIATION FOR STRIPE RUST RESISTANCE IN SPRING WHEAT

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Stripe rust, caused by *Puccinia striiformis f. sp. tritici*, is a major biotic constraint to global wheat production. Stripe rust can be effectively controlled by developing resistant wheat varieties. This, however, requires identification of resistant sources to be used as parents in breeding programs. Molecular markers provide a quick way of detecting rust resistance genes in adapted wheat material. The present study was conducted to investigate genetic variation for markers linked with stripe rust resistance genes in 67 Pakistani adapted spring wheat varieties using 12 pairs of microsatellite and sequence tagged site markers. Seventy nine percent wheat varieties showed marker allele of *Xgwm11* associated with stripe rust resistance gene *Yr26*, whereas 75% varieties had the *Yr26* linked allele of sequence tagged site (STS) marker CYS5. Stripe rust resistance gene *Yr5* was found in 45%, whereas *Yr9* and *Yr10* were present in 28% varieties tested based on the previously reported linked markers. Stripe rust resistance gene *Yr17* was found in 10%, whereas *Yr18* in 15% of varieties only. Three markers *Xwe173, Xbarc181 and Xgwm140* did not produce the expected amplicons associated with the stripe rust resistance genes. Cluster analysis revealed considerable genetic variation for marker alleles linked with stripe rust resistance genes. Results of this study may be useful for wheat breeders in pyramiding stripe rust resistance genes in future wheat varieties of Pakistan through Marker Assisted Selection.

Keywords: DNA Markers, Genetic variation, Marker Assisted Selection, Stripe rust, *Triticum aestivum* L.

INTRODUCTION

Wheat stripe rust is caused by *Puccinia striiformis* f.sp. *tritici*. It adversely affects the yield and quality of wheat grain. Seeds produced from stripe rust damaged crop have low vigour and poor emergence following germination. Stripe rust pathogen can cause 100% yield losses in susceptible cultivars if stripe rust occurs at an early stage and the infection continues to develop during wheat's growth. Stripe rust has caused yield losses ranging from 10-70% in most areas of wheat production (Chen, 2005). The wide range of yield losses depend on the susceptibility of wheat cultivar grown, timing of the initial infection, rate with which stripe rust develops and duration of stripe rust infection (Chen, 2005).

Wheat rusts can either be controlled by spraying chemical fungicides or by developing resistant varieties. Fungicides are expensive, environment non-friendly and need to be applied at a specific growth stage. On the other hand, development of resistant varieties is relatively inexpensive and environment friendly method of controlling rusts. This, however, requires identification of resistant sources to be used as parents in breeding programs. Identification of yellow rust resistance genes and breeding of stripe rust resistant wheat varieties is an efficient approach to minimize wheat yield losses due to stripe rust (Ma *et al*., 1999). Therefore, wheat breeders and pathologists emphasizes on the development of high yielding and stripe rust resistant wheat varieties in order to combat threats created by new stripe rust races through incorporation of durable rust resistance with the help of marker assisted breeding.

Presently, 48 stripe rust resistance genes (designated as *Yr1-Yr48*) and several undesignated genes have been identified (McIntosh *et al*., 2010; McIntosh *et al*., R. A. 2008). Most of the identified *Yr* genes confer resistance to specific stripe rust races at seedling stage. Race-specific *Yr* genes confer short-lived and non-durable resistance in the field due to the evolution of new virulent races of stripe rust (Kilpatrick, 1975). Deployment of multiple stripe rust resistance genes can broaden the genetic base of future wheat cultivars against stripe rust fungus.

Marker assisted selection is a useful tool in combining multiple *Yr* genes in a single genotype and in the development of multi-line cultivars having durable rust resistance. Microsatellites or simple sequence repeats (SSRs) are DNA sequence repeats of 1-6 base pairs. They are abundant in eukaryotic genomes and have been commonly used to facilitate identification and incorporation of durable resistance genes of various diseases in wheat (Gupta *et al*., 1999). Bulos *et al*. (2006) screened 88 Argentinian wheat cultivars with marker Ventriup-LN2 for the presence of *Lr37/Yr17/Sr38* gene complex. Wang *et al*. (2009) screened 226 wheat cultivars and advance lines from Hungary and

other countries with the STS marker csLV34 and concluded that this marker can reliably be used for marker assisted selection. McCallum *et al*. (2012) studied genetic variation for *Lr34/Yr18* gene in Canadian wheat cultivars using csLV34, cam11, caISBP1 and wms1220 markers. There are number of published reports on the use of DNA markers to detect stripe rust resistance in Pakistani adapted spring wheat. This study was conducted to extend such efforts by investigating genetic variation at the major loci controlling stripe rust resistance in Pakistani adapted spring wheat varieties.

MATERIALS AND METHODS

The seeds of sixty Pakistani spring wheat varieties released during 1970-2008 (Table 1) were obtained from the Wheat Program of National Agricultural Research Center (NARC), Islamabad, Pakistan. The seeds of the positive and negative controls were acquired from Crop Diseases Research Program (CDRP), NARC, Islamabad.

Genomic DNA was extracted from mature dry seeds of 67 wheat varieties and controls using the following procedure. Four to five seeds of each variety were ground using mortar and pestle and put in a 1.5 uL eppendorf tube. Then, 750 uL of 2% CTAB solution [(100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4M NaCl, 2% CTAB (w/v), 1% PVP "Polyvinylpyrrolidone Mr.40, 000)] was added to the tube and incubated for 30 min at 65 ºC. Following incubation, 750 uL of Chloroform: Isoamyl Alcohol in the ratio of 24:1 was added to the tube and centrifuged for 10 min at 12,000 rpm. Following centrifugation, supernatant was pipetted into a new 1.5 mL eppendorf tube and 0.8 volume chilled Isopropanol was added to it. The tube was incubated at 4°C for 10 minutes and then centrifuged for 10 min at 12,000 rpm. The supernatant was decanted from tube and DNA pellet was washed 2-3 times with 70 % Ethanol. The DNA pellet was air dried for 15-20 min and dissolved in 50 µL of TE buffer. RNA was removed by treating with 1µL of RNase A (10 mg/mL) and incubating at 37 ºC for 1 hour. Quantity of DNA in samples was determined by electrophoresis using 1% agarose gel.

Polymerase chain reaction was performed in 20uL volume, containing 1X PCR buffer (containing $(NH_4)_2SO_4$), 3mM $MgCl_2$, 0.2 mM dNTPs mix, reverse and forward primers (10 pmol each), 1 U of Taq DNA polymerase (Fermentas, Life sciences) and 25-30 ng template DNA. Twelve SSRs and STS markers were used for detecting the presence/absence of several stripe rust resistance genes (Table 2). Amplification was performed in an automated thermal cycler (Applied Biosystems, Veriti 96 well) at 94ºC for 4 min, followed by 35cycles each consisting of one denaturation step for 40s at 94ºC, an annealing step at 72 ºC for 40s and a step of extension at 72ºC for 1 min. Following last step, a final extension was performed for 10 min at 72ºC. Amplified fragments were separated on 1.5-3% agarose gel (pre-stained with ethidium bromide) and were visualized in Gel Documentation System (UVIpro Platinum, Uvitec, Cambridge, UK). Bands previously proved to be associated with various stripe rust resistance genes were scored based on size. Cluster analysis was performed to group the studied varieties based on similarity of marker alleles using Statistical Analysis Software (SAS) version 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

We used one microsatellite marker *Xgwm120* and two Amplified Fragment Length Polymorphism (AFLP) derived STS marker *S19M93-140* and *S23M41-310* linked with stripe rust resistance gene *Yr5* to screen 67 Pakistani wheat varieties for the presence of this gene. Microsatellite marker *Xgwm120* located 12cM distal to *Yr5* produced 130-150 bp marker alleles associated with the presence of *Yr5* in 45 wheat varieties, whereas, 22 varieties showed no amplification, suggesting the absence of *Yr5* gene. Marker *S19M93-140* produced the expected 100bp fragment in 30 varieties and positive control 'Avocet *Yr5*', indicating the presence of *Yr5* gene while no amplification was observed in negative control and remaining 37 varieties, suggesting the absence of *Yr5* (Fig. 1; Table 1). Marker *S23M41-310* amplified a 275 bp band associated with the presence of *Yr5* in positive control and 30 varieties, whereas 37 varieties and negative control 'Avocet S' showed absence of 275 bp band, suggesting absence of *Yr5* in these varieties (Fig. 1; Table 1).

The STS marker *iag95* was used to detect the presence of *Yr9* in 67 Pakistani wheat varieties. Nineteen varieties and positive control 'Avocet *Yr9*' showed 1100bp fragment associated with the presence of stripe rust resistance gene *Yr9*. The remaining 48 wheat varieties and negative control did not amplify any fragment, indicating the likely absence of *Yr9* (Fig.2; Table 1).

Microsatellite marker *Xpsp3000* was used to determine the presence or absence of *Yr10* gene in Pakistani wheat varieties. Marker *Xpsp3000* amplified a 260 bp fragment in 19 Pakistani wheat varieties and positive control 'Avocet *Yr10*', showing the likely presence of *Yr10* gene. The negative control 'Avocet S' and 27 varieties produced 240 bp fragment associated with the absence of yellow rust resistance gene *Yr10* (Fig.2; Table 1). Two varieties, Yecora-70 and Shahkar-95, showed heterozygous banding pattern. Nine varieties showed a 220 bp fragment, not reported previously, indicating that these varieties may have novel allele at *Yr10* locus. Eight varieties did not produce any fragment, therefore, we could not determine the *Yr10* status of these varieties.

The presence of *Yr17* gene in Pakistani wheat was investigated with the primers VENTRIUP and LN2 (Helguera *et al*., 2003). Seven varieties viz. Zardana-89, Shahkar-95, Suleman-96, MH-97, Fakhar-e-Sarhad, GA-2002, Moomal-

2002 and the positive control 'Avocet *Yr17*' showed a 259bp fragment (Table 1), indicating the presence of *Yr17* gene.

Table 1. Allelic variation at marker loci linked with stripe rust resistance genes in Pakistani spring wheat varieties

*No.	Variety	*Yr5*		*Yr9*	*Yr10*	*Yr17*	*Yr18*		*Yr26*	
		S19M93-140	*S23M41-310*	*iag95*	*Xpsp3000*	Ventriup-LN2	*csLV34*	*Cssfr-5*	*Xgwm11*	*CYS-5*
01	Barani-70	-	-	-	M	-	-	-	+	+
02	Lyallpur-73	-	-	-	-	-	+	+	+	-
03	Yecora-70	+	+	-	+/-	-	+/-	+	+	-
04	Punjab-76	-	-	-	-	-	+/-	+	+	+
05	Lu-26	+	+	-	+	-	+/-	-	+	+
06	Pavon-76	+	+	+	-	-	-	-	?	-
07	Zarghoon-79	-	-	-	N	-	-	-	+	+
08	Pak-81	-	-	+	N	-	-	-	-	+
09	Kohinoor-83	+	+	+	N	-	-	-	-	+
10	Tandojam-83	-	-	-	-	-	+	M	+	-
11	Pirsabak-85	-	-	+	-	-	-	-	+	+
12	Sarsabz	-	-	-	-	-	M	-	+	+
13	Chakwal-86	+	+	-	+	-	-	-	+	+
14	Khyber-87	+	+	+	-	-	+/-	+	-	-
15	Shalimar-88	-	-	-	+	-	-	-	+	+
16	Zardana-89	-	-	-	+	+	-	-	+	+
17	Soghat-90	-	-	-	N	-	-	-	+	+
18	Pasban-90	+	+	+	-	-	-	-	-	+
19	Inqilab-91	+	+	-	-	-	-	-	+	+
20	Sariab-92	+	+	-	+	-	-	-	+	+
21	Bakhtawar-92	+	+	+	-	-	+	+	-	+
22	Pothowar-93	-	-	-	+	-	+	+/-	+	+
23	Kiran-95	+	+	-	-	-	M	-	+	+
24	Shahkar-95	+	+	+	+/-	+	+/-	+/-	+	+
25	Suleman-96	-	-	-	+	+	-	+	+	+
26	Tatara	+	+	+	M	-	-	-	-	+
27	Nowshera-96	+	+	-	+	-	-	-	+	+
28	MH-97	-	-	-	+	+	M	-	+	+
29	Chakwal-97	+	?	-	M	-	+/-	-	+	+
30	Fakhr-e-Sarhad	-	-	-	-	+	+/-	-	+	+
31	Koshistan-97	+	+	+	+	-	M	+/-	+	+
32	Zarlashta-99	-	-	+	-	-	-	-	+	+
33	Auqab-2000	-	-	+	+	-	-	-	+	+
34	Saleem-2000	+	+	-	N	-	+	+	+	+
35	Marvi-2000	+	+	+	-	-	-	-	+	+
36	Wafaq-2001	+	+	-	+	-	-	-	-	+
37	GA-2002	-	-	+	N	+	-	+/-	+	+
38	Moomal-2002	-	-	+	-	+	-	-	-	+
39	SH-2003	+	+	-	M	-	-	-	+	+
40	Raskoh-2005	-	-	-	-	-	-	-	+	-
41	Pirsabak-05	+	+	-	M	-	+/-	-	+	-
42	Sehar-2006	-	-	+	N	-	-	-	+	+
43	Shafaq-2006	+	+	-	+	-	-	-	-	-
44	Sassui	-	-	-	-	-	-	-	+	+
45	NARC-09	+	+	+	N	-	-	-	+	+
46	NR-281	-	-	-	M	-	+/-	+	-	-
47	NR-287	-	-	-	-	-	-	-	+	+
48	Local White	-	-	-	+	-	-	-	+	+
49	S-24	-	-	-	+	-	-	-	+	+
50	Mexi Pak	-	-	-	-	-	-	-	+	+
51	SARC-1	+	+	-	-	-	-	-	+	-
52	Sonalika	+	+	-	-	-	-	-	+	+
53	NR-360	-	-	-	N	-	-	-	+	-
54	Blue silver	+	+	-	M	-	-	-	+	-
55	Faisalabad-08	-	-	-	-	-	-	-	+	+
56	Lasani-08	-	-	-	-	-	-	-	+	M
57	Panjnad	-	-	-	-	-	-	+	+	M
58	Mairaj-08	+	+	+	-	-	-	-	+	M
59	Chakwal-50	-	-	-	M	-	-	-	+	M
60	Fareed-06	+	+	-	-	-	-	+	M	M
61	Millat-2011	+	+	-	+	-	-	-	+	+
62	Dharabi-2011	-	+	-	+	-	-	-	+	+
63	Pakistan-2011	-	-	-	+	-	-	-	+	+
64	Galaxy-2013	-	-	-	-	-	+	-	+	+
65	Benazir-2013	+	+	-	M	-	-	-	+	+
66	Pirsabak-2013	-	-	+	M	-	-	-	-	+
67	NIFA Lalima	-	-	-	+	-	-	-	-	+
	Frequency	30	30	19	19	7	6	10	53	50

* indicates the same number as in the figures, [+]indicates marker allele associated with presence, whereas "–" indicates marker allele associated with absence of gene. M=missing; N= marker allele previously not reported

Table 2 DNA markers linked with different stripe rust resistance genes in wheat

No	Marker	Ta[a]	Linkage	Product (bp)[b]	Distance	Reference
1	*S19M93-100*	62	*Yr5*	P=100	Diagnostic	Smith *et al*. (2007)
2	*S23M41-275*	58	*Yr5*	P=275	0.7 cM	Smith *et al*. (2007)
3	*Xgwm120*	60	*Yr5*	P=120	38.4 cM	Smith *et al*. (2007)
4	*iag95*	55	*Yr9*	P=1100	Diagnostic	Mago *et al*. (2002)
5	*Xpsp3000*	55	*Yr10*	P= 260 or 285 A= 240	2.7/3.5%	Bariana *et al*. (2002)
6	*Ventriup/LN2*	65	*Yr17*	P= 259	Diagnostic	Helguera *et al*. (2003)
7	*csLV34*	58	*Yr18*	P= 150 A= 229	Diagnostic	Lagudah *et al*. (2006)
8	*cssfr-5*	58	*Yr18*	P= 751 A= 523	Diagnostic	Lagudah *et al*. (2009)
9	*CYS-5*	55	*Yr26*	P=348	0.5 cM	Wen *et al*. (2008)
10	*Xgwm11*	55	*Yr15 &Yr26*	P=215	1.9 cM	Ma *et al*. (2001)

[a] Annealing temperature in degrees Celsius

[b] indicates presence whereas A indicates absence of gene.

[c] Recombination frequency

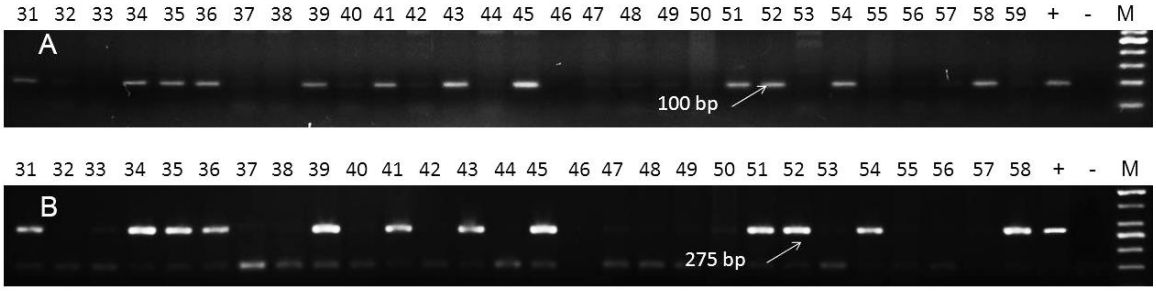

Figure 1. PCR amplification of (A) *S19M93-140* locus in Pakistani spring wheat, (B) Marker *S23M41-310* for *Yr5*. M=50bp; + means positive (Avocet *Yr5*) whereas – means negative control 'Avocet S'.

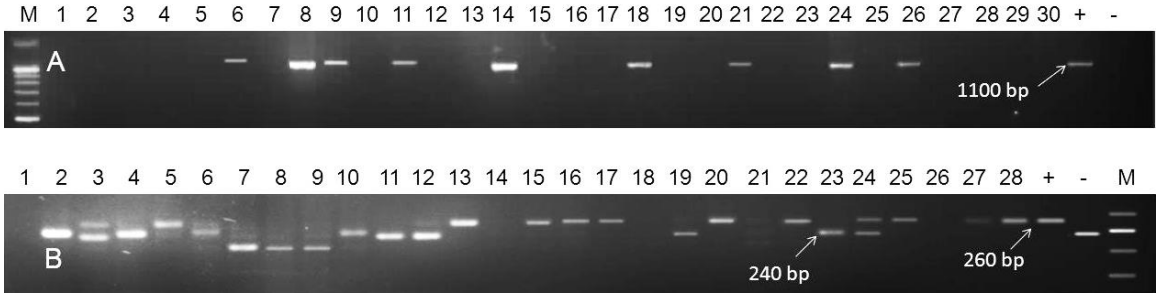

Figure 2. PCR amplification of (A) *Yr9*-linked marker *iag95* in Pakistani wheat for M=100bp, + means positive control 'Avocet *Yr9*', while – means negative control 'Avocet', (B) Markers *Psp3000* for *Yr10* gene M=50bp; + means positive control 'Avocet *Yr10*' while – means negative control 'Avocet S'.

The remaining 60 varieties and 'Avocet S' did not produce the 259 bp fragment, suggesting the absence of *Yr17*.

The presence of stripe rust resistance gene *Yr18* was assayed using a STS marker *csLV34* and gene specific marker *cssfr5*. The STS marker *csLV34* having 79 bp insertion within the intronic sequence of sulphate transporter-like gene (Lagudah *et al*., 2006) produced two allelic variants i.e. *csLV34a* and *csLV34b*. Out of 67 varieties, 48 varieties showed *csLV34a Yr18*-linked allele of 229 bp, indicating absence of *Yr18* gene, whereas, six varieties amplified 150bp fragment, suggesting the presence of *Yr18*-associated allele *csLV34b* (Table 1). Moreover, nine varieties produced both alleles showing heterozygous banding pattern and four varieties did not show any of the two amplicons. The gene specific marker *cssfr5*

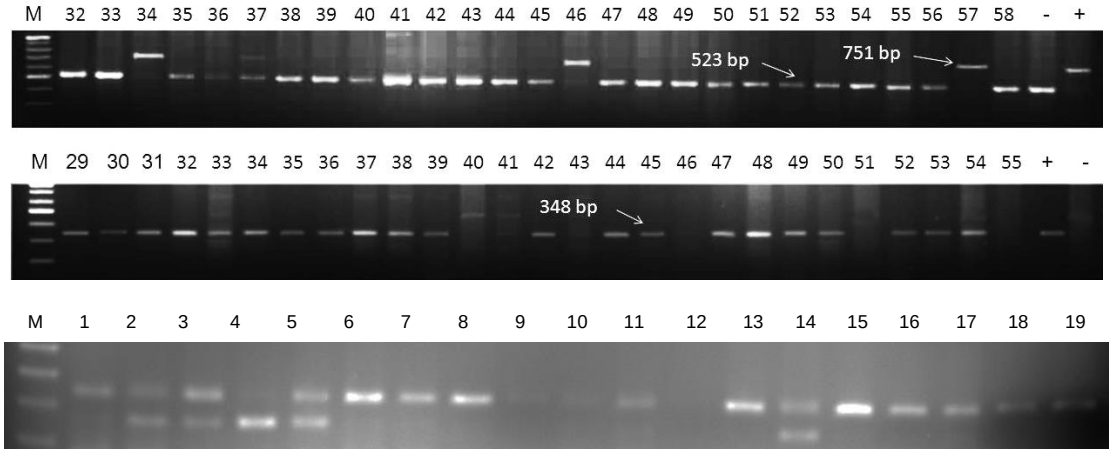

Figure 3. PCR amplification pattern of (A) marker *cssfr-5* linked with *Yr18.* (B) Banding pattern of marker CYS-5 associated with *Yr26,* (C) marker csLV34 linked with Yr18 gene in Pakistani wheat (M=100 bp). + Means positive 'Avocet *Yr18*' and 'Avocet *Yr26*' respectively, while – means negative control 'Avocet S.

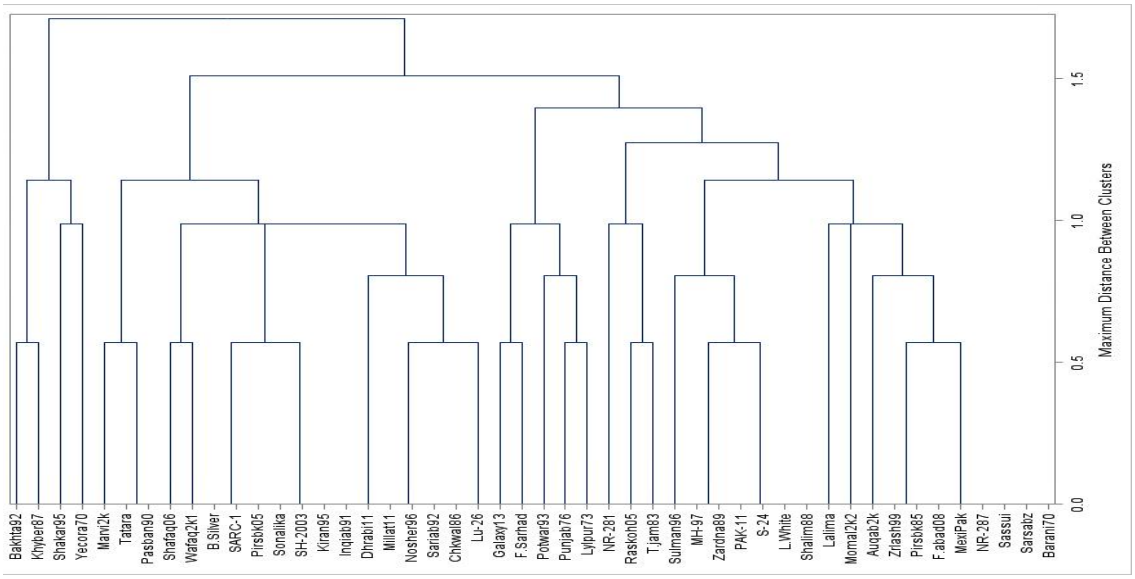

Figure 4. Dendogram showing grouping of Pakistani wheat varieties based on 9 markers linked with stripe rust resistance genes.

showed two alleles of 523 and 751 bp, respectively in this study. Fifty two varieties showed the presence of 523 bp band associated with absence of *Yr18*, whereas 751 bp fragment was amplified only in 10 varieties, suggesting the presence of *Yr18* gene (Fig 3; Table 1). Four varieties exhibited the heterozygous banding pattern, whereas one variety did not show amplification.

We used four markers *Xgwm11, Xwe173, Xbarc181* and CYS-5 to detect the presence/absence of *Yr26*. Microsatellite markers *Xgwm11* and *Xbarc181* are 1.9 and 6.7 cM distal to *Yr26*, whereas EST based STS marker *Xwe173* has been mapped 1.4 cM from *Yr26* (Wang *et al*., 2008). Marker *Xgwm11* amplified a 215bp fragment in 53 Pakistani wheat varieties and positive control, indicating the likely presence of *Yr26*, whereas 13 varieties and negative control did not produce any fragment (Table 1). We could not obtain previously reported results with marker *Xwe173*. STS marker *CYS-5* has been developed from resistance gene analogs and is located 0.5 cM away from *Yr26* (Wen *et al*., 2008). In our study, 50 varieties and positive control produced a 348 bp fragment with marker CYS-5, indicating the presence of *Yr26*, whereas 12 varieties and negative control did not yield any fragment showing the absence of this gene (Fig. 3; Table 1).

Cluster analysis grouped 44 of the 67 wheat varieties into 3 major clusters (Fig. 4), whereas 23 varieties remained ungrouped. Each of the three major clusters had both old and new wheat varieties, indicating that breeding efforts have not

favoured a particular allele over the other over the period of time. The lack of grouping of the 23 varieties suggested that these were genetically more variable with respect to the marker alleles studied.

DISCUSSION

The present study was conducted to detect stripe rust resistance genes in 67 Pakistani adapted spring wheat varieties using 12 pairs of microsatellite and sequence tagged site markers. Nine markers viz: *S19M93-140, S23M41-310, Xgwm120, iag95, Ventriup-LN2, Xgwm11, csLV34, CYS5* and *cssfr5* produced expected results whereas three markers viz: *Xwe173, Xbarc181 and Xgwm140* failed to produce the desired band size associated with the stripe rust resistance genes.

Stripe rust resistance gene *Yr5* is expressed at seedling stage (Macer, 1963), and is mapped on chromosome 2BL (Law, 1976). Our results of marker *S23M41-310* were different from Smith *et al*. (2007) as they reported the presence of 210 bp fragment in *Yr5* non-carriers. The 210 bp was not produced in Pakistani varieties studied. Instead, we observed a faint band of 275 bp fragment in some of the *Yr5* non-carriers. We compared the results of all three markers used to detect the presence/absence of *Yr5* gene. Results for markers *S19M93-140* and *S23M41-310* were 99% similar, demonstrating the likely presence of *Yr5* in 45% of Pakistani wheat varieties studied. On the contrary, results of microsatellite *Xgwm120* were different from these two STS markers and produced variable fragment sizes. Moreover, this marker is 12 cM distal to *Yr5*, which does not make it a diagnostic marker for *Yr5*. Marker *S19M93-140* has been reported to be co-segregating with *Yr5* gene making it more reliable marker compared to *S23M41-310* which is 0.7 mapped cM from *Yr5* (Smith *et al*., 2007). However, both these markers are equally diagnostic and reliable and may be used for the routine screening of wheat germplasm for *Yr5*.

AFLP converted STS marker *iag95* is reported diagnostic for the detection of 1BL.1RS translocation segment that carries *Sr31/Yr9/Lr26/Pm8* genes (Mago *et al*., 2002). This marker has been previously used the detection of stem rust resistance gene *Sr31* in Pakistani wheat varieties by Ejaz *et al*. (2012). Pretorius *et al*. (2010) reported the usefulness of this marker to detect stem rust resistance gene *Sr31* in African wheat germplasm. Despite the fact that marker *iag95* is diagnostic for the detection of *Yr9*, its dominant nature does not allow to distinguish between heterozygous and homozygous dominant varieties. Hence, its usefulness for selection of *Yr9* in segregating generations is limited.

Stripe rust resistant gene *Yr10* was originated from a Turkish wheat line P.I.178383 (Wang *et al*., 2002). It was mapped on chromosome 1BS, 2cM apart from *Rg1* locus that confers brown glume colour (Metzger and Silbaugh, 1970) and 5cM from locus *Gli-1B* (Payne *et al*., 1986). *Rg1* is a phenotypic marker for the selection of *Yr10* but it can only be used at adult plant stage (Metzger and Silbaugh, 1970). Bariana *et al*. (2002) reported two alternative alleles for yellow rust resistant gene *Yr10* i.e. *Yr10* and *Yrvav*. They described the association of marker *Xpsp3000* marker with both genes *Yr10* and *Yrvav*. Our study demonstrated that 28% wheat varieties produced 260 bp fragments, indicating the presence of *Yr10* but none of them amplified 285bp band associated with the *Yrvav* allele. Instead, nine varieties showed a 220bp fragment, which hasn't been reported previously. Therefore, this allele may be a novel allele. As *Yr10* is also linked with locus *Gli-B* on chromosome 1BS (Payne *et al*., 1986), hence, a combine assay of *Xpsp3000* and *Gli-B* would be more reliable to evaluate the germplasm for screening and pyramiding of *Yr10* gene into adapted varieties.

Alien chromosomal segment carrying three rust resistance genes i.e. *Lr37*, *Sr38*, and *Yr17*, was translocated into bread wheat from 2NS chromosome of *Triticum ventricosum* (Maia, 1967). Only 7 of the studied wheat varieties had *Yr17-Lr37-Sr38* gene complex. Although, virulence to *Yr17* has been detected in various parts of the world (Robert *et al*., 2000); J. Kolmer unpublished data), but it can still provide resistance against various stripe rust races if pyramided with other *Yr* genes.

Locus *Yr18/Lr34/Pm38* provides adult plant resistance against stripe rust, leaf rust and powdery mildew (McIntosh, 1992; Singh, 1992; Spielmeyer *et al*., 2005). Moreover, this segment is also associated with barley yellow dwarf virus resistance gene *Bdv1* (Singh, 1993) and tolerance to black rust (Dyck, 1993). STS marker *csLV34* is 0.4cM distal to *Yr18/Lr34* (Lagudah *et al*., 2006) and is diagnostic for the detection of heterozygotes in the segregating population due to its co-dominant nature. Kolmer *et al*. (2008) reported the usefulness of this marker for detecting the presence/absence of *Lr34/Yr18* in a diverse range of wheat germplasm. Allele specific marker *cssfr5* has been developed from 3bp indel sequence polymorphism in *Yr18*-linked gene *Lr34*, first rust resistance gene to be sequenced (Lagudah *et al*., 2009). We compared the results of *csLV34* and *cssfr5* markers in order to determine the reliability of these markers. The marker comparison showed some discrepancies for the presence/absence of *Lr34/Yr18*. However, *cssfr5* is more reliable as it is a gene specific marker and thus should be preferred for the MAS of this gene complex. Based on the results of *cssfr5*, only 10 (15%) Pakistani wheat varieties had *Lr34/Yr18* gene complex. As this gene complex provides durable adult plant resistance, its frequency should be increased in future wheat varieties of Pakistan so that their genetic base can be broadened against the continually evolving new races of *Puccinia striiformis tritici*.

Stripe rust resistance genes *Yr26*, *Yr24* and *YrCH42* are considered to be identical genes as they produced similar infection types against 26 rust isolates (Li *et al*., 2006). *Yr26* is located on chromosome 1B (Ma *et al*., 2001) and is

effective against majority of the *Pst* races. Combined results of *Xgwm11* and *CYS-5* markers exhibited the presence of *Yr26* in 41 varieties, whereas four varieties were found non-carriers of *Yr26* based on the results of both markers. The results of 17 varieties were dissimilar in the marker comparison. Thus, we suggest that use of STS marker *CYS-5* is more reliable compared to microsatellite *Xgwm11,* because the latter is only 1.9cM distal to *Yr26*. Marker assay showed that 75-79% varieties exhibited the presence of *Yr26* gene. Marker *Xgwm11* has also been reported linked with *Yr15.*

Stripe rust is one of the major biotic production constraints in Pakistan. Stripe rust epidemics have occurred from time to time with 35% intensity during 1973, 55% during 1978, 38% during 1995 and 20% during 2003 in Pakistan (Ahmad, I. 2004). Virulent races of *Pst* have broken down the resistance of major deployed genes such as *Yr9* (in Mexipak and Pak-81) and *Yr27* (Inqalab-91) with the passage of time (Khan and Mumtaz, 2004). The breakdown of major stripe rust resistance genes has alarmed wheat breeders for broadening the genetic base of future Pakistani wheat varieties by incorporating multiple stripe rust resistance genes. This objective can be achieved by first determining the status of *Yr* genes of Pakistani adapted wheat varieties through an efficient method such as DNA markers. Marker assisted selection can provide as an efficient means of pyramiding *Yr* genes. In this study, the maximum frequency of stripe rust resistance gene was observed for *Yr26* (75-79%), followed by *Yr5* (45%), *Yr10* (28%), *Yr9* (28%), *Yr18* (9-15%) and *Yr17* (10%), respectively. Results of this study can be useful for wheat breeders in pyramiding rust resistance genes in future wheat varieties using Marker Assisted Selection.

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