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Full Length Research Paper

Effective genes for resistance to stripe rust and virulence of *Puccinia striiformis* f. sp. *tritici* in Pakistan

Hadi Bux[1], Muhammad Ashraf[2]*, Xianming Chen[3] and Abdul Samad Mumtaz[1]

[1]Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan.
[2]NUST Centre for Virology and Immunology, National University for Science and Technology, (NUST), Rawalpindi, Pakistan.
[3]United States Department of Agriculture, Agricultural Research Service, Wheat Genetics, Quality, Physiology and Disease Research Unit and Department of Plant Pathology, Washington State University, Pullman, Washington, USA.

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Virulence patterns of wheat stripe rust were studied under the field conditions across four environmentally different locations: Quaid-i-Azam University (Islamabad), Pirsabak (NWFP), Faisalabad (Punjab) and Sakrand (Sindh) by planting trap nursery of tester lines and Pakistan varieties. The results revealed that stripe rust resistance genes *Yr3*, *Yr5*, *Yr10*, *Yr15*, *Yr26*, *YrSP* and *YrCV* were resistant, while *Yr18* showed moderate susceptibility at all locations. Genes *YrA-*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27* and gene combinations Opata (*Yr27+Yr18*) and Super Kauz (*Yr9*, *Yr27*, *Yr18*) were found susceptible. Among the fifty-one (51) commercial varieties; Barani70, Marvi2000, Iqbal2000, GA2000 and Seher2006 were found resistant. The genes found effective against stripe rust under natural conditions may be deployed singly or in combination to develop high yielding resistant wheat varieties in Pakistan.

Key words: Near-isogenic lines, *Puccinia striiformis* f. sp. *tritici*, stripe rust, *Triticum aestivum*, virulence, *Yr* genes.

INTRODUCTION

Stripe (yellow) rust of wheat, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks prevails in cooler climates and in cooler years (Singh et al., 2002) on all continents except Antarctica (Stubbs, 1985; McIntosh and Brown, 1997). Stripe rust severely damages wheat production worldwide (Roelf et al., 1992; Line, 2002) causing yield losses from 10 to 70% besides affecting the quality of grain and forage (Chen, 2005). In China, India and Pakistan, the top wheat producers in Asia, 59.3 million hectares are under wheat cultivation, stripe rust prevails in 24.8 million hectares that is, ~ 40% of wheat grown area (Singh et al., 2004). In Pakistan, 8.414 million hectares are under wheat cultivation and stripe rust prevails in 70% of the wheat growing area (Singh et al., 2004). Stripe rust has been historically a major production constraint in parts of North West Frontier Province (NWFP), Punjab and Balochistan (Khan et al., 2005).

Frequent outbreaks of stripe rust have caused severe yield losses in wheat (Kisana et al., 2003). The stripe rust epidemics of 1994 to 1995 and 1995 to 1996 caused grain yield losses estimated worth of Pak Rs. 2.0 billion (Ahmed, 2000).

Screening of varieties against stripe rust is a regular activity due to the dynamic evolutionary nature of the pathogen. The fungal pathogen evolves into new races quickly through mutation and somatic hybridization (Stubbs, 1985). Being airborne, local races can migrate to other areas and quickly become regionally and often globally predominant. Thus, virulence has been reported for many *Yr* genes worldwide. However, virulence for certain genes or gene combinations may still be absent regionally (Singh et al., 2002).

The specific interactions between host (wheat) resistance genes and pathogen (rusts) avirulence genes serve as useful markers for characterizing rust populations. Near-isogenic or single-gene wheat lines (Samborski and Dyck, 1976) also referred to here as tester lines, are used to identify virulence patterns of rust pathogens which either mutate locally or introduced through migration. This

*Corresponding author. E-mail: ashrafjahanian@yahoo.com. Tel: + (92)-51-9271579. Fax: + (92)-51-9271593.

helps in devising and managing crops through selecting effective resistance in high yielding wheat varieties. In the present study, a set of tester lines and selected Pakistan varieties have been studied for their response to stripe rust. The objective of the study was to identify the virulence of the stripe rust pathogen and determine the effectiveness of the resistance genes against the rust population in Pakistan.

MATERIALS AND METHODS

Plant materials and field experiments

A set of 20 wheat tester lines received from CIMMYT with known stripe rust resistance genes and 51 commercial varieties (Table 1) well adapted to the field conditions of Pakistan were used to plant a trap nursery at four environmentally different locations: Quaid-i-Azam University (Islamabad), Pirsabak (NWFP), Faisalabad (Punjab) and Sakrand (Sindh) during the cropping seasons of 2007 to 2008 and 2008 to 2009. The entries were planted in an unreplicated single meter row, each 30 cm apart. Susceptible check Morocco was planted in every 20^{th} row and as spread borders around the nursery to ensure uniform infection.

Description of localities and cropping features

Pirsabak is located at 75 miles northwest, Faisalabad 150 miles south and Sakrand is located at 600 miles southwest of Islamabad (Figure 1). Wheat, maize and sugarcane are the most common crops of these localities alongside with cotton, tobacco and rice.

Disease assessment

The rust response was recorded after the heading stage upon natural occurrence of stripe rust on the susceptible check Morocco. Rust severity was also assessed after heading according to 0 to 100% modified cob scale (Peterson et al., 1948).

RESULTS

The field data obtained in both cropping seasons revealed that seven resistance genes, *Yr3*, *Yr5*, *Yr10*, *Yr15*, *Yr26*, *YrSP* and *YrCV* were effective (Table 1). The line with *Yr24* was moderately resistant while that with *Yr18* was moderately susceptible. The lines with *YrA*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17* or *Yr27* and variety Opata (*Yr27*+*Yr18*) and Super Kauz (*Yr9*, *Yr27*, *Yr18*) were susceptible. Among the Pakistani varieties, five varieties (Iqbal2000, Marvi2000, GA2000, Barani70 and Seher2006) that have uncharacterized genes were resistant, one variety (Manthar) showed moderate resistance and the remaining varieties were susceptible. Twelve varieties (Sandal73, Faisalabad83, Rohtas90, Soghat90, Parwaz94, TJ83, Shahkar95, Pak81, Faisalabad85, Rawal87, Khyber87 and Bakhtawar93) carried unique gene combinations: *YrA* and *Yr6*, *Yr2* and *Yr7*, *Yr6* and *Yr7*, *Yr6*+, *Yr9* and *Yr7*, *Yr9* and *Yr4* and *Yr9*+ (Anonymous, 2005) were largely susceptible (that is, S or MS/MSS-type) in the field. The varieties that were resistant may have the effective resistance genes like *Yr3*, *Yr5*, *Yr10*, *Yr15*, *Yr26*, *YrSP* and *YrCV* or unidentified genes. Therefore, the genes in these cultivars need to be studied through marker-assisted gene identification and genetic mapping.

Both the tester lines and commercial varieties showed wide range of rust response at different locations. The tester lines with *Yr5*, *Yr10*, *Yr15*, *Yr24*, *Yr26* and *YrCV* showed 0-type or R-type reaction suggesting these genes to be immune or resistant against the pathogen population's at all four locations. When the variety responses were compared across the four locations, the responses at Pirsabak were more different, while those at three other locations were relatively similar. Based on these observations, the Pirsabak location may be considered as the 'hot spot' of virulence variation for the stripe rust pathogen in Pakistan.

The Pakistan varieties had more variations in reaction across the locations. Sixteen (16) varieties that had varied reactions may be classified into 3 classes (Table 2). Class 1 included nine varieties (BlueSilver, WL711, Kohinoor83, Faisalabad83, Khyber87, Pasban90, Soghat90, Saleem 2000 and AS2002) showing susceptible to moderately susceptible reactions. Class 2 included, moderately resistant to resistant varieties (example, Pirsabak2005, Manthar and Barani70). Varieties in Class 3 included Iqbal2000, Marvi2000, GA2002 and Seher2006, showing R-type or 0-type reaction. More Pakistan varieties were susceptible and their susceptibility levels were higher at the Pirsabak and Faisalabad locations than in the other two locations.

Based on the reactions of the tester lines, the *P. striiformis* f. sp. *tritici* populations in the four regions during the 2007 to 2008 and 2008 to 2009 cropping season had virulences to *YrA*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17* and *Yr27* and avirulences to *Yr3*, *Yr5*, *Yr10*, *Yr15*, *Yr26*, *YrSP* and *YrCV*. *Yr1* virulence was detected at Pirsabak and Faisalabad, while no virulence was recorded at Islamabad and Sakrand. Similarly, partial virulence was recorded at Faisalabad location for *Yr24* only. Among the Pakistan varieties, Faisalabad 83 and Khyber87 showed partial virulence at Faisalabad, while complete virulence was recorded elsewhere. Furthermore, differences in virulence pattern were observed in consecutive years, especially at Sakarand where the varieties, Blue Silver, Kohinoor83, Pasban90, Soghat90 and Saleem2000 showed immunity in 2007 to 2008 while remained partially susceptible in 2008 to 2009.

DISCUSSION

The tester lines received from CIMMYT and selected Pakistan varieties were used to identify virulences and avirulences for the current populations of the stripe rust pathogen in various regions of Pakistan. In this study, we found that *Yr*3, *Yr*5, *Yr*10, *Yr*15, *Yr*26, *Yr*SP and *Yr*CV

Table 1. Stripe rust response of wheat tester lines and Pakistan varieties at different locations during 2007 to 2008 and 2008 to 2009. Stripe rust was not observed in Sakrand during 2007 to 2008.

SN	Genotype	*Yr* gene	2007 to 2008			2008 to 2009			
			QAU	Pirsabak	Faisalabad	QAU	Pirsabak	Faisalabad	Sakrand
1	Morocco	-	70S	100S	80S	60S	100S	80S	40S
2	Avocet-*YrA*	-	60S	80S	40S	80S	90S	40MSS	5MSS
3	Avocet+*YrA*	*YrA*	60S	90S	70S	70S	100S	80S	0
4	Yr1/6Avocet(s)	*Yr1*	0	30MSS	20MS	0	20S	30MS	0
5	Yr2/6Avocet(s)	*Yr2*	30S	60S	30MSS	50S	80S	30MSS	5S
6	Tatara	*Yr3*	0	0	0	0	0	0	0
7	Yr5/6Avocet(s)	*Yr5*	0	5MR	10MR	0	20MR	5R	0
8	Yr6/6Avocet(s)	*Yr6*	20S	20MSS	60S	40S	60S	30S	10S
9	Yr7/6Avocet(s)	*Yr7*	60S	80S	50S	50S	80S	30MSS	5S
10	Yr8/6Avocet(s)	*Yr8*	10MSS	30MSS	50MSS	20S	30S	10MSS	30MSS
11	Yr9/6Avocet(s)	*Yr9*	5MSS	50S	50S	20MSS	80S	30S	5S
12	Yr10/6Avocet(s)	*Yr10*	0	TR	0	0	TR	0	0
13	Yr15/6Avocet(s)	*Yr15*	0	5R	0	0	TR	0	0
14	Yr17/6Avocet(s)	*Yr17*	10MSS	60S	30MSS	40S	70S	50MSS	5MS
15	Yr18/6Avocet(s)	*Yr18*	5MSS	30MSS	20MSS	20MSS	50MSS	10MSS	15MSS
16	Yr24/6Avocet(s)	*Yr24*	0	30MRMS	0	5MRMS	20MRMS	0	0
17	Yr26/6Avocet(s)	*Yr26*	0	5R	20MR	0	TMR	0	0
18	Yr27/6Avocet(s)	*Yr27*	40S	60S	5S	50S	70S	10S	30S
19	YrSP/6Avocet(s)	*YrSP*	0	0	0	0	0	0	0
20	Pavon	*Yr29*, *Yr30*	30MSS	50S	10MSS	0	50S	30S	0
21	Seri	-	0	10S	5MSS	0	20S	10MSS	0
22	Opata85	*Yr27*, *Yr18*	70S	90S	80S	60S	100S	60S	15S
23	Super Kauz	*Yr9*, *Yr27*, *Yr18*	70S	80S	90S	50S	90S	70S	0
24	YrCV/Avocet	*YrCV*	0	0	0	0	0	0	0
25	PBW343	*Yr27*	30S	20S	10MSS	50S	60S	5MSS	15MS
26	Yr28/Avocet	*Yr28*	30MSS	20S	5S	10S	50S	10S	5S
27	Yr29/Avocet	*Yr29*	10S	40S	60S	20S	30S	10MSS	15S
28	Yr31/Avocet	*Yr31*	40S	60S	0	50S	60S	5MSS	30S
29	Mexipak65	*Yr2*	20S	50S	40S	60S	80S	30S	20S
30	Chenab70	-	10S	50S	30S	70S	40S	60MSS	15S
31	Blue Silver	*YrA*, *Yr6*	50S	60S	30MSS	70S	80S	40MSS	30MRMS
32	Sandal73	*YrA*, *Yr6*	5S	50S	70S	60S	40S	50MSS	5S
33	Yacora70	-	5S	30S	60S	50S	50S	70MSS	50MS
34	WL711	*Yr2*	10S	50S	20S	70S	50S	80S	15MR

Table 1. Contd.

35	Pak81	*Yr9, Yr7*	30S	60S	40S	50S	70S	30S	20S
36	Kohinoor83	*Yr7, Yr9*	20MSS	60S	40MSS	50S	60S	40MSS	5MRMS
37	Faisalabad83	*Yr7,Yr2*	30S	20MSS	30MRMS	60S	30MSS	20MRMS	5MS
38	TJ83	*Yr6+*	30S	50S	40S	40S	60S	40S	30MSS
39	Faisalabad85	*Yr9,Yr4*	20S	60S	30MSS	60S	70S	40MSS	15MS
40	Chakwal86	-	10MS	5MS	30MS	30MS	50MS	70MS	0
41	Sarsabz	*Yr7*	40S	50S	20S	50S	60S	20MSS	15S
42	Rawal87	*Yr9+*	30S	40S	15MSS	60S	60S	30S	30MSS
43	Khyber87	*Yr9+*	30MSS	50MSS	20MRMS	50MSS	50MSS	10MRMS	0
44	Pasban90	*Yr9*	20MSS	40MSS	10MSS	30MSS	60MSS	20MSS	40MRMS
45	Inqlab91	*Yr27*	30S	60S	40MSS	60S	80S	40S	20S
46	Mehran89	-	40S	70S	50S	40S	70S	80S	30MSS
47	Rohtas90	*Yr6,Yr7*	10S	30S	20MSS	50S	30S	10MSS	5MS
48	Soghat90	*Yr6,Yr7*	20MSS	60MSS	10MSS	40MSS	70MSS	40MSS	30RMR
49	Bakhtawar93	*Yr9+*	10MSS	50MSS	10MS	20MS	30MSS	5MSS	0
50	Wattan94	*Yr6+*	5S	30S	40S	20S	50S	30MSS	40S
51	Zardana	*Yr7*	10S	50S	60S	30S	40S	20MSS	40MSS
52	Kaghan93	*Yr9*	40S	80S	30S	60S	90S	40S	30S
53	Parwaz94	*Yr6,Yr7*	10MSS	40MSS	20MSS	50MSS	50MSS	30MSS	20S
54	Kohsar95	-	0	20MS	10MS	30MSS	60MSS	0	TR
55	Shahkar95	*Yr6+*	30MS	40MS	10MS	40MSS	50MSS	20MS	20MSS
56	MH97	*Yr27*	30S	60S	60S	40S	70S	50S	30MSS
57	Kohistan97	-	50S	80S	60S	70S	60S	50S	15S
58	Auqab2000	-	5MS	10MS	20MS	30MS	40MS	20MS	5S
59	Iqbal2000	-	0	0	0	0	0	0	0
60	Chenab2000	-	10MS	10S	20MSS	30S	60S	10MSS	5S
61	Saleem2000	-	5MS	20MSS	10MS	30MSS	40MSS	5MS	5MR
62	Marvi2000	-	5R	30R	10R	20R	30R	40R	5R
63	Bakhar2002	-	30MSS	70S	40MSS	20S	40S	30MSS	30S
64	Moomal2002	-	30S	80S	50S	60S	70S	50S	20MSS
65	Haider2000	-	30S	50S	10S	30MSS	60S	40S	20S
66	Wafaq2002	-	20S	80S	40S	40S	70S	60S	30S
67	GA2002	-	5R	0	0	0	0	0	0
68	SH2002	-	30S	70S	20S	50S	80S	30S	30S
69	Pirsabak2004	-	20MSS	50S	30S	20S	60S	50S	40S
70	Pirsabak2005	-	30MRMS	0	0	5MRMS	0	0	TR
71	Seher2006	-	0	0	0	0	0	0	0

Table 1. Contd.

72	Shafaq2006	-	20S	40S	50S	40S	60S	30S	30MS
73	Margalla99	-	30S	60S	50S	20S	60S	70S	15MSS
74	Sonalika	*Yr2, YrA*	10S	40S	30S	60S	60S	20S	5S
75	Zamindar80	-	5S	30S	20MSS	40S	70S	50S	5MSS
76	Manthar	-	5MRMS	30MRMS	50MRMS	30MRMS	50MRMS	60MRMS	5R
77	Barani70	-	5R	10MR	TR	30R	20MR	40MR	0
78	AS2002	-	5MSS	20MSS	30MRMS	10MSS	50MSS	20MS	0
79	Sariab92	*Yr6+*	20S	50S	40S	50S	70S	50S	40MS

The observations were recorded as: 0 = no visible infection; R = no uredinia present, visible chlorosis and necrosis; TR = resistance reaction with below 5% severity; MR = small uredinia surrounded by chlorotic/necrotic areas; MS = medium size uredinia with moderate sporulation and some chlorosis may still be present; S = large uredinia with abundant sporulation

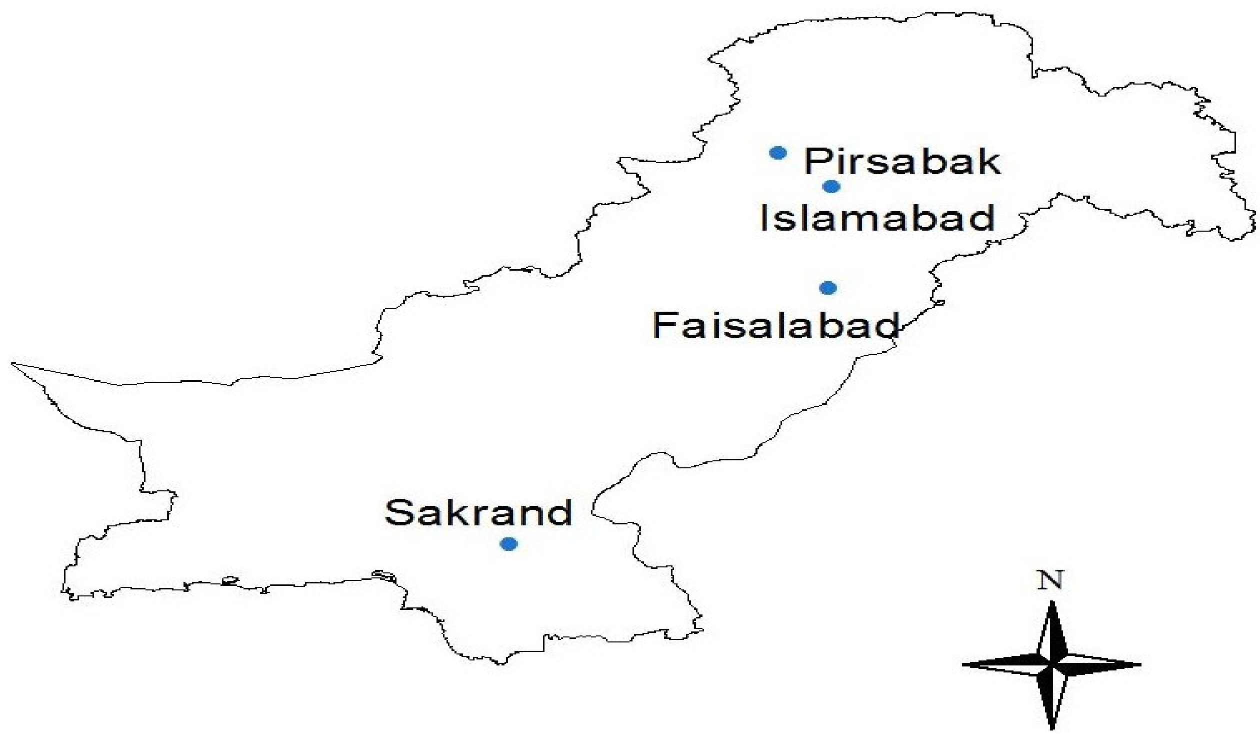

Figure 1. Map of Pakistan showing the locations of planted trap nursery.

Table 2. Response of tester lines and classification of Pakistan varieties at different locations.

Parameter		Location			
		QAU	Pirsabak	Faisalabad	Sakrand
Tester lines	Yr5/6Avocet(s)	0	MR	R	0
	Yr10/6Avocet(s)	0	TR	0	0
	Yr15/6Avocet(s)	0	R	0	0
	Yr24/6Avocet(s)	0	MRMS	0	0
	Yr26/6Avocet(s)	0	R/MR	0	0
	YrCV/Avocet	0	0	0	0
Pak varieties					
Class I	Blue Silver	S	S	MSS	MRMS
	WL711	S	S	S	MR
	Kohinoor83	S	S	MSS	MRMS
	Faisalabad83	S	MS	MRMS	MS
	Khyber87	MS	MS	MRMS	0
	Pasban90	MSS	MSS	MSS	MRMS
	Soghat90	MSS	MSS	MSS	RMR
	AS2002	MSS	MSS	MRMS	0
	Saleem2000	MS	MSS	MS	MR
Class II	Pirsabak2005	MRMS	0	0	TR
	Barani70	R	MR	MR	TR
	Manthar	MRMS	MRMS	MRMS	R
Class III	Iqbal2000	0	0	0	0
	Seher2006	0	0	0	0
	GA2002	R	0	0	0
	Marvi2000	R	R	R	R

were effective against the rust populations and *Yr18*, which is an adult-plant resistance gene, provided some level of resistance (and therefore, moderately susceptible) under the field conditions. Genes such as *Yr5* are previously known to show high level resistance to stripe rust in China, Iran, Turkey, North America and Africa (Macer, 1966; Wang et al., 1996; Zeybeck and Fahri, 2004; Chen 2005; Afshari, 2008). Furthermore, virulences to *Yr5* and *Yr15* genes rarely occur in wheat producing areas of the world (Chen, 2005; Chunmei et al., 2008). These major genes along with five others mentioned earlier have been found to confer resistance in tester lines. However, which of these genes (as single gene or in combinations) are present in the resistant Pakistan varieties are to be studied.

Building breeding programs around major genes pose vulnerability. In contrast, minor genes that work in combination with other (major and/or minor) genes provide durable resistance. Durable resistance for stripe rust in many wheat varieties around the world has been attributed to the presence of *Yr18* (McIntosh, 1992; Singh, 1992). *Yr18* with other minor genes is known to be in resistance germplasm sources such as Jupateco73R, Parula, Trap, Cook, Tonichi81, Sonoita81, Yaco, Chapio, Tukuro, Kukuno, Vivitsi and others (Singh et al., 2004). Virulence on seedlings of varieties with *Yr18* is present in most parts of the wheat growing areas of the world under highly conducive conditions (Ma and Singh, 1996). Our reaction data showed that *Yr*18 provided a low level (moderate susceptible) resistance to stripe rust at various locations, agreeing with the previous reports. For durable resistance in wheat, breeding programs in Pakistan should focus on the use of genes like *Yr18*, *Yr29*, *Yr36*, *Yr39* and many other QTLs for adult-plant resistance or high-temperature adult-plant (HTAP) resistance (Chen, 2005).

A number of Pakistan varieties (Iqbal2000, Marvi2000, GA2000, Barani70 and Seher2006) have been found resistant under field conditions, however, their resistance genes are unknown. One of the possibilities is that varieties carry major gene(s) as in resistant tester lines: *Yr3*, *Yr5*, *Yr10*, *Yr15*, *Yr26*, *YrSP* and *YrCV*. However, further characterization is required especially through molecular markers, using the gene postulation approach and genetic analysis.

Spatial variation in virulence pattern among locations indicated the distribution of different pathotypes at different locations besides differences in prevailing environmental conditions (Torabi and Nazari, 1998). We have always observed higher disease incidence at Pirsabak location. The differences of the genotype reactions from other locations suggest that this location is a hotspot for the pathogen variation in Pakistan. Such hot spots serve as the centre of diversity providing a major over-summering area and an inoculum base for the pathogen (Wan et al., 2007). In addition to these attributes, we found environmental conditions, for example temperature range, 7 to 19°C, which is optimum for the development of stripe rust. This is the reason why Pirsabak is frequently used for wheat germplasm screening against stripe rust resistance in Pakistan.

Conclusions

Based on this study, genes *Yr3*, *Yr5*, *Yr10*, *Yr15*, *Yr26*, *YrSP* and *YrCV* are still effective in Pakistan and can be used in developing new resistant wheat cultivars. Approaches like gene pyramiding, gene deployment and multiline cultivars should be considered for the incurporation of race-specific major resistance genes. *Yr18* showed moderate level of resistance. This gene can be used for pyramiding with other genes in breeding programs to produce durable resistant cultivars. The results of this study will assist in devising a strategy for stripe rust management, using the well characterized wheat germplasms carrying effective resistance genes in the breeding programs in Pakistan.

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