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Identification of seedling resistance to stem rust in advanced wheat lines and varieties from Pakistan

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Abstract

Stem rust is a major disease of wheat (*Triticum aestivum* L.) worldwide and new *Puccinia graminis* f. sp. *tritici* (*Pgt*) races including TTKSK (Ug99) pose a serious threat to wheat production. The protection of new varieties against *Pgt* races can be increased by identifying and combining several types of stem rust resistance genes (*Sr*). We screened a set of 707 wheat lines and cultivars against 11 *Pgt* races under glass house conditions. Of the tested lines, groups of 99, 513, 289, and 515 exhibited low infection type (IT < 3) to races TTKSK, TRTTF, TTTTF, and RRTTF, respectively. Screening against *Pgt* races (QFCSC, QTHJC, MCCFC, RCRSC, RKRQC, TPMKC, and QCCSM) showed that most of the tested lines were resistant. These lines were screened with eight DNA markers for the presence of *Sr2*, *Sr9a*, *Sr24*, *Sr25*, *Sr31*, *Sr36*, *Sr38*, and *Sr57* genes. *Sr36* was absent from all the tested lines, whereas *Sr9a* was detected in four lines. The marker *Sr2_ger93p* predicted the presence of *Sr2* in 40 lines, and marker *barc71* suggested the presence of *Sr24* in 12 lines. *Sr25* and *Sr38* were present in 13 and 54 lines, respectively. The highest frequency of *Sr* genes was observed for *Sr57* (199 lines) and *Sr31* (177 lines). Except for lines

Abbreviations: APR, adult plant resistance; BRS, baseline resistance study; CDL, Cereal Disease Laboratory; CIMMYT, International Maize and Wheat Improvement Center; IT, infection type; *Pgt*, *Puccinia graminis* f. sp. *tritici*.

carrying *Sr25* and/or *Sr24* genes, most lines were susceptible to *Pgt* race TTKSK. Since Ug99 is overcoming *Sr* genes worldwide, including *Sr24* and *Sr36*, a strategy to pyramid multiple *Sr* genes in new cultivars should be pursued to achieve a durable control of stem rust. The effectiveness of lines such as NRL0902, 11050, B-2(RF)-11, and CCRI-6, found in the current study and featuring other *Sr* genes, warrants further investigation to identify the source of their resistance and use it in Pakistan wheat breeding programs.

1 | INTRODUCTION

Wheat stem rust caused by *Puccinia graminis* Per. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*) is an important and widespread disease of wheat (*Triticum aestivum* L.) (Manninger, 2002). The most effective, economical, and environmentally safe protection against rusts is the use of resistant wheat varieties. Wheat stem rust had largely been under control for about 30 yr (the second half of the 20th century) due to the widespread use of resistant varieties, such as those featuring the major-effect resistance gene *Sr31* (Singh, Huerta-Espino, & Rajaram, 2000). However, Ug99, a *Pgt* race that appeared first in Uganda (Pretorius, Singh, Wagoire, & Payne, 2000) and was later designated race TTKS (Wanyera, Kinyua, Jin, & Singh, 2006) and TTKSK (Jin et al., 2008), has overcome *Sr31* and many other resistance genes deployed in wheat varieties (Singh et al., 2006). TTKSK, which is considered the most devastating *Pgt* race, constitutes a major threat to global wheat production and has evolved rapidly, spawning multiple variants such as the TTKST strain, which caused severe epidemics in Kenya in 2007. Grain loss from stem rust epidemics can reach 50% and, for infections by Ug99 variants, as high as 90% (Ejaz et al., 2012). A number of variants of Ug99 have spread throughout eastern Africa and across Yemen, Sudan, Iran, and, most recently, to Egypt (Patpour et al., 2016). Though not yet detected in Pakistan, Ug99 variants could easily travel to South Asia, as occurred with wheat stripe rust (*Puccinia striiformis* f.sp. *tritici*) (Singh et al., 2011), and could cause severe yield losses in Pakistan and India, where some 40 million ha of wheat is grown each year (USDA-FAS 2017). In stem-rust-prone areas of Pakistan such as southern Punjab and Sindh provinces, local *Pgt* races could also evolve to generate more virulent races.

Fungicides can reduce stem rust severity and associated yield losses in susceptible wheat cultivars (Wanyera, Macharia, Kilonzo, & Kamundia, 2009), but they are expensive and can pose environmental and human health hazards. To ensure food security in Pakistan, where farmers produce some 25 Tg of wheat grain each year and average annual per-capita consumption of wheat is 120 kg (Abbas, Sheikh, Shahbaz, & Afzaal, 2007), the identification and effective deployment of resistance genes for wheat stem rust are critical.

Achieving this requires extensive screening of wheat genetic resources against prevalent races of *Pgt*, as well as genetic analyses using molecular markers that are linked to Ug99 effective *Sr* genes (Kolmer et al., 2013). Over 50 *Sr* genes have been described in wheat, and a number of those are used in breeding programs. Molecular markers have been developed for many of the stem rust resistance genes including *Sr2*, *Sr6*, *Sr8a*, *Sr9a*, *Sr9h*, *Sr11*, *Sr12*, *Sr13*, *Sr15*, *Sr21*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr28*, *Sr31*, *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr38*, *Sr39*, *Sr40*, *Sr42*, *Sr43*, *Sr44*, *Sr45*, *Sr46*, *Sr47*, *Sr50*, *Sr51*, *Sr52*, *Sr53*, *Sr57*, *Sr59*, *Sr60*, *Sr8155B1*, *SrCad*, *SrTmp*, and *SrND643* (Spanic, Rouse, Kolmer, & Anderson, 2015; https://maswheat.ucdavis.edu/). *Sr* genes effective at the seedling stage are usually race specific, whereas adult plant resistance (APR) genes generally are effective to all *Pgt* races tested. Although APR genes generally confer a relatively small resistance effect compared with many seedling genes, with the accumulation of four to five APR genes, a resistance level near immunity can be achieved in wheat lines (Nisha et al., 2015). As new virulent *Pgt* races appear, knowledge of the stem rust resistance genes in available germplasm is important for wheat breeders (Purnhauser, Bona, & Lang, 2011).

National wheat breeding programs in Pakistan are conducting multilocation tests in hotspot areas of Pakistan to assess the level of resistance to stem rust and other diseases in newly developed wheat lines. However, race-specific tests have not been conducted, so there is limited information regarding which major resistance genes are responsible for the resistance in Pakistan-adapted spring wheat cultivars. To address this, the Wheat Production Enhancement Program (WPEP) for Pakistan funded by the USDA initiated efforts to identify and characterize stem rust resistance genes in Pakistani wheat lines. In this study, we extended those efforts by identifying genes controlling stem rust resistance in a set of 707 spring wheat lines and cultivars. Our findings provide information about the presence or absence of *Sr* genes in Pakistani wheats and their seedling response under glasshouse conditions to prevalent

TABLE 1 Sequence and number of lines in baseline resistance study (BRS) sets screened for *Puccinia graminis* f. sp. *tritici* at the seedling stage

Year	BRS designation	No. of lines	No. of contributing Pakistan institutes
2010–2011	First BRS	195	9
2011–2012	Second BRS	271	8
2012–2013	Third BRS	241	19
Total	3 BRS	707	–

Pgt races in order to help breeders in gene pyramiding and deployment.

2 | MATERIALS AND METHODS

2.1 | Seed source

Baseline resistance study (BRS) sets were prepared by collecting spring wheat breeding lines and cultivars from national and provincial wheat breeding programs across Pakistan. Three sets designated as 1BRS, 2BRS, and 3BRS having 195, 271, and 241 wheat lines assembled during 2010–2011, 2011–2012, and 2012–2013, respectively, were used for this study (Table 1). These sets comprised old and newly released wheat varieties and advanced breeding lines, as well as germplasm introduced to Pakistan from the International Maize and Wheat Improvement Center (CIMMYT).

2.2 | Seedling evaluation

The BRS sets were tested for seedling resistance at the USDA Cereal Disease Laboratory (CDL) at the University of Minnesota (St. Paul, MN) using eight North American *Pgt* races (QFCSC, QTHJC, MCCFC, RCRSC, RKRQC, TPMKC, TTTTF, and QCCSM) and one race each from Yemen (TRTTF), Pakistan (RRTTF), and Kenya (TTKSK = Ug99) (Table 2). Lines resistant to race TTKSK were further screened for variant race TTKST, which has virulence for the *Sr24* gene. Five to six untreated seeds of each line were planted in pots at a depth of 1 cm, along with seeds of the susceptible control McNair701 at the CDL during 2010–2011 (1BRS), 2011–2012 (2BRS) and 2012–2013 (3BRS). Procedures for inoculation and disease assessment were as described by Jin et al. (2007).

Plants were scored on the 14th day after inoculation using a 0-to-4 scale (Stakman, Stewart, & Loegering, 1962). Infection types (ITs) with extensive necrosis or chlorosis were designated as "N" and "C," respectively. Infection types 0 to 2^{2+} were considered low, indicating the presence of host plant resistance, whereas ITs 3 to 4 were considered high, indicating host susceptibility. When low and high ITs were present on the same leaf, the plant was considered resistant. Lines were classified as heterogeneous when both resistant and susceptible plants were present.

2.3 | DNA marker analysis

The three BRS sets were screened with molecular markers linked to *Sr2* (Mago et al., 2011), *Sr24* (Olson et al., 2010), *Sr31* (Olson et al., 2010; Saal & Wricke, 1999), *Sr25* (Prins, Groenewald, Marais, Snape, & Koebner, 2001), *Sr36* (Tsilo, Jin, & Anderson, 2008), *Sr38* (Helguera et al., 2003), and *Sr57* (Lagudah et al., 2009). Sets 2BRS and 3BRS were also screened with a marker linked to *Sr9a* (Tsilo, Jin, & Anderson, 2007). All marker work took place at the USDA-ARS Eastern Regional Genotyping Laboratory, Raleigh, NC. Genotyping followed the methods described by Olson et al. (2010).

3 | RESULTS AND DISCUSSION

3.1 | Status of resistance in the baseline resistance study

The three sets of spring wheat breeding lines and cultivars evaluated for seedling reactions at the CDL displayed a wide range of ITs to the 11 *Pgt* races used (QFCSC, QTHJC, MCCFC, RCRSC, RKRQC, TMPKC, TTTTF, QCCSM, TRTTF, RRTTF, and TTKSK). The number of wheat lines with resistance (IT < 3) to the *Pgt* races used in this study are displayed in Table 3. Out of 707 lines and cultivars screened, 99 showed seedling resistance to race TTKSK, 515 to race RRTTF, and 513 to race TRTTF. More than 90% of the lines screened proved resistant to races QFCSC, MCCFC, RKRQC, TPMKC, and QCCSM. Among the eight North American *Pgt* races, TTTTF was the most virulent, causing high infection scores (IT > 2^{2+}) in 59% of the wheat lines screened. The identification of 99 wheat lines resistant to race TTKSK in this study (Table 3) represents considerable progress from the 15 known resistant lines in 2010 (Rehman et al., 2018).

3.2 | Seedling resistance in three baseline resistance study sets

The results of this study clearly demonstrate the high diversity of responses to infections by *Pgt* races in the lines screened. Nearly all of the wheat lines that showed resistance to Ug99 race TTKSK were also resistant to a majority of the other races used, indicating either that the resistance

TABLE 2 *Puccinia graminis* f. sp. *tritici* races used to screen Pakistani wheat lines under controlled conditions and their avirulence and virulence to stem rust resistance genes

Race	Origin	Avirulence	Virulence
QFCSC	USA	6, 7b, 9b, 9e, 11, 24, 30, 31, 36, 38, Tmp	5, 8a, 9a, 9d, 9 g, 10, 17, 21, McN
QTHJC	USA	7b, 9a, 9e, 24, 30, 31, 36, 38, Tmp	5, 6, 8a, 9b, 9d, 9 g, 10, 11, 17, 21, McN
MCCFC	USA	6, 8a, 9a, 9b, 9d, 9e, 11, 21, 24, 30, 31, 36, 38	5, 7b, 9 g, 10, 17, Tmp, McN
RCRSC	USA	6, 8a, 9e, 11, 24, 30, 31, 38, Tmp	5, 7b, 9a, 9b, 9d, 9 g, 10, 17, 21, 36, McN
RKRQC	USA	9e, 10, 11, 17, 24, 30, 31, 38, Tmp	5, 6, 7b, 8a, 9a, 9b, 9d, 9 g, 21, 36, McN
TPMKC	USA	6, 9a, 9b, 24, 30, 31, 38	5, 7b, 8a, 9d, 9e, 9 g, 10, 11, 17, 21, 36, Tmp, McN
TTTTF	USA	24, 31	5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9 g, 10, 11, 17, 21, 30, 36, 38, Tmp, McN
QCCSM	USA	6, 7b, 8a, 9b, 9e, 11, 30, 31, 36, 38, Tmp	5, 9a, 9d, 9 g, 10, 17, 21, 24, McN
TTKSK	Kenya	24, 36, Tmp	5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9 g, 10, 11, 17, 21, 30, 31, 38, McN
TRTTF	Yeman	8a, 24, 31	5, 6, 7b, 9b, 9d, 9e, 9 g, 10, 11, 17, 21, 30, 36, 38, McN
RRTTF	Pakistan	8a, 9e, 24, 31	5, 6, 7b, 9a, 9b, 9d, 9 g, 10, 11, 17, 21, 30, 36, 38, Tmp, McN

TABLE 3 Number of Pakistani wheat lines showing seedling resistance to races of *P. graminis* f. sp. *tritici* (*Pgt*)

	Wheat lines resistant to *Pgt* races in each BRS[a] set			
Pgt races	1BRS (2010–2011)	2BRS (2011–2012)	3BRS (2012–2013)	Total
QFCSC	184	268	240	692
QTHJC	156	247	227	630
MCCFC	183	270	234	687
RCRSC	153	247	206	606
RKRQC	173	259	226	658
TPMKC	163	261	235	659
TTTTF	89	115	85	289
QCCSM	182	267	237	686
TRTTF	121	206	186	513
RRTTF	123	200	192	515
TTKSK	15	35	49	99

[a]BRS, baseline resistance study.

effective against TTKSK also confers resistance to other races, or that the lines contain multiple race-specific resistance genes. Using an appropriate crop improvement strategy such as interspecific and wide crosses or even the direct transfer of these resistances through backcrosses, the resistance level of the adapted but highly susceptible widely grown wheat varieties could be improved, as proposed by Nzuve, Bhavani, Tusiime, Njau, and Wanyera (2012).

A total of 515 wheat lines were found to be effective (IT < 3) against race RRTTF from Pakistan, 423 of which were susceptible to the Ug99 race TTKSK and 92 of which were resistant, indicating a weak association among the lines tested for resistance to races RRTTF and TTKSK (Rouse, Olson, Gill, Pumphrey, & Jin, 2011). The 92 lines with resistance to multiple *Pgt* races including TTKSK can provide useful sources of resistance for breeding to pyramid resistance sources and postulated gene(s) against both exotic and Pakistan indigenous *Pgt* race(s).

The identified sources of resistance against TTKSK and other *Pgt* races with postulated *Sr* genes are presented in Table 4. This table shows the ITs of 39 wheat lines to 11 *Pgt* races. Gene *Sr25* was identified in lines NR-378, NR-392, NR-395, NR-399, NR-407, NR-410, B-1(RF)-11, NR-356, CCRI-1, CCRI-2, and CCRI-4. Ten lines [B-2 (RF)-17, CCRI-12, 11B2106, IEYT (2011-12)10, SD-998, 11C021, AZRC-18, 99108, NR-383, and SD-4085/3] carried *Sr24*. Another eight of these resistant lines carried *Sr57* singly, and the *Sr57* + *Sr38* gene combination was detected in line 09FJ34. *Sr57* and *Sr24* were both present in line CCRI-12. Wheat lines 11050, NRL 0902, B-2 (RF)-11, and CCRI-6 showed resistance to the *Pgt* races used, but we did not detect the presence of any *Sr*-linked marker allele in them. It is possible that these wheat lines may carry one or few of the resistant genes *Sr9h*, *Sr15*, *Sr28*, *Sr42*, or *SrTmp*, which were not postulated in the present study. Further genetic study of resistance in these lines with support of available diagnostic molecular

TABLE 4 Responses of 39 wheat lines against 11 *Pgt* races including those carrying *Sr24* and/or *Sr25* genes with seedling resistance to the Ug99 race group of *Puccinia graminis* f. sp. *tritici*

Name	Postulated *Sr* genes	Race[a]										
		QFCSC	QTHJC	MCCFC	RCRSC	RKRQC	TPMKC	TTTTF	QCCSM	TTKSK	TRTTF	RRTTF
11B2024	*Sr38*	0;	0;	0;	0;1	0;	;1	3+	0;	0	2	2
11B2057	*Sr57*	0;	2	0;	;2–	;2–	2	33+	0;	2	2	2
11B2058	*Sr57*	1	22+	0;	;2–	0;	22+	3+	0;	2	2	2
11050	–	0;	2–	0;/22+	;2–	2–	2	2–/3+	12–	2	;	;2–
11092	*Sr57*	2	2	;2–	;2–	2–	2–	2	;12–	2	2–	2–
NRL 0902	–	2	22+	–	–	0;	2–	3+	2–	2	2–	2–
NR-378	*Sr25*	;12–	2	2–	2–	0;	2	0;/3+	;2–	2/3–	2–	;2–/3
NR-392	*Sr2*, *Sr25*	;	2–/3–	1–;	;	1–1	2–	;/13–	;1–	2	2	2–
NR 389	*Sr24*, *Sr25*, *Sr38*	2–	2–	;1–	;1–	2–	2–	2–	1–/3	2	2–	2
NR 395	*Sr25*	;	2–	;	;1–	1–;/;	2	2–;/3	;	2–;	;2–	2–
AYT(2011-12)37	*Sr57*	0;	;1–	0;	;	1–;	1–	0;	;1–	2	1	;
MPT(N)-6	*Sr57*	0;	2–;	;1–	;/;1–	1–;/0;	2–	;1–	;	2/3+	3/3	2–/3
B-1(RF)-11	*Sr25*	;	2–	;	;	;	2–	;	;	2	2–	;2–
B-2 (RF)- 11	–	;	2–	1–;	;	1–;	2–	;1–	;	2/3	2–	;2–
B-2 (RF)- 17	*Sr24*, *Sr38*	0;	;	0;	;1–/;	0;	1;	1–;/4	0;	1	2–	2–;
B-3 (RF)- 19	*Sr38*	;1–	;	0;/;1–	;/1	;/1	1–;	4	;	2	2	2
NR-408	*Sr2*, *Sr38*	;	;	;	;/1–	;	1–;	13″Y”	;	0;/3	2+3	2+3
NR-356	*Sr25*	1–	1–/1	;1–	1–	1–	1–	1–	1–;	2/3+	2–	2–
09FJ34	*Sr57*, *Sr38*	0;	0;	0;	;1–	;/;1–	1–;	3–1	0;	2+/3	2	2
CCRI-1	*Sr9a*, *Sr25*	1;	2	;1	1;	;	2	2+	1;	0/23–	2	;2–
CCRI-4	*Sr25*	2–	2	;	2–	;	2+	3	2–	2	2–	2–
CCRI-6	–	0;	2	;	2–	2–	2–	2–;	0	23 LIF	0;	;2–
CCRI-10	*Sr57*	;1	0	;	;/;2	;1	2–	;1	;/2	2/3+	;1	;
CCRI-11	*Sr57*	2–	2–	1;	;	;1	2/3	;2–	;1	2	2–	2–
CCRI-12	*Sr24*, *Sr57*	0	;	0/1	;1	;1	2–	2	0	0;	;	;
CCRI-13	*Sr57*	0;/2–	2	0;	2–	2–	2	3	0	2	2–	2–
NR 407	*Sr25*	;	;	;	;/1	;	1–1;	31	;	0/3 LIF	2	;3 LIF
NR 410	*Sr25*	;	2–	;1–	2–	1–;	2–	13	1–;	2+	;1	2
NR-399	*Sr25*	;	;1	;	2–	2–	;2–	22+	;	2+	;2–	;2–
CCRI-2	*Sr25*	0	2–	;	2–	;1	2	2+	0	23–	2	2–
11B2106	*Sr24*	;2–	2+	0;1	2–	0;1	2	3+	12–	2/3 LIF	2	2–
IEYT(2011-12)10	*Sr24*, *Sr31*	1–1;	1–;/3	1–;	;1–	;	2–	1–;	1–	2–	2–	2–
SD-998	*Sr24*, *Sr57*	;1–	1–	;1–	1–	1–	1–	1–	;	;	;1–	;1
11C021	*Sr24*, *Sr57*	;	2–	2–;	;	2–	2–	2–	;	;2–	2–;	0
11C022	*Sr24*, *Sr25*, *Sr57*	2–	2–	2–	2–	2–	2–	2–	2+	2–	;2–	2–
AZRC-18	*Sr24*, *Sr38*	1;	;1+	;1	2–	;1	2+	2	;13–	2–;	2–;	2–;
99108	*Sr24*	;	2	1–;	2–	;	2	2–	;	2–	2–	2–
NR 383	*Sr24*	;/2–	2–	;	2/;	;	2+	;1/4	;/2	;	;1	2–
SD-4085/3	*Sr24*	2-	2-	2-	2	2-	2N	2	2-	;2-	2-	2-

[a]0 = immune, ; = nearly immune, 1 = very resistant, 2 = moderately resistant, 3 = moderately susceptible, and 4 = susceptible (Stakman et al., 1962). LIF = low infection frequency (few pustules), / = heterogeneous reaction with the predominant type given first. Symbols + and − indicate slightly larger and smaller pustule sizes, respectively.

TABLE 5 Details of wheat lines postulated for the presence of *Sr* genes by linked markers and their responses against *Puccinia graminis* f. sp. *tritici* (*Pgt*) races

Sr genes	Marker used	Wheat lines with marker loci	Lines susceptible to *Pgt* races
Sr2	Sr2_ger93p	40	22 (TTTTF), 3 (TPMKC), 1 (QTHJC), 2 (RKRQC), 12 (TRTTF), 15 (RRTTF), 40 (TTKSK).
Sr9a	gwm47	04	2 (TTTTF), 3 (TTKSK), 1 (TRTTF/RRTTF)
Sr24	barc71	12	1 (TTTTF)
Sr25	Gb	14	1(TTTTF)
Sr31	scm9	177	143 (TTKSK), 30 (TTTTF), 12 (TRTTF/RRTTF)
Sr36	wmc477	0	–
Sr38	Lr37	54	1 (MCCFC/RCRSC), 2 (TPMKC), 36 (TTTTF), 47 (TTKSK), 16 (TRTTF), 14 (RRTTF)
Sr57	Lr34	199	10 (QTHJC/RKRQC/TPMKC), 4 (MCCFC/RCRSC), 91 (TTTTF), 169 (TTKSK), 44 (TRTTF/RRTTF)

markers and *Pgt* races could result in the identification of new sources of resistance for stem rust in wheat.

3.3 | Seedling resistance in lines with known genes linked to molecular marker(s)

Based on molecular marker data, we postulated that the lines had the particular genes (Table 5). We used eight molecular markers—*Sr2ger93p*, *gwm47*, *barc71*, *Gb*, *Scm9*, *wmc477*, *Lr37*, and *Lr34*—to detect the stem rust genes *Sr2*, *Sr9a*, *Sr24*, *Sr25*, *Sr31*, *Sr36*, *Sr38*, and *Sr57*. Molecular markers predicted the presence of *Sr57* in 199 lines and *Sr31* in 177 lines, followed by *Sr38* in 54 lines and *Sr2* in 40 lines. Of the 177 that tested positive for *Sr31*, 164 wheat lines were resistant to races RRTTF and TRTTF, whereas all were resistant to the North American races MCCFC, RCRSC, QTHJC, RKRQC, and TPMKC. Previous research suggests the effectiveness of *Sr31* to the local race RRTTF (Ejaz et al., 2012). Another 34 lines that were predicted to carry *Sr31* showed low ITs (IT < 3) against TTKSK and might carry additional genes that complement *Sr31*; for example, 16 of these wheat lines also carried at least one of the following genes: *Sr2*, *Sr24*, *Sr25*, *Sr38*, and *Sr57*. The presence of *Sr2*, *Sr31*, and *Sr57* in the tested lines could be attributed to the significant use of CIMMYT germplasm in Pakistan wheat breeding programs.

Developing wheat lines that feature a combination of resistance genes is the most effective way to combat the threat posed by the emerging races. It is expected that with the combination of other stem rust resistance genes, these susceptible genes can provide resistance, a phenomenon known as residual resistance (Knott, 2008). The 199 lines postulated to carry *Sr57* gene had low ITs against races QFCSC, QTHJC, MCCFC, RCRSC, RKRQC, TPMKC, and QCCSM. Out of the 199, 29 wheat lines had low ITs to TTKSK, and 155 lines displayed low IT to races TRTTF and RRTTF. Three of the 29 TTKSK-resistant lines tested positive for the presence of *Sr57* + *Sr24*, 11 were postulated to carry *Sr57* + *Sr31*, and one tested positive for *Sr57* + *Sr38*. The remaining 14 TTKSK-resistant lines tested negative for other *Sr* gene(s) and may carry some other gene(s) not postulated in the present study, warranting further investigation. Of the 54 lines postulated to possess *Sr38*, seven had an IT of 0 and 40 had ITs of 1 or 2 to races TTKSK and RRTTF, respectively. In addition to *Sr38*, two of the seven TTKSK-resistant wheat lines, AZRC-18 and B-2 (RF)-17, also tested positive for the presence of *Sr24*, and line 09FJ34 was postulated to carry *Sr31* and *Sr57*. Gene *Sr38* is also linked with stripe and leaf rust resistance genes *Yr17* and *Lr37* and the *Lr37/Yr17/Sr38* gene cluster could be incorporated into Pakistani wheat varieties to confer resistance to multiple avirulent races of the three rust pathogens.

All *Sr2*-carrying lines had IT > 2 against TTKSK, whereas 25 lines had low ITs to RRTTF. The slow-rusting gene *Sr2*, which provides a nonhypersensitive response at the adult plant stage, may not substantially reduce yield losses under severe epidemics, so combining *Sr2* with other minor genes (*Sr2*-complex) could provide resistance to the Ug99 race group (Singh et al., 2006). None of the tested wheat lines were predicted to possess *Sr36*, whereas *Sr9a* was postulated to be present in four lines—NR 420, V-103 (V-11153), V-111 (10B9346), and CCRI-1—that exhibited low ITs against all races except TTTTF and TTKSK. Eleven and ten wheat lines postulated to carry the TTKSK resistance genes *Sr25* and *Sr24*, respectively. Two other lines (NR389 and 11C022) were postulated to carry both major genes *Sr25* and *Sr24*. According to our seedling tests, wheat lines with *Sr25*, *Sr24*, and their combinations were resistant to all *Pgt* races used in this study (Table 4). However, wheat line 11B2106 with *Sr24* and lines CCRI-4 and NR 407 with *Sr25* showed IT > 2 against TTTTF, but these genes are not known to be susceptible to TTTTF (Jin et al., 2008; Newcomb et al., 2016).

The high ITs for most wheat lines against TTKSK are not surprising, given that stem rust was of low importance in Pakistan and thus had not been a priority in breeding programs until recently, and prior studies using simple sequence repeat (SSR) and sequence-tagged sites (STS) on the 117 Pakistani wheat varieties found that none carried *Sr24* or *Sr25* (Ejaz et al., 2012). The emergence and spread of new *Pgt* races (TTKSK/Ug99) with virulence for *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9b*, *Sr9e*, *Sr9g*, *Sr11*, *Sr17*, *Sr30*, *Sr31*, and *Sr38* makes stem rust a grave threat to wheat production worldwide, including Pakistan, where farmers sow ~9 million ha of wheat each year.

Characterizing Pakistan wheat lines for stem rust resistance genes constitutes a crucial underpinning for preemptive breeding. Our results showed that most wheat lines are susceptible to Ug99, which agrees with the outcomes of screening of Pakistani germplasm in Kenya under natural Ug99 infections during 2005 to 2010 (Ejaz et al., 2012). It is important to broaden the genetic base of stem rust resistance in future wheat cultivars by pyramiding multiple stem rust resistance genes. Marker-assisted selection can greatly facilitate the transfer of the needed *Sr* genes; the use of sources of stem rust resistances identified in this study, particularly those effective against Ug99, can speed the development of cultivars able to mitigate present and future challenges from *Pgt* in Pakistan and ensure food security. Some of the lines tested have been released as commercial varieties and are grown on farmers' fields in the country, like NARC-2011 (NR-356) and Borlaug-2016 (NR-399).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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