Rust resistance in exotic wheat germplasm tested through molecular genotyping and field trials across Himalayan region of Pakistan.

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ABSTRACT

Stem rust, caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks and E. Henn, poses an important risk for wheat production, worldwide. Though its infestation is low in Pakistan, screening of germplasm should be done for preparedness against the novel emergence as observed in for Ug99 (strain TTKS) and its variants. In this study, a set of 30 exotic wheat lines were screened in field (during 2018 to 2022) for stem rust disease in the Himalayan region of Pakistan. The field surveillance across these 38 environments (as location x years combinations) revealed that no stem rust was observed at any of the tested locations and therefore necessitated to use molecular markers to screen for stem rust resistance. Molecularly genotyping was done with eight DNA markers linked to resistance genes (*Sr34*, *Sr31*, *Sr38*, *Sr57*, *Sr58*, *Sr39*, *Sr24*, and *Sr21*). The molecular marker-based screening revealed that *Sr57* gene linked marker was detected in 100% of the wheat lines followed by *Sr34* and *Sr58*-linked marker which was detected in 97% of the tested wheat lines. 93% of the wheat lines showed the presence of *Sr38* linked marker. The *Sr31*linked marker was detected in 30% of lines whereas 27% had *Sr31* linked marker; 23% had *Sr21*; and 13% had *Sr24* gene. The Exotic wheat line SA-Wht-14 showed the presence of the maximum seven *Sr* resistance genes (*Sr34*, *Sr31*, *Sr38*, *Sr57*, *Sr58*, *Sr39*, and *Sr24*). whereas the wheat line SA-Wht-4 showed the presence of the minimum three *Sr* resistance genes (*Sr34*, *Sr38*, and *Sr57*). The variability observed could be utilized for breeding resistant wheat varieties in the context of potential invasions in future.

Keywords: Wheat, stem rust, breeding, resistance, European

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops and has great impact on the economy of Pakistan (Li and Ali, 2022). Three different rust species are stem rust (caused by *Puccinia graminis* pers. f. sp. *tritici* Eriks, and E. Henn (*pgt*), leaf rust (by *Puccinia triticina* Eriks.), and stripe rust (by *Puccinia striiformis* West. f. sp. *tritici* Eriks.) (Saunders *et al*., 2019; Sandiswa *et al*., 2014; Bariana *et al*., 2007). Stem rust has been an important disease in many parts

of the world for centuries, imparting significant losses to wheat production. Stem rust epidemics have been reported to cause yield losses of up to 50% (Zhao and Kang, 2023; Dean *et al*., 2012). The disease remained under control for a long period of time due to incorporation of the resistance genes *Sr31*, which protected wheat from this disease. However, the resistance was overcome by the pathogen, first reported from Uganda in 1999, named Ug99 (Pretorius *et al*., 2012).

The *Puccinia graminis* f. sp. *tritici* race Ug99 and its variants remains the most dangerous pathogen and poses a serious threat to wheat production. The Ug99 can cause yield losses of up to 90% (Pretorius *et al*., 2007). It originally emerged in Uganda in 1999 (Pretorius *et al*., 2012), and since then, it has spread to Yemen, Sudan, Iran, and East Africa. It was expected to spread to North Africa, the Middle East, Asia, and beyond, causing epidemics leading to wheat destruction in various areas of the country (Singh *et al*., 2008). In Kenya, two mutant strains (TTKST and TTSSK) of Ug99 were detected in 2006 and 2007, indicating further diversification of Ug99 (Terefe *et al*., 2016). TTKST caused a serious epidemic in some parts of Kenya in 2007, and it looked that half of the world's Ug99-resistant wheat germplasm was susceptible to this variant (Singh *et al*., 2008). Although Ug99 has not yet been found in Pakistan, the migration pattern indicated the possibility that it may enter the country via Iran (Hodson and DePauw, 2010). In addition, some regional stem rust infestation has been reported in Sindh and southern Punjab with more prevalence and higher severity than usual infestation (Atiq and Rajput, 2022). Hence, a risk exists in Pakistan that a potential re-emergence of stem rust may occur in Pakistan and pose a threat to wheat production in Pakistan. Therefore, to achieve the food security, it is necessary to develop effective strategies to explore resistance against *Pgt* in both exotic and indigenous wheat germplasm of Pakistan (Wanyera *et al*., 2006).

Limited studies have been done to explore the status of resistance in wheat germplasm (Shah *et al*., 2007). Though in wheat and its wild relatives, more than 80 stem rust resistance genes have been discovered so far (Online *Sr* gene catalog, Singh, 2017). Many of the genes are specific to pathogens except *Sr2*, which is race-nonspecific and confers long-lasting resistance (Singh *et al*, 2006), which confers slow rusting, however under severe outbreaks, this may not significantly minimize yield losses (Singh *et al*., 2007). Thus, the Sr2-complex, which combines *Sr2* with other minor rust resistance genes, can confer resistance to several stem rust races, including Ug99 (Singh *et al*., 2007). Using conventional approaches to screen breeding material for disease resistance genes takes time because certain genes don't express until later in plant's life cycle. The conventional approach also has the risky requirement that disease inoculum be applied to plants, which is dangerous in areas where a specific pathogen race is not available. Similarly, the natural inoculum may not be present in the testing areas and thus special greenhouses may be required. For the postulation of resistance genes in the host plant, gene-for-gene specificity between the host resistance genes and various avirulence genes in the pathogen can be used under such greenhouse-controlled conditions. The interaction between resistance genes and the stage of plant development at which these genes express can make the gene postulation difficult to determine, hence this strategy works best for seedling resistance genes (Kolmer, 1996). These problems can be resolved by identifying potential resistance genes using DNA-based markers (McCartney *et al*., 2005).

Molecular markers offer an effective tool to complement conventional breeding techniques to identify sources of resistance against wheat diseases (Iqbal *et al*., 2020, Ismail *et al*., 2019). Selection based on tightly linked DNA markers that can identify rust resistance genes and increases the effectiveness of breeding programs (Todorovska *et al*., 2009). Gene pyramiding, in which genes from various genotypes are deployed in a single cultivar that possesses desired alleles at several loci, has become effective with the development of marker-assisted selection (MAS) (Joshi and Nayak, 2010). There are several DNA markers that have been developed and connected to different wheat stem rust resistance genes. The genes include *Sr2* (Spielmeyer *et al*., 2003; Hayden *et al*, 2004), *SrlR* (Olson *et al*., 2010), *Sr6* (Tsilo *et al*., 2009), *Sr9a* (Tsilo *et al*, 2007), *Sr24* (Mago *et al*., 2005; Olson *et al*, 2010), *Sr25* (Liu *et al*., 2010), *Sr26* (Mago *et al*., 2002; Liu *et al*., 2011), *Sr31* (Das *et al*., 2006), *Sr35* (Zhang *et al*., 2010), *Sr36* (Tsilo *et al*., 2008), *Sr38* (Helguera *et al*., 2003), *Sr39* (Gold *et al*, 1999), *Sr40* (Wu *et al*, 2009), *SrCad* (Hiebert *et al*., 2011), *SrWeb* (Hiebert *et al*, 2010), *Sr51* (Liu *et al*., 2011b), *Sr52* (Qi *et al*., 2011), and *Sr53* (Liu *et al*., 2011a). Very few studies have been made to explore the status of stem rust resistance in the breeding material and varieties of Pakistan, using molecular markers (Ejaz *et al*., 2012).

There is a no information available on the presence and absence of stem rust resistance genes in Exotic wheat germplasm used in breeding material across Pakistan. The goal of this work was to identify the stem rust resistance genes in Exotic wheat germplasm tested across muti location of Pakistan using DNA markers to aid in result resistance breeding in the future.

MATERIALS AND METHODS

The objective of the current study was to describe the field-based disease screening and molecular genotyping of the Exotic wheat germplasm against stem rust in the Himalayan regions of Pakistan, which was based on a five-year experiment with up to 15 locations. For the study, a total of 30 Exotic wheat lines were chosen (Table 1).

Table 1. The set of 30 Exotic wheat lines along with three Pakistani checks selected for testing against wheat stem rust disease.

S.No.	Genotype	Detail	S.No.	Genotype	Detail
1	SA-Wht-1	Exotic Line	18	SA-Wht-18	Exotic Line
2	SA-Wht-2	Exotic Line	19	SA-Wht-19	Exotic Line
3	SA-Wht-3	Exotic Line	20	SA-Wht-20	Exotic Line
4	SA-Wht-4	Exotic Line	21	SA-Wht-21	Exotic Line
5	SA-Wht-5	Exotic Line	22	SA-Wht-22	Exotic Line
6	SA-Wht-6	Exotic Line	23	SA-Wht-23	Exotic Line
7	SA-Wht-7	Exotic Line	24	SA-Wht-24	Exotic Line
8	SA-Wht-8	Exotic Line	25	SA-Wht-25	Exotic Line
9	SA-Wht-9	Exotic Line	26	SA-Wht-26	Exotic Line
10	SA-Wht-10	Exotic Line	27	SA-Wht-27	Exotic Line

11	SA-Wht-11	Exotic Line	28	SA-Wht-28	Exotic Line
12	SA-Wht-12	Exotic Line	29	SA-Wht-29	Exotic Line
13	SA-Wht-13	Exotic Line	30	SA-Wht-30	Exotic Line
14	SA-Wht-14	Exotic Line			
15	SA-Wht-15	Exotic Line			
16	SA-Wht-16	Exotic Line			
17	SA-Wht-17	Exotic Line			

Multilocation field experimentation and stem rust scoring

The field testing was done during the crop seasons of 2017-18 to 2021-22 at multilocation, where Peshawar Mansehra, Shangla and Skardu remained the same across all years, making an overall of 38 environments in terms of location x year combinations. The exotic wheat lines were planted in RCB design at the selected sites, with two replications at each site. Each plot within each replication had two rows, each measuring 2 meters in length, with a row-to-row distance of 0.3 meters (30 cm). Blocks next to each other were maintained apart by a meter. The crop production techniques were implemented at each location in accordance with the location-specific best practices (Iqbal *et al*., 2020; Ismail *et al*., 2021). The fields were screened for the presence of stem rust infestation at all the trial locations at least at the grain anthesis to grain filling stage (Table 1).

Molecular characterization for *Sr* resistance genes

To characterize the Exotic wheat lines molecularly, leaves (1 – 2 g) from each of the wheat lines were collected for DNA extraction. Molecular markers linked to resistance genes were then used for genotyping (Table 2). After that, these leaves were individually crushed using a modified DNA extraction technique (Ali *et al*., 2017), based on CTAB (Cetyltrimethylammonium bromide) protocol. After the DNA had been isolated, 50 – 60 µL of TE buffer were added to dilute it (Ali *et al*., 2017). The purity and concentration of the DNA were checked using the nano-drop technique, while the isolated DNA was maintained at -20°C for PCR based genotyping.

Molecular marker amplification

Eight SSR markers in total, which are associated with genes for disease resistance i.e., *Sr34* (Blaszczyk *et al*., 2004), *Sr31* (Mago *et al*., 2002), *Sr38* (Khan *et al*., 2017), *Sr57* (Lagudah *et al*., 2006), *Sr58* (Sharma *et al*., 2015), *Sr39* (Gold *et al*., 1990), *Sr24* (Schachermayer *et al*., 1994), and *Sr21* (Dadkhodaie *et al*., 2011), were amplified for molecular genotyping. First, using a Thermo Scientific PCR kit, several polymerase chain reactions (PCR) were carried out to establish the correct annealing temperatures, number of cycles, DNA concentrations, and primer concentrations (Iqbal *et al*., 2020). The final PCR reactions were run to genotype each of the 30 Exotic wheat lines after the optimum conditions were identified (Table 2). Following the successful completion of the PCR reactions, the amplified products were separated on an agarose gel, and the bands obtained from various samples were compared with the expected bands described in the original articles describing the development/application of the relevant resistance gene marker (Table 3).

Statistical analyses of the data

The data was compiled in MS Excel, and then was analyzed, primarily by R statistical environment to assess the clustering of the lines based on presence of different resistance genes-based markers, as described previously (Ali *et al*., 2009; Iqbal *et al*., 2020; Ismail *et al*., 2021).

Table 2. Amplification parameters for the stem rust resistance genes linked molecular markers in Exotic wheat germplasm.

S.No.	Primer	Denaturation		Annealing	Extension	
		Initial	Final		Initial	Final
1	CSLV-34	95ºC for 5 minutes	95ºC for 30 seconds (40 Cycles)	58.0ºC	72ºC for 45 seconds	72ºC for 7 minutes
2	SCY-15	95ºC for 5 minutes	95ºC for 30 seconds (40 Cycles)	54.0ºC	72ºC for 45 seconds	72ºC for 7 minutes
3	IAG95-STS	95ºC for 5 minutes	95ºC for 30 seconds (40 Cycles)	55.8ºC	72ºC for 45 seconds	72ºC for 7 minutes
4	Lr28-01 Lr28-02	95ºC for 5 minutes	95ºC for 30 seconds (40 Cycles)	56.9ºC	72ºC for 45 seconds	72ºC for 7 minutes
5	SR39 F2/R3	95ºC for 5 minutes	95ºC for 30 seconds (40 Cycles)	57.2ºC	72ºC for 45 seconds	72ºC for 7 minutes
6	J09	95ºC for 5 minutes	95ºC for 30 seconds (40 Cycles)	59.9ºC	72ºC for 45 seconds	72ºC for 7 minutes
7	XGWM259	95ºC for 5 minutes	95ºC for 30 seconds (40 Cycles)	55.8ºC	72ºC for 45 seconds	72ºC for 7 minutes
8	Xgwm88	95ºC for 5 minutes	95ºC for 30 seconds (40 Cycles)	58.4ºC	72ºC for 45 seconds	72ºC for 7 minutes

Table 3. SSR Primers and expected amplicon sizes used for molecular characterization of Exotic wheat Lines tested across various environments of Pakistan during cropping season 2018-2022

S. No	Primer	Name of Gene/QTL/Locus	Nucleotide Sequence (5' to 3')	Annealing Temp. (°C)	Amplicon Size (bp)	Reference
1	csLV-34	Sr57	F: GTTGGTTAAGACTGGTGATGG R: TGCTTGCTATTGCTGAATAGT	58.0	250	Lagudah *et al*., 2006
2	SCY-15	Sr38	F: AGGGGCTACTGACCAAGGCT R: TGCAGCTACAGCAGTATGTACACAAAA	54.0	290	Khan *et al*., 2017
3	IAG95-STS	Sr31	F: CTCTGTGGATAGTTACTTGAT R: CCTAGAACATGCATGGCTGTTACA	55.8	1100	Mago *et al*., 2002
4	Lr28-01 Lr28-02	Sr34	F: CCCGGCATAAGTCTATGGTT R: CAATGAATGAGATACGTGAA	56.9	378	Blaszczyk *et al*., 2004
5	SR39 F2/R3	Sr39	F: AGAGAGAGTAGAAGAGCTGC R: AGAGAGAGAGCATCCACC	57.2	900	Gold *et al*., 1990
6	J09	Sr24	F: TCTAGTCTGACATGGGGGC R: TGGCACATGAACTCCATACG	59.9	310	Schachermayr *et al*., 1995
7	XGWM259	Sr58	F: AGGGAAAAGACATCTTTTTTTC R: CGACCGACTTCGGGTC	55.8	105	Sharma *et al*., 2015
8	Xgwm88	Sr21	F: GCTGCATGAGCTCTGCAAT R: TCTGTGAGGCATGACAGAA	58.4	282	Dadkhodaie *et al*., 2011

RESULTS

Over the years across locations, the stem rust disease scoring was made several times (Table 4). According to the field scoring, no stem rust disease was present during the years across all the tested locations. According to the molecular markers-based screening, the 30 Exotic wheat lines that were tested for *Sr* resistance (*Sr34, Sr31, Sr38, Sr57, Sr58, Sr39, Sr24*, and *Sr21*) showed variation in the presence and absence of *Sr* genes.

Stem rust disease scoring across the tested environments.

The disease scoring details across the tested environments during the cropping season 2018 to 2022 are shown in the Table 4. The field observation enabled to do scoring from a single to a maximum of three scores, during the years. Field observation confirmed the lack of stem rust at all these locations over the studied years (2018 to 2022), as no disease was observed.

Table 4. Details of the stem rust scoring made at multilocation during the cropping years 2018-2022, to assess the prevalence of stem rust on 30 exotic wheat lines.

Location	Cropping season									
	2017-18		2018-19		2019-20		2020-21		2021-22	
	Scoring made	Stem rust severity	Scoring made	Stem rust severity	Scoring made	Stem rust severity	Scoring made	Stem rust severity	Scoring made	Stem rust severity
Peshawar	3	0	3	0	3	0	3	0	3	0
Mansehra	2	0	2	0	3	0	3	0	3	0
Shangla	1	0	1	0	1	0	-	-	-	-
Skardu	1	0	1	0	-	-	1	0	1	0
Parachinar	-	-	-	-	-	-	2	0	2	0
Bajaur	-	-	-	-	-	-	2	0	2	0
Chitral	-	-	-	-	-	-	2	0	2	0
Nagar	-	-	-	-	-	-	1	0	1	0
Ghizer	-	-	-	-	-	-	1	0	1	0
Gilgit	-	-	-	-	-	-	1	0	1	0
Lakki Marwat	-	-	-	-	-	-	-	-	2	0
Kasur	-	-	-	-	-	-	2	0	-	-
Jhang	-	-	-	-	-	-	-	-	2	0
Sargodha	-	-	-	-	-	-	-	-	2	0
Quetta	-	-	-	-	-	-	1	0	-	-
Zhob	-	-	-	-	-	-	-	-	1	0

Molecular marker-based screening for disease resistance

The presence of several *Sr* genes (*Sr34, Sr31, Sr38, Sr57, Sr58, Sr39, Sr24,* and *Sr21*) in these Exotic wheat lines was determined using gene specific markers.

A band of 250 bp was produced by the marker linked with *Sr57* and was present in all the 30 tested wheat lines. Similarly, a specific band of 378 bp was produced by marker which is linked with *Sr34*. This gene was present in all the 29 lines except a single wheat line "SA-wht-1". A band of 105 bp was produced by the marker linked with *Sr58* detected in all the 29 wheat lines except SA-Wht-4.

A band of 1100 bp was produced for the marker linked with *Sr31*, which was present in nine wheat lines (i.e., SA-Wht-2, SA-Wht-5, SA-Wht-12, SA-Wht-14, SA-Wht-15, SA-Wht-17,

SA-Wht-19, SA-Wht-23, and SA-Wht-24), while it was absent in 21 wheat lines (i.e., SA-Wht-1, SA-Wht-3, SA-Wht-4, SA-Wht-6, SA-Wht-7, SA-Wht-8, SA-Wht-9, SA-Wht-10, SA-Wht-11, SA-Wht-13, SA-Wht-16, SA-Wht-18, SA-Wht-20, SA-Wht-21, SA-Wht-22, SA-Wht-25, SA-Wht-26, SA-Wht-27, SA-Wht-28, SA-Wht-29, and SA-Wht-30).

A band of 290 bp was produced for the marker linked with *Sr38*, which was present in 28 wheat lines (SA-Wht-1, SA-Wht-2, SA-Wht-3, SA-Wht-4, SA-Wht-5, SA-Wht-6, SA-Wht-7, SA-Wht-8, SA-Wht-9, SA-Wht-10, SA-Wht-12, SA-Wht-13, SA-Wht-14, SA-Wht-15, SA-Wht-16, SA-Wht-17, SA-Wht-18, SA-Wht-19, SA-Wht-20, SA-Wht-21, SA-Wht-22, SA-Wht-23, SA-Wht-25, SA-Wht-26, SA-Wht-27, SA-Wht-28, SA-Wht-29, and SA-Wht-30) and was absent in wheat line SA-Wht-11, and SA-Wht-24.

A band of 900 bp was produced by the marker linked with *Sr39* which was present in eight wheat lines (SA-Wht-1, SA-Wht-3, SA-Wht-5, SA-Wht-7, SA-Wht-8, SA-Wht-10, SA-Wht-11, and SA-Wht-14) and was absent in 22 wheat lines (SA-Wht-2, SA-Wht-4, SA-Wht-6, SA-Wht-9, SA-Wht-12, SA-Wht-13, SA-Wht-15, SA-Wht-16, SA-Wht-17, SA-Wht-18, SA-Wht-19, SA-Wht-20, SA-Wht-21, SA-Wht-22, SA-Wht-23, SA-Wht-24, SA-Wht-25, SA-Wht-26, SA-Wht-27, SA-Wht-28, SA-Wht-29, and SA-Wht-30).

A band of 310 bp was produced by the marker linked with *Sr24* which was detected in four wheat lines (SA-Wht-13, SA-Wht-14, SA-Wht-15, and SA-Wht-16) and was absent in 26 wheat lines (SA-Wht-1, SA-Wht-2, SA-Wht-3, SA-Wht-4, SA-Wht-5, SA-Wht-6, SA-Wht-7, SA-Wht-8, SA-Wht-9, SA-Wht-10, SA-Wht-11, SA-Wht-12, SA-Wht-17, SA-Wht-18, SA-Wht-19, SA-Wht-20, SA-Wht-21, SA-Wht-22, SA-Wht-23, SA-Wht-24, SA-Wht-25, SA-Wht-26, SA-Wht-27, SA-Wht-28, SA-Wht-29, and SA-Wht-30).

A band of 282 bp by the marker linked with *Sr21* which was present in seven wheat lines (SA-Wht-2, SA-Wht-7, SA-Wht-9, SA-Wht-16, SA-Wht-18, SA-Wht-19, and SA-Wht-24) and was absent in 23 wheat lines (SA-Wht-1, SA-Wht-3, SA-Wht-4, SA-Wht-5, SA-Wht-6, SA-Wht-8, SA-Wht-10, SA-Wht-11, SA-Wht-12, SA-Wht-13, SA-Wht-14, SA-Wht-15, SA-Wht-17, SA-Wht-20, SA-Wht-21, SA-Wht-22, SA-Wht-23, SA-Wht-25, SA-Wht-26, SA-Wht-27, SA-Wht-28, SA-Wht-29, and SA-Wht-30) (Table 4).

Table. 4 A set of 30 Exotic wheat lines tested for the presence and absence of stem rust resistance genes to describe their resistance at the molecular level.

Code	Sr34	Sr31	Sr38	Sr57	Sr58	Sr39	Sr24	Sr21
SA-Wht-1	-	-	+	+	+	+	-	-
SA-Wht-2	+	+	+	+	+	-	-	+
SA-Wht-3	+	-	+	+	+	+	-	-
SA-Wht-4	+	-	+	+	-	-	-	-
SA-Wht-5	+	+	+	+	+	+	-	-
SA-Wht-6	+	-	+	+	+	-	-	-
SA-Wht-7	+	-	+	+	+	+	-	+

SA-Wht-8	+	-	+	+	+	+	-	-
SA-Wht-9	+	-	+	+	+	-	-	+
SA-Wht-10	+	-	+	+	+	+	-	-
SA-Wht-11	+	-	-	+	+	+	-	-
SA-Wht-12	+	+	+	+	+	-	-	-
SA-Wht-13	+	-	+	+	+	-	+	-
SA-Wht-14	+	+	+	+	+	+	+	-
SA-Wht-15	+	+	+	+	+	-	+	-
SA-Wht-16	+	-	+	+	+	-	+	+
SA-Wht-17	+	+	+	+	+	-	-	-
SA-Wht-18	+	-	+	+	+	-	-	+
SA-Wht-19	+	+	+	+	+	-	-	+
SA-Wht-20	+	-	+	+	+	-	-	-
SA-Wht-21	+	-	+	+	+	-	-	-
SA-Wht-22	+	-	+	+	+	-	-	-
SA-Wht-23	+	+	+	+	+	-	-	-
SA-Wht-24	+	+	-	+	+	-	-	+
SA-Wht-25	+	-	+	+	+	-	-	-
SA-Wht-26	+	-	+	+	+	-	-	-
SA-Wht-27	+	-	+	+	+	-	-	-
SA-Wht-28	+	-	+	+	+	-	-	-
SA-Wht-29	+	-	+	+	+	-	-	-
SA-Wht-30	+	-	+	+	+	-	-	-

Distribution of resistance genes markers in Exotic wheat lines

Our findings showed that one *Sr* resistance gene associated marker linked with *Sr57*, was present in all the tested Exotic wheat lines followed by the marker linked with *Sr34*, and the one linked with *Sr58* which were present in 29 Exotic wheat lines. The *Sr34* was absent in exotic wheat line SA-Wht-1 whereas the *Sr58* was absent in wheat line SA-Wht-4. The Exotic wheat line SA-Wht-14 showed the presence of the maximum seven *Sr* resistance genes (*Sr34, Sr31, Sr38, Sr57, Sr58, Sr39,* and *Sr24*) (Table 4), while the wheat line SA-Wht-4 showed the presence of the minimum number of three *Sr* resistance genes (*Sr34, Sr38*, and *Sr57*). The *Sr57*

gene linked marker was detected in all the 30 Exotic wheat lines (100%) followed by *Sr34* and *Sr58* (detected in 29 wheat lines i.e., 97% of tested wheat lines) (Fig. 2).

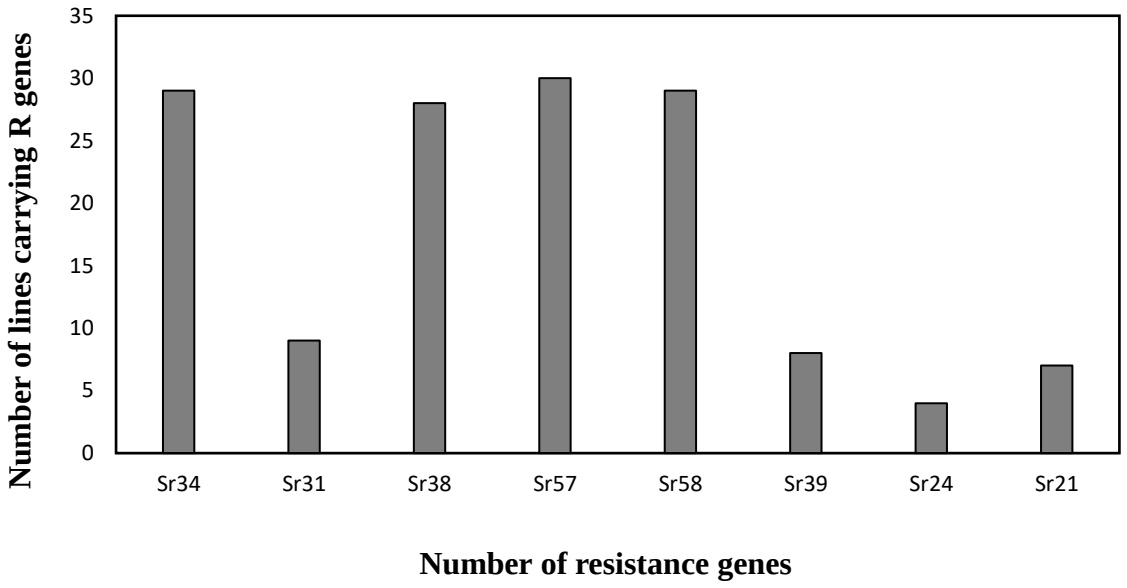

Figure 1. Distribution of *Sr* resistance genes in Exotic wheat lines, as revealed through molecular markers-based screening.

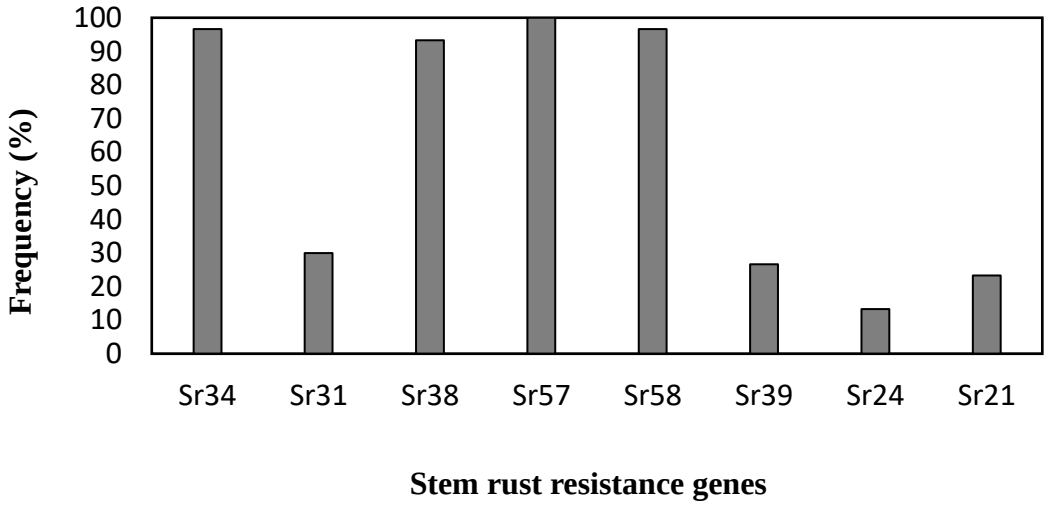

Figure 2. Relative distribution of *Sr* resistance genes in the Exotic wheat lines, as revealed by molecular genotyping.

Clustering of lines based on the presence of resistance genes.

Cluster analysis based on molecular genotyping resulted in the formation of four groups. In the 1st group there was only one wheat line SA-Wht-24 which contained five *Sr* genes (*Sr34, Sr31*, *Sr57, Sr58,* and *Sr 21*). The 2nd group contained five exotic wheat lines (SA-Wht-14, SA-Wht-5, SA-Wht-23, SA-Wht-12, and SA-Wht-17), which showed the presence of five *Sr* genes (*Sr34*, *Sr31*, *Sr38*, *Sr57*, and *Sr58*) except for SA-Wht-5 which also contained *Sr39* and SA-Wht-14 which also contained *Sr39* and *Sr24*. The 3rd group contained 17 wheat lines (SA-Wht-1, SA-Wht-3, SA-Wht-4, SA-Wht-6, SA-Wht-7, SA-Wht-8, SA-Wht-10, SA-Wht-11, SA-Wht-20, SA-Wht-21, SA-Wht-22, SA-Wht-25, SA-Wht-26, SA-Wht-27, SA-Wht-28, SA-Wht-29, and SA-Wht-30). All these wheat lines contained *Sr* genes (*Sr34, Sr38, Sr57*, and *Sr58*) except for wheat lines (SA-Wht-11, SA-Wht-7, SA-Wht-1, SA-Wht-10, SA-Wht-3, and SA-Wht-8) which also contained *Sr39* whereas in the wheat line SA-Wht-11 the *Sr38* gene was absent. The 4th group consisted of seven exotic wheat lines (SA-Wht-13, SA-Wht-15, SA-Wht-16, SA-Wht-2, SA-Wht-19, SA-Wht-9, and SA-Wht-18) which contained the *Sr* genes (*Sr34, Sr31, Sr38, Sr57, Sr58, Sr24,* and *Sr21*). In this group, the wheat line SA-Wht-13 did not contain the *Sr31* and *Sr21* whereas the wheat line SA-Wht-15 did not contain the *Sr21*. The *Sr* gene i.e., *Sr31* was absent in wheat line SA-Wht-16. In the wheat lines SA-Wht-19, SA-Wht-9, and SA-Wht-18 the *Sr24* was absent whereas in the wheat lines SA-Wht-9, and SA-Wht-18 the *Sr31* gene was also absent.

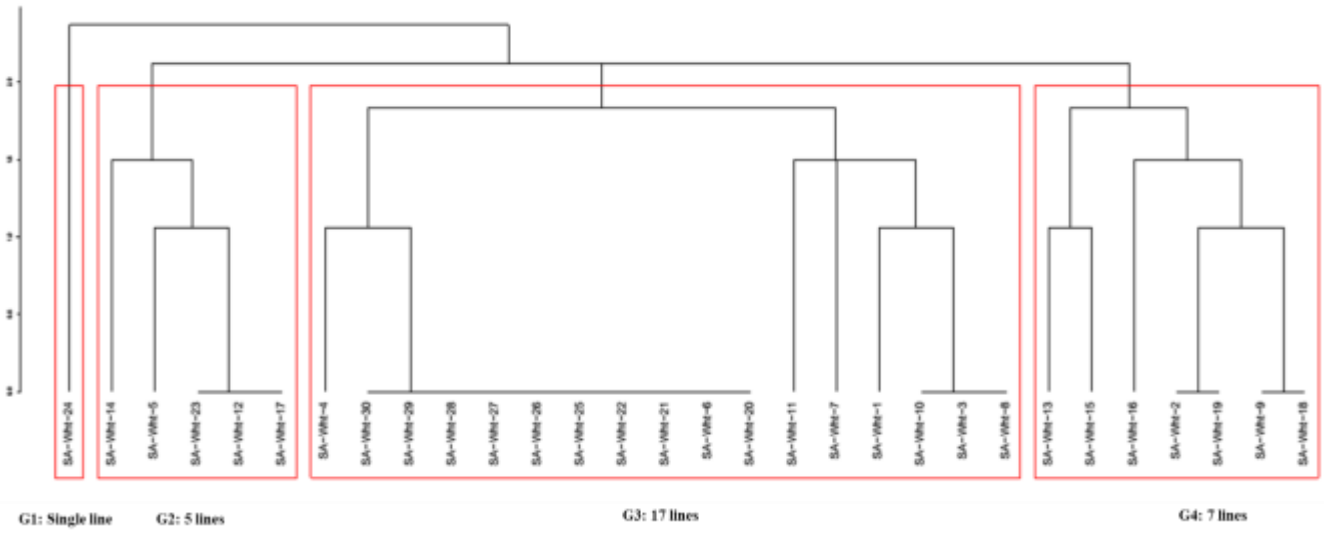

Figure 3. Clustering of 30 exotic wheat lines, based on the presence of stem rust resistance genes linked markers.

DISCUSSION

The current study revealed the response of 30 Exotic wheat lines, against the wheat stem rust disease in Pakistan's Himalayan region from 2018 to 2022 across several locations, scoring at a total of 24 environments (location x year). Additionally, the Exotic lines were screed using molecular markers.

Overall, the stem rust disease was totally absent during the years in the studied environments. Stem rust has been shown to be present only in the southern part of the country with rare reports in the southern Punjab (Ali *et al*., 2022). Stem rust is favored by the relatively warm temperature, which is not present in most of the wheat growing areas of the country during the crop season (Singh *et al*., 2007). The disease requires warm days and mild temperature at night times (Schuman and Leonard, 2000). The reason for disease might be the non-availability of the favorable climatic conditions for the stem rust pathogen in the tested environments/locations.

The molecular markers-based screening showed the presence of the markers linked with eight resistance genes i.e., *Sr34, Sr31, Sr38, Sr57, Sr58, Sr39, Sr24,* and *Sr21*. The stem rust resistance gene *Sr57* linked marker was detected in 30 of the tested Exotic wheat lines followed by *Sr34* linked marker and *Sr58* linked marker was detected in 29 wheat lines. The stem rust resistance gene *Sr38* linked marker was detected in 28 lines whereas in nine of the tested wheat lines the gene *Sr31* linked marker was detected. The eight wheat lines showed the presence of *Sr39* linked with marker, seven lines showed the presence of *Sr21* linked with marker and four lines showed the presence of *Sr24* linked with marker. The germplasm evaluated had an average frequency of resistance between 15 and 25 percent, which is equivalent to or even greater than other parts of the world (Rouse *et al*. 2011).

The molecular marker linked with *Sr57* produced a specific band of 250 bp in 100% of the tested Exotic wheat lines. In 26 genotypes (16.5%), including eight with the *Sr57* gene, adult plants' resistance to stem rust was observed (Shamanin *et al*., 2016). Wheat chromosome 7DS has the *Sr57* gene, which is linked to the csLV34 marker (Lagudah *et al*., 2006). Initially inserted into the wheat cultivar "Frontana," the *Sr57* gene connected to *Lr34/Yr18/Pm38* (Dyck *et al*., 1966). The gene is linked with *Yr18, Lr34* and *Pm38*. Dyck (1977) found the *Sr57* gene for the first time in wheat line PI58548, and chromosome 7D was later found to contain the gene (Dyck 1987). According to further research, *Sr57* is an APR gene and is present in many wheat varieties (Singh and Rajaram 1993). The effectiveness of this gene depends upon the genetic backgrounds and growth conditions (Lagudah *et al*., 2006). In breeding programs around the world, the *Sr57/Lr34/Yr18/Pm38* genes confer long-lasting resistance to rust and powdery mildew (Singh *et al*., 2012).

The molecular marker linked with *Sr34* and *Sr58* produced a specific band of 378 bp and 105 bp respectively in 97% of the tested wheat lines. According to reports, *Sr34* is ineffective against 19 different pathotypes of Indian stem rust in seedling stages (Patil and Deokar 1996). As a result of analysis of the resistance spectra of the varieties Yunmai 47, Yunmai 48, Yunmai 51, Yunmai 53, Yunmai 54, and Yunmai 56, it was concluded that these wheat lines may contain the *Sr24* and/or *Sr34* resistance genes (Li *et al*., 2016). *Sr58*, an adult plant resistance gene, is

independent of the seven QTL on chromosome 1BS and was distantly mapped on 1BL (McIntosh *et al*., 2013). The *Lr46/Yr29* and *Pm39* genes are connected to the *Sr58* gene at the 1BL locus (Kolmer, 2015). This marker exhibits resistance to powdery mildew and rust diseases after being crossed with CIMMYT lines expressing the effective resistance genes *Sr58/Lr46/Yr29/Pm39* and others (Lillemo *et al*., 2008).

The molecular marker linked with *Sr38* produced a specific band of 290 in 93% of the tested wheat lines. In the DUP2015 Nebraska winter wheat, Mourad *et al*. (2019) discovered a group of 17 SNPs that were found on 2A and clinked to *Sr38* and were related with higher resistance to the same race. Ten of the 17 important SNPs that had previously been found were shared by the two studies and associated to the QFCSC stem rust race. The two populations (DUP2015 and DUP 2017) shared many of the same parents while being genetically distinct and resulting from different crossings. The placement of the *Sr38* gene in the 2A chromosome was confirmed as expected by the LD between the SNP markers and *Sr38* gene (Mourad *et al*., 2019).

The molecular marker linked with *Sr31* produced a specific band of 1100 bp and was present in 30% of the tested wheat lines. The stem rust resistance gene *Sr31* is also linked with *Yr9* and *Lr26*. In various regions of the world, including Pakistan, India, and other South Asian nations, the yellow rust resistance gene *Yr9* associated with *Lr26* and *Sr31* has been manipulated (de Vallavieille-Pope *et al*., 2012). With the introduction of CIMMYT-based varieties, the *Yr9/Lr26/Sr31* gene was one of several that increased wheat yield in Pakistan (Singh *et al*., 2004). This transformation from rye has given a long-lasting protection against the stem rust, though the linked Yr9 and Lr31 had become susceptible earlier. The gene became only ineffective when the Ug99 race and its variants were observed in Uganda and spread later to other parts of the world.

The molecular marker linked with *Sr39* produced a specific band of 900 bp and was detected in 27% of the tested wheat lines. The leaf rust resistance gene *Lr35* linked with *Sr39* was initially inserted into chromosome 2B from chromosome 2S of the diploid wild relative *T. speltoides* (Kerber and Dyck, 1990). Specific SCAR markers were applied to identify the leaf rust resistance gene *Lr35* linked with *Sr39* in wheat lines. The negative control and other Iranian wheat genotypes did not exhibit this band; however, this marker amplified a specific band of 900 bp TcLr35. When this gene is infected by a virulent race, it results in a hypersensitive response (Kolmer, 1997). In contrast to Europe, where the effectiveness of the gene is quite low, the Caucasian region and Russia appear to have a more virulent strain of the gene. Following the discovery of virulence against this gene in Iran and its northern neighbors, this gene cannot be employed alone in breeding (Volvoka *et al*., 2020).

The molecular marker linked with *Sr21* produced a specific band of 282 bp and was detected in 23% of the tested wheat lines. The stem rust resistance gene *Sr21* linked with *Lr36* is a seedling resistance gene against leaf rust disease in the development of resistant varieties (Vanzetti *et al*., 2011). The molecular marker J09 linked with *Sr24* produced a specific band of 310 bp and was detected in 13% of the studied wheat lines. The leaf rust resistance gene *Lr24* is

linked to the stem rust resistance gene *Sr24*, which together shows race non-specific resistance against stem rust (Schachermayr *et al*., 1995). The molecular marker used to identify wheat lines with the leaf rust resistance gene *Lr24* can also provide us with additional information about the presence of the *Sr24* gene (Singh *et al*., 2003; Stepien *et al*., 2003).

CONCLUSION

The work resulted that stem rust was absent in most of the Pakistani environments and thus screening would not be easy in these locations for stem rust resistance, thus molecular markers would be more useful. The current study also concluded that molecular marker-based screening revealed potential diversity in the resistance of the Exotic wheat lines against stem rust disease. The stem rust resistance gene *Sr57* was detected in 30 of the tested Exotic wheat lines followed by *Sr34* and *Sr58* which was detected in 29 wheat lines. The stem rust resistance gene *Sr38* was detected in 28 lines whereas in nine of the tested wheat lines the gene *Sr31* was detected. Eight wheat lines showed the presence of *Sr39*, seven lines showed the presence of *Sr21*, and four lines showed the presence of *Sr24*. These lines demonstrated a promising source for additional breeding and wheat improvement for long-term disease management via genetic resistance.

Acknowledgements

We are thankful to the research scientists and field working staff at IBGE and Hazara University Mansehra, particularly Dr. Zakir H. Facho, Dr. Ijlal Hussain, Mr. Minjhaj, Dr. Muhammad Rameez Khan Marwat, Dr. Zainab Iftikhar, Mr. Zia Ur Rehman, Dr. Qasim, Mr. Safi Ullah, Mr. Amir Muhammad Khan, Gohar Zaman, Shahid Ullah Khan, Muhammad Arif, Mr. Shahzad Ahmad, and Mr. Aizaz Arshad. Special thanks to Exotic colleagues who sent their material for testing in Pakistan. The work received financial support from the Exotic Union's Horizon 2020 research and innovation program under grant agreement No 773311 (RustWatch) and the CIMMYT funding through Agriculture Innovation Program (AIP GRANT number PIO grant # AID-BFS-G-11-00002 and ICC W0266.02).

Conflict of interest

The authors declare that there is no conflict of interest.

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