Seedling Stage Resistance of Iranian Bread Wheat Germplasm to Race Ug99 of *Puccinia graminis* f. sp. *tritici*

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Abstract

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Following emergence of Ug99, the new virulent race of *Puccinia graminis* f. sp. *tritici* in Africa, a global effort for identification and utilization of new sources of Ug99-resistant germplasm has been undertaken. In this study, we conducted replicated experiments to evaluate the resistance of Iranian wheat germplasm to the TTKSK lineage of the Ug99 race of *P. graminis* f. sp. *tritici*. We also evaluated for presence of stem rust resistance genes (i.e., *Sr2*, *Sr24*, *Sr26*, *Sr38*, *Sr39*, *Sr31*, and *SrIRS^{Amigo}*) in wheat cultivars and breeding lines widely cultivated in Iran. Our phenotyping data revealed high levels of

susceptibility to Ug99 in Iranian bread wheat germplasm. Our genotyping data revealed that Iranian cultivars do not carry *Sr24*, *Sr26*, or *SrIRS^{Amigo}*. Only a few salt-tolerant cultivars and breeding lines tested positively for *Sr2*, *Sr31*, *Sr38*, or *Sr39* markers. In conclusion, the genetic basis for resistance to Ug99 in Iranian wheat cultivars was found to be vulnerable. Acquiring knowledge about existing resistance genes and haplotypes in wheat cultivars and breeding lines will help breeders, cereal pathologists, and policy makers to select and pyramid effective stem rust resistance genes.

Approximately 630 million tons of wheat (*Triticum aestivum*) is produced in over 200 million ha of cultivation area globally (22). Wheat contributes 21% of the food calories and 20% of the protein consumed by more than 4.5 billion people in 94 developing countries (2). However, wheat productivity is adversely affected by many biotic and abiotic stresses, and rust diseases are of prime importance worldwide.

Severe wheat yield losses due to stem rust, caused by *Puccinia graminis* f. sp. *tritici*, have been reported in Europe, Asia, Australia, and the United States before the middle of the 20th century (20,24), making stem rust the most feared wheat disease (25). Since 1970, yield losses caused by stem rust have been minimal due to the successful deployment of stem rust resistance (*Sr*) genes (e.g., *Sr31*) in commercial wheat cultivars (9). A new virulent race of *P. graminis* f. sp. *tritici* was identified in Uganda wheat nurseries and was observed to be able to infect wheat lines carrying *Sr31*. This new race became popularly known as Ug99 after the country and year of discovery, and was reported to disperse over large distances due to windborne spores. Similarly, Ug99 was observed in 2001 in Kenya, in 2003 in Ethiopia, and in 2007 in Yemen and Iran. Therefore, stem rust is again becoming a threat to wheat production worldwide (18).

More than 50 *Sr* genes have been identified and assigned to specific chromosome locations (17). Some of these resistance genes are deployed in commercial cultivars worldwide and have remained effective individually or in combination with other *Sr* genes until recently. Phenotyping tests after the emergence of the new race revealed that only a few are effective against Ug99 (5,6,26) and some of these are associated with adverse effects on agronomically important traits (16). The genetically characterized *Sr* genes belong to two categories; seedling resistance genes (major genes) and adult plant resistance (APR) genes. The APR genes, also referred to as minor genes, show intermediate responses, and combinations of more than three genes are usually required to at-

tain a commercially acceptable level of resistance. Most characterized genes are race specific but *Sr2* confers durable non-race-specific slow-rusting adult plant resistance (16,27,28).

Genes *Sr24*, *Sr26*, *Sr38*, and *Sr39* are derived from various translocations (1,3,7,8,11). *Sr26* was first transferred to wheat by translocation of the long arm of a group 6 *Agropyron elongatum* chromosome to wheat chromosome 6A (8); the segment was recently shortened through a distal translocation known as 6AL/6Ae#1 (3). Gene *Sr38*, located at a 2NS/2AS translocation (1), was initially sourced from a bread wheat called 'VPM1' (*T. ventricosum*). Although virulence against cultivars possessing this gene has been reported in many countries, *Sr38* is tightly linked with *Yr17* and *Lr37*, and such a gene cluster is believed to provide useful resistance to a wide range of races when combined with other rust resistance genes. Gene *Sr39* originated from *Aegilops speltoides* and was transferred to 'Marquis' wheat (7). This gene is located on the translocated segment of *A. speltoides* chromosome 2S/2B. This translocation also carries the adult plant hypersensitive leaf rust resistance gene, *Lr35*.

To combat the threat posed by Ug99, breeders require knowledge about existing sources of resistance to this race. Such information would enable wheat breeders to carefully design crosses to combine individual resistance sources into one breeding line and enhance germplasm for Ug99 resistance. Yu et al. (30) characterized resistance genotypes of a diverse and widely distributed collection of germplasm originating from the International Maize and Wheat Improvement Center (CIMMYT). *Sr* genes, including *Sr2*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr36*, *Sr40*, and *1A.1R*, were selected for using linked microsatellite or simple-sequence repeat (SSR) and sequence-tagged site (STS) markers. Because it is not always possible to obtain breeding lines resistant to specific races, we believe that such haplotyping can be useful for strategic crossing and selection (30). Here, we report on the resistance of Iranian bread wheat germplasm, including cultivars widely adopted by farmers. We also used established polymerase chain reaction (PCR) markers to diagnose the presence of *Sr* resistance alleles, including *Sr2*, *Sr24*, *Sr26*, *Sr31*, *Sr38*, *Sr39*, and *SrIRS^{Amigo}*, in the most commonly cultivated wheat germplasm in Iran. Developing such information would allow wheat breeders and pathologists to become aware of existing sources of resistance to Ug99 in wheat germplasm and plan for better germplasm enhancement. Because wheat is the main crop in Iran, knowledge of the distribution of

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resistance genes and levels of Ug99 resistance in Iranian wheat germplasm across the country might be of interest to regional scientists and policy makers and within the global wheat industry.

Materials and Methods

Plant materials. The germplasm investigated in this study included bread wheat cultivars grown in Iran (Table 1). These genotypes were either developed at CIMMYT or were products of wheat crosses and general germplasm enhancement practices conducted in Iran. In some cases, cultivars were the product of pure line selection practice from landraces. Most of the landrace accessions were commercially used cultivars in the past. These included ‘Omid’, ‘Roshan’, ‘Khalij’, ‘Tabassi’, ‘Azar’, ‘Shahi’, and ‘Sefid’—some with a history of cultivation going back to the 1940s. Other cultivars included leading and widely adopted genotypes such as ‘Chamran’, ‘Falat’, and ‘Alvand’. We also included elite wheat entries that will soon be used in the major agroclimatic regions, such as ‘Arta’, ‘N-86-3’, ‘C-85-3’, ‘DN-11’, ‘Sivand’, ‘Parsi’, and ‘Oroom’. The last group of genotypes included the salt-tolerant entries, which were either national cultivars (e.g., ‘Arg’ and ‘Bam’) or advanced breeding lines (e.g., MS-84-13, MS-85-17, MS-87-8,

MS-87-9; Table 1). Differential cultivars and lines possessing known *Sr* genes were used as positive controls (Table 1).

DNA extraction. We adopted a cost-effective, rapid, and small-scale nucleic acid isolation method for marker-assisted selection procedures. Fresh leaf tissue of wheat grown for 2 weeks in a greenhouse was flash-frozen in liquid nitrogen and ground into a fine powder. Extraction was performed in 2-ml sterile tubes. A quarter of each 2-ml tube was filled with frozen leaf powder, to which 0.9 ml of preheated (up to 65°C) cetyltrimethylammonium bromide (CTAB) buffer (2% [wt/vol] CTAB, 200 mM Tris/HCl [pH 8.0], 20 mM EDTA [pH 8.0], 1.4 M NaCl, and 1% [vol/vol] freshly added β-mercaptoethanol) was added. Samples were incubated for 45 min at 65°C. Precooled 24 chlorophorm/1 isoamyl-alcohol mixture (known as 24:1) (0.9 ml) was added to the heated tubes. The extraction mixes were then centrifuged at 12,000 rpm for 15 min at 4°C. The upper layer was then aspirated (approximately 700 µl) and transferred into a new tube, where it was supplemented with 0.7 volume (approximately 490 µl) of pre-cooled (–20°C) isopropanol and 0.1 volume (approximately 70 µl) of 3 M ammonium acetate. DNA was allowed to precipitate at –20°C for at least 1 h. The precipitates were pelleted by centrifuga-

Table 1. Stem rust wheat differential lines and wheat cultivars of unknown stem rust resistance (*Sr*) genotype and their seedling infection type (IT) responses to Iranian isolates of *Puccinia graminis* f. sp. *tritici*, CPU01 and CPU02

Germplasm	Growth habit ^a	<i>Sr</i> genes	Year ^b	Origin ^c	Pedigree	CPU01 ^d	CPU02 ^d
Differential cultivars							
Selkirk	S	<i>Sr2</i>	1953	Canada	MCMURACHY/EXCHANGE//3*REDMAN	4	4
Hope/CS	S	<i>Sr9d</i>	NRY	ICARDA	Hope/CS	4	4
SWSr22TB	...	<i>Sr22</i>	...	Australia	SWSr22TB	2	2
Tam200	W	<i>Sr24</i>	1986	United States	TX-71-A-1039-V-1*3/AMIGO	0	;
Sage	W	<i>Sr24</i>	2005	United States	AGENT/4*SCOUT	;	;
Agatha	S	<i>Sr25</i>	1970	Canada	THATCHER*5/AGRUS(M)//THATCHER	;	;1
Eagle	S	<i>Sr26</i>	1969	Australia	THATCHER/AG.EL//3*FALCON	;1	;1
Trident	S	<i>Sr38</i>	2006	Australia	VPM-1/5*COOK//4*SPEAR	3	4
RL5711	S	<i>Sr39</i>	1990	Canada	MARQUIS-K*8/RL-5347	0	;2
RL6087	S	<i>Sr40</i>	1992	Canada	RL-6071*7/(TR.TI)PGR-6126	2	22+
Amigo	W	<i>SrAmigo</i>	2006	United States	OK-66-C-3190/6/GAUCHO(M)/4/TASCOSA/3/WICHITA//WICHITA(M)/TEEWON/5/2*TEEWON	;	;1
Fleming	W	<i>SrAmigo</i>	2002	United States	(S)LV-RUS	;	;
MV-17	W	<i>IB.1R</i>	1993	Hungary	Martonvasar-17	3	4
AC Cadillac	S	<i>SrCad</i>	1996	Canada	BW-90*3/BW-553	0	0
McNair-701	W	Susceptible	1973	United States	(S)MCNAIR-2203	3	4
Germplasm tested							
Tabassi	S	...	1951	Iran	Landrace	4	3
Arvand	S	...	1973	Iran	Rsh//Mt-Ky/My48	4	4
Moghan1	S	...	1973	CIMMYT	LR-N10B/AN3E	3	4
Karaj1	S	...	1973	Iran	200H/Vfn/Rsh	4	4
Moghan2	S	...	1974	India	Choti Lerma	3	4
Chenab	S	...	1975	Pakistan	C-271,IND//WILLET-DWARF/SONORA-64	4	3
Bayat	S	...	1976	Iran	C271/Wte-Son64//CIR	4	4
Vernstein	S	...	1977	Australia	W-2691*2//STEINWEDEL*3/CI-1778[117]; LITTLE-CLUB//GABO*3/CHARTER*2/3/STEINWEDEL*3/CI-7778	4	3
Alborz	S	...	1978	CIMMYT	CM-2182 FRONTANA/MIDA//KENYA-117-A/3/2*COLLAFEN/4/SONORA-64/KLEIN-RENDIDOR/3/CIANO-67(SIB)//LERMA-ROJO-64*2/SONORA-64	3	4
Azadi	S	...	1979	Iran	4820/1-32-15409/Mexp	4	4
Falat	S	...	1981	CIMMYT	Seri 82 (Kvz/Buho "s"//Kal/Bb)	4	4
Golestan	S	...	1986	CIMMYT	Alondra "s"	4	4
Maroon	S	...	1991	Iran	Avd/Pchu	4	4
Niknejad	S	...	1995	ICARDA	F13471/Cow "s"	3	3
Darab2	S	...	1995	CIMMYT	Maya "s"//Nac	4	3
Atrak	S	...	1995	CIMMYT	Kauz "s"	3	4
Chamran	S	...	1997	CIMMYT	Attila, (CM85836-50Y-OM-OY-3M-OY)	4	4
Kavir	S	...	1997	Iran	Stm/3/ Kal//V534/Jit716	4	3

(continued on next page)

^a S, F, and W stand for spring, facultative, and winter type growth habit. “;” means unspecified growth habit.

^b NRY stands for “not released yet”.

^c ICARDA = International Center for Agricultural Research in Dry Areas, and CIMMYT = International Maize and Wheat Improvement Center.

^d IT, where 0, ;, ;1, ;2, 22+, and 2 indicate resistance and 3, 33+, or 4 indicate susceptibility.

tion at more than 11,000 rpm for 10 min at 4°C. The pellets were washed at least once with 300 µl of precooled 70% (vol/vol) ethanol. DNA was finally dissolved in 70 µl of nuclease-free water.

PCR markers and reactions. We employed available PCR methods to assess the presence of *Sr* genes, including *Sr2*, *Sr24*, *Sr26*, *Sr38*, and *Sr39*, as well as the presence of the rye translocation in Iranian wheat germplasm. For *Sr2*, the microsatellite marker *gwm533*, which is closely linked to *Sr2* on 3BS, was used (28).

Two important *Sr* genes (*Sr24* and *Sr26*) are derived from *Agropyron elongatum*. Presence or absence of *Sr24* was examined in wheat germplasm with amplified fragment length polymorphism-derived PCR marker *Sr24#12* (Table 2), following the protocols reported by Mago et al. (11). Distribution of *Sr26* in the germplasm was examined with a codominant marker system by simultaneous PCR reactions (multiplexing) of two dominant PCR markers, as described by Liu et al. (10). In such a system, *Sr26#43* was used as a dominant marker for the presence of *Sr26*, whereas BE518379 was used as a dominant marker for the absence of *Sr26*. Primer sequences for *Sr26#43* and BE518379 are given in Table 2. The presence or absence of the short arm of rye chromosome one

(1RS) in wheat germplasm was determined with the rye-specific SSR marker SCM9 (Table 2) described by Saal and Wricke (23). Presence or absence of *Sr38* in wheat germplasm was examined by using a dominant marker system of VENTRIUP-LN2 reported by Helguera et al. (4).

We used the STS marker *Sr39#22r* developed by Mago et al. (14) to test for the presence or absence of the *Sr39* gene. PCR reaction mixes of 25 µl of final volume contained 1 µl of DNA, 2.5 µl of 10× Taq buffer, 1.5 mM MgCl₂, 1 µl of dNTP mix (2.5 mM each dNTP), 0.4 pmol of each primer, and 1 unit of Taq polymerase. An Eppendorf Mastercycler (Eppendorf AG) was used for all PCR amplifications. PCR products were stained by ethidium bromide and separated by 1, 1.5, or 2% agarose gel electrophoresis. For imaging and photography, a UVItec Gel Document system (UVItec Limited) was used.

Disease phenotyping. Disease phenotyping at seedling stage was conducted in the Cereals Research Department Campus in Karaj, Tehran. Two isolates were used: CPU01 collected from Borujerd in 2011 and CPU02 from Khuzestan in 2009. Standard protocols (21) were used to characterize the race using the 20 North American differential lines. Disease phenotyping for Iranian

Table 1. (continued from previous page)

Germplasm	Growth habit ^a	<i>Sr</i> genes	Year ^b	Origin ^c	Pedigree	CPU01 ^d	CPU02 ^d
Shiroodi	S	...	1997	CIMMYT	Attila, (CM85836-4Y-OM-OY-8M-OY-OPZ)	3	4
Zagross	S	...	1999	CIMMYT	Tan/Vee "s"/Opata 85	4	4
Marvdasht	S	...	1999	Iran	HD2172/Bloudan//Azadi	4	4
Shiraz	S	...	2002	Iran	Gv/D630//Ald "s"/3/ Azd	4	4
Pishtaz	S	...	2002	Iran	Alvand//Aldan/Ias58	4	4
Arta	S	...	2005	CIMMYT	Seri 82 derivative	4	4
Bam	S	...	2006	Iran	Vee"s"/Nac//1-66-22	4	33+
Naishabour	S	...	2006	Iran	1-63-31/3/12300/Tob// Cno/Sx	4	4
Thatcher	S	...	2006	United States	MARQUIS/(TR.DR)IUMILLO//MARQUIS/ KANRED	33+	3
Wangshuibai	S	...	2006	China	LV-Suyang	4	4
Arg	S	...	2009	Iran	1-66-22/Inia	4	4
N-85-5	S	...	2011	CIMMYT	Atrak/Wangshuibai	3	3
Parsi	S	...	2011	Iran	Dov "s"/Buc "s"/Darab	4	3
Sivand	S	...	2011	Iran	Kauz "s"/Azadi	4	4
MS-87-8	S	...	NRV	Iran	1-66-22/3/Alvd//Aldan/Ias	4	4
N-86-3	S	...	NRV	Iran	Nanjing 82149/Kauz/3/PFAU/Seri//Bow (DH)	4	4
DN-11	S	...	NRV	CIMMYT	Attila * 2/PBW65	3	33+
MS-87-9	S	...	NRV	Iran	Desperz80/Rsh//1-66-22/Inia	4	3
MS-84-13	S	...	NRV	Iran	GF-gy54/Attila	3	3
Ws-86-14	S	...	NRV	CIMMYT	Kauz/Pastor	4	3
MS-85-17	S	...	NRV	Iran	Sakha8/Darab#2 //1-66-22	4	4
Red Egyptian/CS	S	...	NRV	ICARDA	Red Egyptian/CS	4	4
Azar	F	...	1956	Iran	Landrace	4	4
Roshan	F	...	1958	Iran	Landrace	4	4
Adl	F	...	1962	Iran	Fr * 3/MM/Mt-Y50	3	4
Inia	F	...	1968	CIMMYT	Lr64/Sn65	3	3
Rashid	F	...	1969	Iran	N.P.788/Azar2-588	4	4
Karaj2	F	...	1973	Iran	Fa/Th-Mt/Omid	4	4
Kaveh	F	...	1980	CIMMYT	Fta-P1	3	4
Ghods	F	...	1989	Iran	Rsh/5/Wt/4/Nor10/K54 *2//Fn/3/Ptr/6/Omid//Kal/Bb	4	3
Navid	F	...	1990	United States	Kirkpinar 79 (63-112/66-2*&C)	4	4
Alvand	F	...	1995	Iran	1-27-6275/CF1770	4	3
Zarin	F	...	1995	CIMMYT	PK15841	4	33+
Tous	F	...	2002	United States	Spn/Mcd/Cama/3/Nzr	4	4
Pishgam	F	...	2008	Iran	Barakat/90 Zhong 87	4	4
Oroom	F	...	2010	Iran	Alvand//NS732/Her	4	3
Omid	W	...	1956	Iran	Landrace	4	4
Shahi	W	...	1967	Iran	Landrace	2	22+
Kavkaz	W	...	1972	Russia	LUTESCENS-314-h-147/BEZOSTAYA-1	4	4
Sabalan	W	...	1981	Iran	908/FnA12/1-32-4382	4	4
Tam-107	W	...	1984	United States	TAM-105*4/AMIGO	3	4
Alamoot	W	...	1995	Iran	Kvz/Ti71/3/Maya "s"/Bb/Inia/4/Karaj 2/5/Anza/3/Pi/Nae//Hys	4	4
Azar2	W	...	1999	Iran	Sardari derivative	4	4
Shahriar	W	...	2002	Iran	Kvz/Ti71/3/Maya "s"/Bb/Inia/4/Karaj 2/5/Anza/3/Pi/Nae//Hys	4	33+
Sefid	W	...	1340s	Iran	Landrace	4	4
Entry 520	...	...	NRV	CIMMYT	Quaiu#2=2nd EBWYT Sr2+SrTmp	4	4

wheat lines and checks was conducted in a completely randomized design with a minimum of two and maximum of four replicates for each isolate. Urediniospores of *P. graminis* f. sp. *tritici* isolates stored in a -80°C freezer were heat-shocked at 40°C for 10 min. After incubation in a rehydration chamber for 2 h at approximately 80% relative humidity, urediniospores were mixed with talc powder in a 1-to-3 ratio. The mixture was used for inoculation of fully expanded primary leaves of 7-day-old (two-leaf stage) seedlings of

wheat. Seedlings were immediately transferred to a dark chamber and incubated overnight (approximately 14 to 16 h) at 18°C . Seedlings were transferred into a greenhouse set at $18 \pm 2^{\circ}\text{C}$ with a photoperiod of 16 h. We assessed infection types (ITs) as described by Stakman et al. (29) 14 days post inoculation.

As for statistical analysis of ITs, we developed a data transformation protocol (Table 3) by converting the nominal IT types gained after Stakman et al. (29) into ordinal and interval data that

Table 2. Oligonucleotide primer sequences for stem rust resistance (*Sr*) genes and their thermal cycling conditions

<i>Sr</i> gene	Chr. ^a	Marker	Primer sequence	Cycling conditions used in this study
<i>Sr2</i>	3BS	GWM533	F: 5'-GTTGCTTTAGGGGAAAAGCC-3' R: 5'-AAGGCGAATCAAACGGAATA-3'	94°C, 4 min → 35 × [94°C, 35 s; 58°C, 40 s; 72°C, 25 s] → 72°C, 5 min
<i>Sr24</i>	3DL	Sr24#12	F: 5'-CACCCGTGACATGCTCGTA-3' R: 5'-AACAGGAAATGAGCAACGATGT-3'	94°C, 4 min → 35 × [94°C, 30 s; 65°C, 30 s; 72°C, 40 s] → 72°C, 7 min
<i>Sr26</i>	6AL	Sr26#43	F: 5'-AATCGTCCACATTGGCTTCT-3' R: 5'-CGCAACAAAATCATGCACTA-3'	94°C, 3 min → 35 × [94°C, 60 s; 60°C, 60 s; 72°C, 120 s] → 72°C, 10 min
<i>Sr31/SrIRS^{Amigo}</i>	1R	SCM9	F: 5'-AGCCGCGAAATCTACTTTGA-3' R: 5'-TTAAACGGACAGAGCACACG-3' F: 5'-TGACAACCCCTTTCCCTCGT-3'	94°C, 4 min → 35 × [94°C, 35 s; 55°C, 35 s; 72°C, 25 s] → 72°C, 5 min
<i>Sr38</i>	2A	VENTRIUP	R: 5'-TCATCGACGCTAAGGAGGACCC-3' F: 5'-AGGGGCTACTGACCAAGGCT-3'	94°C, 2 min → 35 × [94°C, 45 s; 65°C, 30 s; 72°C, 25 s] → 72°C, 7 min
<i>Sr39</i>	2B	LN2 Sr39#22r	R: 5'-TGCAGCTACAGCAGTATGTACACAAAA-3' R: 5'-AGAGAAGATAAGCAGTAAACATG-3' R: 5'-TGCTGTCATGAGAGGAACCTCTG-3'	94°C, 4 min → 35 × [94°C, 30 s; 58°C, 30 s; 72°C, 40 s] → 72°C, 10 min

^a Chromosome.

Table 3. Data transformation scheme used for converting nominal wheat stem rust infection types (ITs) to ordinal data

Response	Resistant				Intermediate		Susceptible			
IT reaction	;	;1	;2	2	22+	2+3	3	33+	3+4	4
Ordinal	1	1	1	2	2.33	2.67	3	3.33	3.67	4

Table 4. Infection types (ITs) for North American standard genotypes and additional set of supplemental cultivars used for race typing two Iranian stem rust isolates, CPU01 and CPU02^a

Set number	Set, line number	Line	<i>Sr</i> gene ^b	IT, CPU01	Pgt code	IT, CPU02	Pgt code
1	1	ISr5-Ra	<i>Sr5</i>	4	T	4	T
	2	Cns_T_mono_deriv	<i>Sr21</i>	4		4	
	3	Vernstein	<i>Sr9e</i>	4		4	
	4	ISr7b-Ra	<i>Sr7b</i>	4		4	
2	5	ISr11-Ra	<i>Sr11</i>	4	T	4	T
	6	ISr6-Ra	<i>Sr6</i>	4		4	
	7	ISr8a-Ra	<i>Sr8a</i>	33+		4	
	8	Acme	<i>Sr9g</i>	4		4	
3	9	W2691SrTt-1	<i>Sr36</i>	0;	K	0;	K
	10	W2691Sr9b	<i>Sr9b</i>	4		4	
	11	Festiguay	<i>Sr30</i>	3+		4	
	12	Renown	<i>Sr17</i>	33+		4	
4	13	ISr9a-Ra	<i>Sr9a</i>	4	S	4	S
	14	ISr9d-Ra	<i>Sr9d</i>	4		33+	
	15	W2691Sr10	<i>Sr10</i>	4		4	
	16	CnsSrTmp	<i>SrTmp</i>	22+		22+	
5	17	LcSr24Ag	<i>Sr24</i>	;2	K	;2	K
	18	Sr31/6*LMPG	<i>Sr31</i>	4		3	
	19	Trident	<i>Sr38</i>	4		4	
	20	McNair 701	<i>SrMcN</i>	4		4	
Supplemental ^c	21	Roughrider	<i>Sr36</i> , <i>Sr6</i>	0;		0;	
	22	Enkoy	<i>Sr36</i>	0;		0;	
	23	Siouxland	<i>Sr31</i> , <i>Sr24</i>	;1		;1	
	24	Sisson	<i>Sr31</i> , <i>Sr36</i>	;1		;	
	25	Morocco	Susceptible	4		4	

^a Race designation for CPU01 and CPU02: IT of 0; , ; , ; 1, ; 2, 22+, or 2 indicates resistance and IT of 3, 33+, or 4 indicates susceptibility.

^b Stem rust resistance (*Sr*) gene.

^c Supplemental test cultivars.

could be handled by parametric and nonparametric tests. We analyzed converted ITs using a generalized linear model (a parametric significance test) and a Kruskal-Wallis one-way analysis of variance test of significance (a nonparametric significance test). The statistical tests were all implemented by using PROC GLM and PROC NPAR1WAY in SAS (SAS Institute Inc.).

Results

Stem rust phenotyping. We determined the reaction of 79 wheat genotypes, including cultivars currently widely grown in Iran, to two locally collected isolates of *P. graminis* f. sp. *tritici*. Both isolates, CPU01 and CPU02, were characterized as belonging to race TTKSK, with virulence to *Sr31* (Table 4). The most common seedling reactions (IT scores) observed on the genotypes are presented in Table 1. There were significant differences in ITs among genotypes ($P \leq 0.0001$; Table 5).

Investigations revealed that all Iranian wheat genotypes except Shahi were susceptible to CPU01 and CPU02 when assessed at seedling stage. Of the differential cultivars possessing known *Sr* resistance genes, 'Selkirk' (*Sr2*), 'Hope/CS' (*Sr9d*), and 'Trident' (*Sr38*) were shown to be susceptible where $IT > 3$ (Table 1). 'SWSr22TB' (*Sr22*) and 'RL6087' (*Sr40*) were moderately resistant ($IT = 3$) whereas 'Tam200' and 'Sage' (*Sr24*), 'Agatha' (*Sr25*), 'Eagle' (*Sr26*), 'RL5711' (*Sr39*), 'AC Cadillac' (*Sr^{Cad}*), and 'Amigo' and 'Fleming' (*Sr^{Amigo}*) were resistant to both isolates tested (Table 1).

Genotyping for *Sr* genes. The microsatellite marker *gwm533* was used to characterize the presence or absence of *Sr2*: a 120-bp PCR fragment was diagnostic for the presence of *Sr2*, whereas a null allele or 155-bp PCR fragment was diagnostic for the absence of *Sr2* (28). Out of 67 Iranian bread wheat genotypes tested using *gwm533*, 22 and 11 genotypes possessed null and 155-bp alleles, respectively, indicating that they do not carry the *Sr2* gene. There were 34 genotypes, along with 'Kavkaz' and Selkirk, that had 120-bp alleles, indicating that they are likely to carry the *Sr2* gene. Genotypes, breeding lines, and cultivars 'Wangshuibai', 'N-85-5' ('Gonbad'), 'Navid', 'Tous', 'WS-86-14', 'Inia', 'Kaveh', 'Moghan1', 'Shiroodi', Chamran, 'Alborz', 'Entry 520', Omid, 'MS-84-13', 'Alamoot', Arta, Alvand, 'Shiraz', 'Pishtaz', Sivand, 'Kavir', 'Azadi', 'Maroon', N-86-3, 'Karaj1', 'Naishabour', and 'Marvdasht' were all positive for *gwm533*, indicating the likely presence of *Sr2* gene in these genotypes.

No genotype except for the positive control Tam200 and Sage was identified as carrying the *Sr24* resistance gene. The molecular marker *Sr26#43* produced a 207-bp product (positive response) only for the control genotype Eagle, and a 303-bp PCR product for marker BE518379 (negative response) for all other genotypes. None of the Iranian germplasm possessed *Sr26*.

The presence of the gene cluster *Yr17-Lr37-Sr38* was assayed by using the PCR method developed by Helguera et al. (4). The genetic marker, VENTRIUP-LN2 (4) produced 262 bp of product in six Iranian cultivars and breeding lines (Alborz, Bam, MS-85-

17, Alamoot, Arta, and Alvand), indicating the presence of the *Yr17-Lr37-Sr38* gene cluster.

Three genotypes (Arg, Bam, and 'MS-85-17'), along with the RL5711 control, produced an 850-bp allele, indicating the presence of the *Sr39* resistance gene. However, only the RL5711 control line produced a typical $IT = 1$ reaction to isolates CPU01 and CPU02. This indicates that there is potential to get a false-positive result using the molecular marker.

The presence of a rye chromosomal segment can be diagnosed by the rye-specific SSR marker *Xscm9* (23). This is a dominant marker and does not differentiate heterozygous from homozygous individuals. This marker amplifies a 208-bp fragment in lines carrying the T1RS•1BL chromosome, which contains the *Sr31* resistance gene. Also, the same marker is used to amplify a 224-bp fragment in lines carrying the T1RS•1AL translocation, which include *Sr1RS^{Amigo}* (19). We used SSR marker SCM9 to confirm the presence of the T1RS•1BL translocation and the *Sr31* resistance gene in seven cultivars (N-85-5, Atrak, Shiroodi, Falat, Alamoot, Arta, and N-86-3). No breeding materials amplified a 224-bp allele, indicating the absence of *Sr1RS^{Amigo}*.

Discussion

The emergence of virulence on *Sr31* in 1998 has increased the vulnerability of wheat to stem rust worldwide after decades of widespread use of this effective gene in global wheat-breeding programs. The occurrence of virulence to *Sr31* in Iran (18) has highlighted the need for germplasm enhancement to develop cultivars resistant to the Ug99 race of *P. graminis* f. sp. *tritici*. We determined cultivar reactions to Ug99 at seedling stage and furthered our knowledge about the presence of known and effective genes for resistance to Ug99 in Iranian germplasm. Inclusion of internationally recognized resistant germplasm in this study resulted in a reasonable discrimination between resistant and susceptible germplasm with Ug99 isolates. This study revealed that genes *Sr26*, *Sr24*, *Sr25*, *Sr39*, *Sr40*, *Sr^{Cad}*, and *Sr^{Amigo}* exhibited resistant seedling phenotypes against Iranian isolates CPU01 and CPU02 of *P. graminis* f. sp. *tritici*, which both typed to race TTKSK. Unfortunately, all except one Iranian genotype were susceptible to both isolates at seedling stage, indicating the magnitude of vulnerability of wheat germplasm grown in Iran to this new race of stem rust. The wheat cultivar identified as resistant to Ug99 at seedling stage was Shahi. This cultivar is a pure-line selected land-race from Kermanshah province, west Iran, released in 1967. Shahi appears to possess an unknown gene that conditions a moderate $IT = 2$ to CPU01 and $IT = 22+$ to CPU02.

Gene *Sr2*, located on the short arm of wheat chromosome 3B, has been effective against all known pathotypes of *P. graminis* f. sp. *tritici*. This APR gene restricts the number and size of uredinia and is linked to the pseudoblack chaff trait (13). *Sr2* originated from tetraploid emmer wheat and was introgressed into common wheat in 1930 (15). Although the test with molecular marker *gwm533* revealed that a number of wheat cultivars likely possess *Sr2*, a more precise molecular diagnostic test such as that developed by Mago et al. (12) seems to be necessary to further investigate the presence of *Sr2*.

Our results indicated that cultivars widely adopted in Iran, including Chamran, 'Azar2', Alvand, Roshan, Pishtaz, 'Zarin', 'Zagros', 'Sabalan', Falat, and Omid, do not carry any effective *Sr* genes and would be vulnerable to Ug99 and its derivatives in case of epidemic. It is strongly advisable to actively deploy effective genes in wheat germplasm in Iran. This will require extensive crossing of adapted germplasm with international cultivars and breeding materials that possess the effective resistance genes. Once crossed, procedures such as marker-assisted selection or marker-assisted backcross selection would be the methods of choice.

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Table 5. Tests of significance of differences among genotypes in response to Iranian stem rust isolates CPU01 and CPU02, performed on converted data by means of Kruskal-Wallis and generalized linear model procedures in SAS

Significance test ^a	CPU01	CPU02
Kruskal-Wallis Test		
χ^2 statistic	198.61	200.45
Degrees of freedom	78	78
<i>P</i> value	<0.0001	0.0001
The GLM Procedure		
<i>F</i> value (genotype effect)	18.24	17.86
Degrees of freedom	[78,247]	[78,255]
<i>P</i> value	<0.0001	<0.0001
<i>R</i> ²	0.85	0.84
CV	12%	11.5%

^a Tests included three to seven replications. *R*² = coefficient of determination and CV = coefficient of variation.

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