

RESEARCH PAPER

Physiological races of *Puccinia graminis* f. sp. *tritici* in Iran and evaluation of seedling resistance to stem rust in Iranian wheat cultivars

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Summary. Seedling assessment of 13 stem rust samples collected from southwest of Iran revealed that three collections were virulent for *Sr31* and *Sr38*. Physiological races of RTRTC, HRCTC, RRTTF, TPTTC, TTTQF, JTHTC and TTKSK were identified for nine collections tested against the north American differential lines. The virulence pattern of TTKSK previously identified in east Africa was identical to that of TTKSK in the present study, providing evidence for the migration of this race from East Africa to Iran. Low/ high infection type for the genes *Sr22*, *Sr26*, *Sr27*, *Sr29*, *Sr32*, *Sr33*, *Sr35*, *SrNin*/ *Sr7a*, *Sr8b*, *Sr9f*, *Sr12*, *Sr15*, *Sr16*, *Sr17*, *Sr18*, *Sr20*, *Sr23*, *Sr34*, *SrPL*, *SrTt3+10*, *SrWld-1*, and *Sr42* was shown by nine collections. In evaluations of seedling resistance, high infection types of 3 to 4 were shown by all the 29 Iranian wheat cultivars tested with TTKSK. Presence of TTKSK, susceptibility of wheat cultivars and conducive conditions pose serious threats of stem rust to wheat production in Iran.

Key words: *Puccinia graminis*, physiological races, seedling resistance, Iranian wheat cultivars.

Introduction

Wheat (*Triticum aestivum* L. and *T. durum* L.) is the most important crop in Iran. It is grown on approximately seven million ha with an annual production of 14 million t. The three wheat rust diseases, stripe rust, stem rust, and leaf rust, are the most important constraints to wheat production in this country. Stem (black) rust of wheat, caused by the biotrophic fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*), was once the most devastating disease of wheat worldwide (Pretorius *et al.*, 2000; Singh *et al.*, 2006). Severe crop losses occur, particularly when susceptible cultivars are grown in warm and humid areas (Roelfs *et al.*, 1992). The use of resistant cultivars and eradication of the alternate host have brought stem rust epidemics under control in almost all wheat-growing areas of the world for more than four decades. In most of

the world, resistance to stem rust is based on a few important resistance genes, including *Sr31* (Pretorius *et al.*, 2000, Singh *et al.*, 2006), which was effective against *Pgt* populations worldwide. In 1998, a severe wheat stem rust infection was observed on wheat genotypes containing *Sr31* in Uganda – this *Sr31*-virulent race of *Pgt* is commonly known as Ug99 (Pretorius *et al.*, 2000). Subsequently, Ug99 was designated as race TTKSK using the North American stem rust differential set (Jin *et al.*, 2007). Race TTKSK was found in Kenya in 2004 (Jin and Singh, 2006; Wanyera *et al.*, 2006), in Ethiopia in 2005 (Singh *et al.*, 2006), in Yemen and Sudan in 2006 (Singh *et al.*, 2008), and virulence for *Sr31* was detected in Iran in 2007 (Nazari *et al.*, 2009). This spread of Ug99 from East Africa to the Middle East is in parallel to the suspected migration pathway of the *Yr9*-virulent race of the stripe rust pathogen *P. striiformis* f. sp. *tritici* (*Pst*) during the 1980s (Singh *et al.*, 2006). This race/race group caused severe crop losses in all countries in its pathway (Singh *et al.*, 2004) including 2.5 million t of grain loss of wheat during 1992–1994 in Iran (Torabi

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et al., 1995). It was predicted that Ug99 would follow the migration pathway from East Africa throughout Asia according to the historical step-wise movement of the Yr9-virulence race/race group of *Pst* in the 1980s (Saari and Prescott, 1985; Stubbs, 1988; Singh *et al.*, 2006). The wheat-growing areas in Iran are primary risk areas for Ug99 and may serve as a pathogen bridge between East Africa and South Asia.

Since the first documentation of wheat stem rust in Iran in 1939 (Esfandiari, 1946), several devastating epidemics have occurred in major wheat production areas, particularly in the southern and northern humid warm zones (Esfandiari, 1946; Bamdadian, 1971; Scharif *et al.*, 1971; Khazra and Bamdadian, 1974; Bamdadian and Torabi, 1978). Before the introduction of semi-dwarf wheat genotypes from the International Maize and Wheat Improvement Centre (CIMMYT) to Iran in the 1970s, almost all local varieties were susceptible to stem rust. In the years since, the disease has been under control, with occurrence reduced to occasional infections on local varieties. Similar to those in other parts of the world, most current Iranian cultivars originated from CIMMYT and possess the 1B.1R translocation carrying *Sr31* (McIntosh *et al.*, 1995; Pretorius *et al.*, 2000; Singh *et al.*, 2006). The frequency of the 1B.1R translocation in CIMMYT germplasm was once greater than 70% (Singh *et al.*, 2006).

Stem rust has not been a major problem in wheat production in Iran in recent decades and therefore pathogenicity of the *Pgt* population has not been studied; there is thus a paucity of information on the likely evolution of the *Pgt* population in Iran during the last four decades. Little is known about the pathogenic variability of *Pgt* in Iran. Using the international stem rust race analysis system (Stakman *et al.*, 1962), ten physiological races of *Pgt* were reported by Scharif and coworkers in 1970 (Scharif *et al.*, 1971). Using the international stem rust race analysis system (Stakman *et al.*, 1962), ten physiological races of *Pgt* (21, 34, 40, 75, 100, 194, 213, 226, 320, and 321) were reported in Iran in 1970 (Scharif *et al.*, 1971). Nasrollahi *et al.*, (2001) reported very unusual virulence combinations for *Sr24*, *Sr25*, *Sr26*, *Sr28*, *Sr29*, *Sr31*, *Sr32*, *Sr36*, and *Sr38* from 20 *Pgt* collections obtained during 1996-99.

Field evaluations of Iranian wheat cultivars and advanced breeding lines to Ug99 in Kenya in 2006 and 2007 showed that more than 95% of the common wheat varieties and advanced breeding lines were

susceptible to stem rust race TTKSK (Ug99), indicating the potential threat of this race to wheat production in Iran. The objectives of present study were to (1) obtain detailed characterization of the physiological specialization of an unusual *Pgt* population in wheat growing areas in southwest of Iran in 2007 (Nazari *et al.*, 2009) and (2) assess the vulnerability of Iranian wheat varieties and advanced breeding lines to Iranian races of *Pgt*.

Materials and methods

Sample collection, isolation, and spore multiplication

A field survey was conducted during late June 2007 in wheat growing areas in southern Iran. The survey was conducted over 800 km in the upper Karkheh basin, including Poldokhtar, Khorram Abad, Boroujerd, Malayer and Hamadan. Eleven samples were collected from agricultural research stations and commercial fields (Table 1). Samples were air-dried at room temperature for at least 24 h and then stored at 4°C. The susceptible wheat cultivar Morocco was planted as eight seeds per 9 cm diam. pot filled with potting mix. Seedlings were grown in a greenhouse at 20°C with 16 h supplementary light using high-pressure sodium lighting. To retard plant growth and encourage spore production, seedlings were treated with 40 mL of 0.25 gL⁻¹ maleic hydrazide acid (C₄H₄N₂O₂; Sigma-Aldrich®, Taufkrichen, Germany) per pot at 1 cm coleoptile length emergence. When the first leaves of susceptible plants were fully expanded, infected stems were washed under tap water and then placed in a dew chamber at room temperature for 4 h. Fresh urediniospores were scraped from re-sporulated single pustules using a sterile spatula and transferred to 7- to 9- day- old leaves of cv. Morocco plants. Inoculated seedlings were incubated in a sealed dew chamber for 24 h at 20° C and 90% relative humidity (R.H.). Plants were then placed in greenhouse bench at 20 ± 2° C and 16 h light. At 14 days post inoculation, urediniospores were collected and dried in a container with silica gel at 4°C for 48 h and then stored in an ultra-low freezer at -80°C.

Confirmation of virulence for *Sr31*

Since some of the samples were collected from genotypes with Yr9 (also carrying *Sr31*) in Borou-

Table 1. Isolate code, site and year of collection, name of host cultivar for 15 *Puccinia graminis* f. sp. *tritici* collections.

Pgt-Code	Location	Province	Year	Host	Cultivar
SR10	Boroujerd	Lorestan	1997	Bread wheat	unknown
SRI2	Karaj	Alborz	1997	Bread wheat	unknown
86-11	Karaj	Alborz	2007	Bread wheat	Bolani
86-20	Poldokhtar	Lorestan	2007	Bread wheat	unknown
86-28	Kelardasht	Mazandaran	2007	Bread wheat	unknown
86-29	Kelardasht	Mazandaran	2006	Barley	Fasih
86-31	Boroujerd	Lorestan	2007	Bread wheat	Aristocrat
86-33	Boroujerd	Lorestan	2007	Barley	unknown
86-35	Boroujerd	Lorestan	2007	Bread wheat	Banks
86-42	Hamadan	Lorestan	2007	Bread wheat	TP 67
86-43	Hamadan	Hamadan	2007	Bread wheat	TP 55
86-47	Hamadan	Hamadan	2007	Bread wheat	Clement
86-48	Hamadan	Lorestan	2007	Bread wheat	TP 10
86-55	Atashgah	Hamadan	2007	Bread wheat	unknown
86-58	Boroujerd	Lorestan	2007	Bread wheat	unknown

Table 2. Seedling infection types of wheat lines in the ‘Quickset’ used to assay the effectiveness of *Sr31* to 13 *Puccinia graminis* f. sp. *tritici* collections.

No.	Name/ pedigree	Sr gene/s	Pgt SR10	Pgt SRI2	Pgt 86-11	Pgt 86-20	Pgt 86-28	Pgt 86-29	Pgt 86-31	Pgt 86-33	Pgt 86-35	Pgt 86-42	Pgt 86-48	Pgt 86-55	Pgt 86-58
1	Line E/ Kavkaz	<i>Sr31</i>	;C1= ^a	;1=	;C1=	;C1-	;C1-	;C1=	3+4	1C	;C1=	1-C	1	4	3+
2	Clement	<i>Sr31</i>	12-	;C	1-	2=	;C1=	1-C	4	;C	;C1=	;C1=	;C1=	4	33+
3	Federation*4/ Kavkaz	<i>Sr31</i>	;C1=	0;	12=	12=	;C1-	;C1-	4	;1=	1CN	;	1C	4	33+
4	Kavkaz	<i>Sr31</i>	;C1=	;	;C1=	12=	12=	1C	4	;	;C1=	2-	2	4	33+
5	Mildress	<i>Sr31</i>	;C1=	;1=	1C	12-	12=	0;1-	3+	2	12=	12=	12-	4	3+
6	Bt <i>Sr24</i>	<i>Sr24</i>	1C	;C1=	1-2-	1-2=	2	1-	12=	;C1=	2-	1	11-	;1=	1-C
7	Agent	<i>Sr24</i>	;C1=	1-C	12- CN	;C1=	;C1=	;C1=	;1=	;C1=	;C1=	2	1C	;1=	;1=

^a Infection types were recorded according to ; (fleck), and 0- to - 4. Symbols “-” and “+” were used to describe deviation from the pustule size of major infection type classes. C and N were used to describe extensive chlorosis and necrosis, respectively, associated with infection types and X was used for mesothetic infection types on same genotype.

jerd and Hamadan, and showed highly compatible infection responses, a small set, “Quickset”, of differential lines was used to quickly assay for viru-

lence to *Sr31* (Table 2). These lines included Line E/ Kavkaz (*Sr31*), Clement (*Sr31*), Federation *4/ Kavkaz (*Sr31*), Kavkaz (*Sr31*), Mildress (*Sr31*), Mar-

ton Vászár 17 (*Sr31*), BtSr24 (*Sr24*), Trident (*Sr38*), and Iranian commercial cultivars known to carry 1B.1R translocations: Falat (Seri 82; *Sr31*, *Sr2*) and Shiroudi (Attila 4Y; *Sr31*). Morocco, Federation, and Local Red were used as susceptible checks in this test (Table 2). Two *Pgt* collections obtained in 1997 were recovered from -80° C storage from the Cereal Pathology Unit at the Seed and Plant Improvement Institute (SPII), Karaj, and were included for further comparisons. According to Nasrollahi *et al.*, (2001), *Pgt* SR10 was avirulent on *Sr31*, and *Pgt* SR12 was avirulent on this gene.

Urediniospores from the long-term storage at -80°C were heat shocked at 42°C for 4 min and then suspended in distilled water (DW) with 1 mL Tween 20®. Seedling leaves of the "QuickSet" genotypes were inoculated with the urediniospore suspensions using a small inoculator at 20 kPa pressure. Inoculated seedlings were incubated in dew chamber at 18°C in the dark for 20 h followed by 4 h at 20°C and fluorescent light. Plants were then placed in a greenhouse at 18-20°C with 16 h of supplementary light. Seedling infection types (ITs) were recorded 14 d after inoculation using the 0 – 4 scale described by Stakman *et al.*, (1962) (McIntosh *et al.*, 1995). ITs of 0–2 were considered low (i.e. LITs), indicating host resistance and pathogen avirulence; ITs of 3–4 were considered high (i.e. HITs).

Race analyses and virulence phenotyping

The physiological races of seven *Pgt* collections (Table 1) obtained from Iran in 2007 and the two *Pgt* collections obtained in 1997 were determined using the North American stem rust differential set (Jin *et al.*, 2008, Table 3). The nine races were also used in seedling assessments of additional sources of gene combinations (Table 4). In addition to the stem rust resistance genes assayed in the North American race analysis system, the same nine *Pgt*-collections were used in seedling assessments of an expanded set of stem rust monogenic lines and selected sources of *Sr*-genes from the Australian stem rust differential set (McIntosh *et al.*, 1995; Park and Wellings, 1992; Zwer *et al.*, 1992; Table 5). Expected LITs for individual genes are presented in the first column of Table 5. ITs equal to or lower than the expected LITs for each *Sr* gene were considered as LITs, and ITs higher than the expected LITs or equal to the susceptible checks were considered as HITs.

Assessment of seedling resistance of Iranian varieties to stem rust

Twenty-nine Iranian wheat varieties were screened with four stem rust collections including two *Sr31*-virulent *Pgt* races (Table 6). The cultivar Morocco was used as the susceptible control. Resistance assessments were carried out under the same conditions described for race analyses. To determine relationships among cultivars based upon seedling reactions to the four collections, unweighted pair-group average linkage Agglomerative Hierarchical Clustering (AHC) was used for the Jaccard's dissimilarity coefficient of converted low and high seedling ITs to binary data using XLSTAT software ver. 2011.2.08 (XLSTAT, Addinsoft. <http://www.xlstat.com>).

Results and discussion

Stem rust occurrence

During the field survey in mid-June 2007, a severe stem rust epidemic was observed in experimental plots in Boroujerd and Hamadan Research Stations and commercial fields, particularly on the Silakhor plateau in southern Boroujerd. Late stem rust infections in Lorestan and Hamadan Provinces in Iran (upper Karkheh basin) were considered unusual, particularly because stem rust had not been previously recorded in the Hamadan area where winter wheat cultivars are grown. Additional surveys in other wheat-growing areas were conducted and occasional stem rust infections were recorded. There were high levels of infection on wheat genotypes known to carry the 1B.1R chromosome translocation that were included in the Iranian Yellow Rust Trap Nursery planted in experimental plots in Boroujerd and Hamadan Research Stations. A range of susceptible ITs with 30–80% severity were recorded in some commercial fields in Boroujerd.

Confirmation of virulence for *Sr31*

When the 'QuickSet' was inoculated with the 13 *Pgt* collections, *Pgt* 86-31 collected from winter wheat cultivar Aristocrat, *Pgt* 86-55 and *Pgt* 86-58 collected from bread wheat varieties planted within the yellow rust trap nurseries in Boroujerd and Hamadan research stations, respectively, showed HITs of 3+ to 4 on differential genotypes carrying *Sr31* (Line E/Kavkaz, Clement, Federation*4/Kavkaz, Kavkaz and Mildress). This indicated presence of virulence

Table 3. Seedling infection types produced by nine *Puccinia graminis* f. sp. *tritici* collections on the North American set of differential lines and race designation according to letter code race nomenclature system.

Set	Line	Sr gene	Expected low IT	Pgt SR10	Pgt SRI2	Pgt 86-11	Pgt 86-20	Pgt 86-28	Pgt 86-29	Pgt 86-33	Pgt 86-31	Pgt 86-55
I	ISr5-Ra	Sr5	0, 0; ^a	33+	0;	33+	33+	3+	33+	0;	3+	3+
	CnS_T. <i>monococcum</i> deriv	Sr21	1, 2-	33+	33+	2++	2++3	3	33+	3+	3+	33+
II	Vernstine	Sr9e	;1+	;C1-	1-	1C-2-C	4	2+	1C-2-C	4	3+4	4
	ISr7b-Ra	Sr7b	2	3+	3+4	3+4	4	3+	3+	2-	4	4
	ISr11-Ra	Sr11	;2-, 2+3-	4	3+	3+4	4	3/3+	3+	3+4	4	4
	ISr6-Ra	Sr6	0;	33+	3+4	33+	4	4	3+	4	4	4
	ISr8a-Ra	Sr8a	2	33+	11C	11C	4	3+4	1C	2+/3+	3+	4
	CnSr9g	Sr9g	2-	4	4	4	4	3+	4	4	4	4
III	W2691SrTt-1	Sr36	0, 0; X	33+	0;	4	3+	33+	3+	0;1=	0;1	0;
	W2691Sr9b	Sr9b	22+	33+	;C1-	4	1+	33+	3+4	4	3+4	4
	BtSr30Wst	Sr30	2	2	1C	3+	33+	33+	2++3	12-	33+	4
	Combination VII	Sr13Sr17	0; 1	33+	22+	22+	4	22+	22+	22+	3	3
IV	ISr9a-Ra	Sr9a	2-, 23	33+	4	4	4	3+4	33+	4	4	4
	ISr9d-Ra	Sr9d	;2-	4	3+4	4	4	4	4	4	3+4	4
V	W2691Sr10	Sr10	;1+	33+	33+	4	4	11+	33+	2++	4	4
	CnsSrTmp	SrTmp	2-	22+	22+3	2+3	2+3	12=	2+3	23	2-	2-
	LcSr24Ag	Sr24	2	1C	;C1=	1+2-	1+2-C	2	1+	;C1=	12-C	;C1=
	Sr31/6*LMPG	Sr31	;1+	;C1=	;1=	;C1=	;C1-	;C1-	;C1=	1C	3+4	4
	Trident	Sr38	1	1C2=	1CN	2+	;CN1=	22+	22+	1-C	33+	2++/33+
McNair 701	SrMcN	;1	4	4	4	4	33+	4	4	4	4	
Race		RTRC	HRCIC	RRTIF	TTPIC	TTTQF	RRTIF	JTHIC	TTKSK	TTKSK	TTKSK	TTKSK

^a Infection types were recorded according to ; (fleck), and 0- to -4. Symbols “-” and “+” were used to describe deviation from the pustule size of major infection types classes. C and N were used to describe extensive chlorosis and necrosis, respectively, associated with infection type and X was used for mesothetic infection types on same genotype. Recorded range of infection types were separated by comma (,).

Table 4. Seedling infection type of nine *Puccinia graminis* f. sp. *tritici* collections on additional sources of stem rust resistance genes.

No.	Genotype	Sr- gene	<i>Pgt</i> SR10	<i>Pgt</i> SR12	<i>Pgt</i> 86-11	<i>Pgt</i> 86-20	<i>Pgt</i> 86-28	<i>Pgt</i> 86-29	<i>Pgt</i> 86-33	<i>Pgt</i> 86-31	<i>Pgt</i> 86-55
1	Sage	<i>Sr24</i>	12= ^a	;N1=	1=	;N1=	;C1=	;1=	1=	12-	12-
2	Custer	<i>Sr31</i>	2-	2=	2-	1=	1-CN	1=	1-	3-4	3-4
3	Siouxland	<i>Sr24,Sr31</i>	;C1=	;N1=	;N1=	;N1=	;N1=	;N1=	;N1=	12-	12-C
4	TAM 107	<i>Sr1RS-Am</i>	1C2-	12=	1=	12-	12-	12-	2=	2-	12-
5	Amigo	<i>Sr24, Sr1RS-Am</i>	12-	12-	12-	;C1=	12=	;N1=	;N1=	1=	11-
6	Fleming	<i>Sr6,Sr24,Sr36,Sr1RS-Am</i>	;C1=	;N1=	;N1=	0;	12=	;N1=	1=	;C1=	;C1=
7	Sisson	<i>Sr6,Sr31,Sr36</i>	;	1=	;N1=	0;	12=	12=	1=N	1-C	;;
8	McNair 701	<i>SrMcN</i>	4	4	4	4	4	3+	4	4	4
9	Red Chief	-	4	4	4	4	4	4	4	4	4

^a Infection types were recorded according to ; (fleck), and 0- to -4. Symbols “-” and “+” were used to describe deviation from the pustule size of major infection types classes. C and N were used to describe extensive chlorosis and necrosis, respectively, associated with infection type and X was used for mesothetic infection types on same genotype. Recorded range of infection types were separated by comma (,).

to *Sr31* in these collections (Table 2). HITs of 3+ to 4 on the wheat commercial varieties Falat (#Seri 82), Shiroudi (CIMMYT name Attila 4Y and Indian name PBW343) and MV17, all carrying the 1BL.1RS translocation, further confirmed virulence for *Sr31* in *Pgt* 86-31, *Pgt* 86-55 and *Pgt* 86-58.

The other 11 *Pgt* collections showed LITs of ;C1= to 22- on *Sr31* differential lines and varieties with 1BL.1RS, indicating avirulence for *Sr31*. LITs of ; to 2 on *Sr24* were produced on differential line Bt*Sr24* and Agent when tested against the 13 collections, indicating that all *Pgt* collections were avirulent for *Sr24*. Trident was used as a source of *Sr38*. Except for the three *Sr31* virulent *Pgt* collections (*Pgt* 86-31, *Pgt* 86-55 and *Pgt* 86-58) which were also virulent to *Sr38*, the other 10 collections showed LITs of 1CN to X on Trident, indicating avirulence for *Sr38*. The three susceptible varieties Federation, Morocco and Local Red were susceptible to all collections.

Determination of physiological races and virulence spectra

According to the revised North American race nomenclature system (Jin *et al.*, 2008), the two collections from Boroujerd and Karaj obtained in 1997 were assigned thus; *Pgt* SR10 as RTRTC and *Pgt* SR12 as HRCTC.

Collection *Pgt* 86-11 was obtained from a local susceptible variety Bolani used as a rust spreader in a disease screening nursery planted at an experimental field at SPII. This experiment was planted for leaf rust resistance screening and was intended to be inoculated with a leaf rust collection. Unexpected severe stem rust infections occurred on those nurseries that were inoculated with leaf rust. Since *Pgt* 86-29 was collected from the spreader rows inoculated with leaf rust, and stem rust had not been observed at this station for a minimum of 15 years (K. Nazari, personal observations), we suggest that *Pgt* 86-11 is a result of greenhouse contamination of the leaf rust collection used in field inoculation with *Pgt* 86-29. High stem rust contamination of the same leaf rust collection preserved in a cereal pathology laboratory was confirmed when tested on the North American differential set. In addition, the virulence spectrum of *Pgt* 86-29 was identical to the *Pgt* 86-11 collection obtained from barley cultivar Fasih in Kelardasht in 2006. The second sample collected from bread wheat in Kelardasht (*Pgt* 86-28) was designated as race TTTQF.

Collection *Pgt* 86-20 was designated as race TT-PTC and was originally collected from a bread wheat variety in southwest Poldokhtar. Collections originally obtained from Boroujerd in 2007, *Pgt* 86-31 and *Pgt* 86-33, were designated as races TTKSK and

Table 5. Expected low infection types (LITs) of stem rust resistance gene and reaction of stem rust differential lines when inoculated with nine *Puccinia graminis* f. sp. *tritici* collections

Entry No.	Name/pedigree	<i>Sr gene/s</i>	Expected LIT	<i>Pgt</i> SR10	<i>Pgt</i> SRI2	<i>Pgt</i> 86-11	<i>Pgt</i> 86-20	<i>Pgt</i> 86-28	<i>Pgt</i> 86-29	<i>Pgt</i> 86-33	<i>Pgt</i> 86-31	<i>Pgt</i> 86-55
Effective <i>Sr</i>-genes												
1	SWSR22T.B.	<i>Sr22</i>	1+ to 2- ^a	2-	;N1=	1-	11+C	1-C	1	;1=	0;	;1=
2	Kite	<i>Sr26</i>	1	1	1	1	1	12-C	0;	2	;1=	1
3	Eagle	<i>Sr26+Sr9g</i>	0; to 2-	11+2	;N1=	1-	1-	;1-	1	;1=	1	;1-
4	Coorong	<i>Sr27</i>	0;	;	0;	;1=	1=	12-	;1=	;	1=	;
5	Pusa/Edch	<i>Sr29</i>	2-	1	;1=	1+2	2	2-	1+2	1	11+2-C	2
6	C77.19	<i>Sr32</i>	1+ to 2C	11+2	;1=	1	11+	11-	11+2-	1-N	2-	1-
7	Tetra Canthatch/ <i>Ag. squarrosa</i>	<i>Sr33</i>	2	2	1	12-CN	1	;1=	1C	;1=N	12	11-C
8	W3763	<i>Sr35</i> (<i>SrTm1</i>)	0; to ;	0;	0;	0;	0;	0;	0;	0;	0;	0
9	Ningadhu	<i>SrNin</i>	;1=	0;	;C1=	;;	;C1=	;C1=	0;	;1=	0	;1=
Ineffective <i>Sr</i>-genes												
1	LINE G	<i>Sr7a</i>	1 to 3C	3+	33+	33+/3+	4	3+	3+	3+	3+4	4
2	Barleta Benvenuto	<i>Sr8b</i>	X ⁼	4	3+4	33+	4	3+	3+	33+	3+4	4
3	ISR5SB	<i>Sr9f</i>	2	3+4	4	4	4	3+4	4	4	4	4
4	CH.SP.(TC3B)	<i>Sr12</i>	; to X	4	33+/3+	33+	4	3+	3+	2++	4	4
5	W2691SR15NK	<i>Sr15</i>	; to X	4	33+	33+	4	3	3+	3+4	33+	4
6	Norka	<i>Sr15</i>		4	33+	4	4	3+	3+	4	4	4
7	ISR16RA	<i>Sr16</i>	2	33+	2++3	2+3+	4	3/3+4	33+	2++3/3	3+4	33+
8	LC/Kenya Hunter	<i>Sr17</i>	;1	33+	22+	22+	4	22+	2+	22+	3+4	4

^a Infection types were recorded according to ; (fleck), and 0- to - 4. Symbols “-” and “+” were used to describe deviation from the pustule size of major infection types classes. C and N were used to describe extensive chlorosis and necrosis, respectively, associated with infection type and X was used for mesothetic infection types on same genotype. Recorded range of infection types were separated by comma (,).

JTHTC, respectively. Race TTKSK was also designated for *Pgt* 86-55 collected from Hamadan.

The nine collections were also used in seedling assessments of an expanded set of differentials including *Sr24*, *Sr31*, *Sr1RS-Am* and gene combinations of *Sr24-Sr1RS-Am*, *Sr24-Sr31*, *Sr6-Sr24-Sr36-Sr1RS-Am* and *Sr6-Sr31-Sr36* present in cultivars Sage, Cruse, Tam107, Amigo, Siouxland, Fleming and Sission, respectively (Table 4). McNair 701 and Red Chief were used as susceptible checks in this test. Custer (*Sr31*) was only susceptible to the two *Sr31*-virulent races.

Seedling ITs of ;N1= to 2- for Sage, Tam107, Amigo, Siouxland and Fleming indicated that all nine races were avirulent on *Sr24* and *Sr1RS-Am* when presented singly in Sage and Tam107, respectively, or in gene combination in Amigo (*Sr24-Sr1RS-Am*), and also in combinations with other *Sr*-genes in Siouxland (*Sr24-Sr31*) and Fleming (*Sr6-Sr24-Sr31-Sr1RS-Am*). The *Sr6-Sr31-Sr36* gene combination in Sission was effective against the nine races. Effectiveness of Sission against races RTRTC, RRTTF, TTPTC and TTTQF was due to avirulence to *Sr31*, whereas ef-

Table 6. Name, pedigree, year of release, country of origin and growth habit of 29 Iranian wheat cultivars evaluated for seedling resistance to four *Puccinia graminis f. sp. tritici* collections at seedling stage.

Name	Pedigree Cross number and selection history	Year of release	Growth habit	Pgt SR10	Pgt 86-20	Pgt 86-31	Pgt 86-55
Moghan 1	LR/N10B//3*ANE II8739-4R-1M-1R-0IRN	1974	Spring	1C ^a	;C1 ⁻	3	4
Moghan 2	LR64A/HUAR 'II15929-1M-4R-2M-0IND-0IRN	1974	Spring	1 ⁻	;C1 ⁻	4	4
Sabalan	908//FN/A12/3/1-32-4382 -0IRN	1981	Facultative	1C	;	3	4
MV17	[Slaviya × (Krasnodari 1 × Bezostaya)] × Zg.4431 Marton Vasar, Hungary	1987	Winter	2-	;1 ⁻	3	3
Ghods	RSH/5/WT/4/N10/K 54*2//FN/3/PTR/6/OMI//KAL/ BB -0IRN	1988	Spring	;N1 ⁻	1C	4	4
Falat	KVZ/BUHO//KAL/BB (SERI 82) = VEERY 5 CM33027-F-15M-500Y-0M-87B-0Y-0IRN	1990	Spring	X ⁻	22 ⁻	4	4
Hirmand	BYT/5/JAR/3/CFN//CNO/SR/4/JUP -0ZBL	1992	Spring	X ⁻	1-2	4	4
Shiroudi	ND/VG9144//KAL/BB/3/YACO/4/VEE#5 CM85836-4Y-0M-0Y-8M-0Y-0PZ-0IRN	1999	Spring	2 ⁻	2	4	4
Atrak	JUP/BJY//URES CM67458-4Y-1M-3Y-1M-3Y-0B-0K	1995	Spring	4	4	4	3
Alamout	KVZ/TI71/3/MAYA'S//BB/INIA/4/KARAJ2/5/ ANZA/3/PI/NDR//HYS 0K	1995	Winter	X ⁻	2 ⁻	4	4
Tajan	BOW/NKT CM67428-6M-1Y-05M-3Y-0B-0K	1995	Spring	4	2 ⁻	4	4
Niknejad	F134.71/CROW SWM11147-1AP-2AP-4AP-1AP-0AP-0MRGH	1995	Spring	33 ⁻	3	4	4
Mahdavi	TI/PCH/5/MT48/3/WTE*3//NAR59/TOTA/4/MUS -0K	1995	Spring	;C1 ⁻	1C	4	4
Zarin	NAI60/HN7//BUC/3/F59.71/GHK SWO791095-0MDB	1995	Facultative	4	2 ⁻	4	4
Darab 2	MAYA/NAC CM39424-1Y-1M-4Y-1M-1Y-1M-0Y-0K	1995	Spring	0,,	33 ⁻	4	4
Alvand	1-27-6275/CF 1770 0K	1995	Facultative	X ⁻	33 ⁻	4	3
Chamran	ND/VG9144//KAL/BB/3/YACO/4/VEE#5 CM85836-50Y-0M-0Y-3M-0Y-0IRN	1997	Spring	X ⁻	3 ⁻	4	4
Kavir	STM/3/KAL//V534/JIT716 -0IRN	1997	Spring	X ⁻	23 ⁻	4	4
Marvdasht	HD2172/AZADI -0SHZ	1999	Spring	4	22 ⁻	4	4

(Continued)

Table 6. Continues.

Name	Pedigree Cross number and selection history	Year of release	Growth habit	Pgt SR10	Pgt 86-20	Pgt 86-31	Pgt 86-55
Shahryar	KVZ/TI//MAYA/26591-1T-7M-OY-115Y-OM/3/1-44-21863/4/ANZA/3/PI/NAR59//HYS-0IRN	2002	Winter	3 ⁻	2 ⁻	4	4
Toos	SPN/MCD//CAM/3/NZR-17H-4H-1H-0H-0IRN	2002	Facultative	4	2 ⁻	4	4
Shiraz	GV/D6301//ALD/3/AZADI-0IRN	2002	Spring	3 ⁻	X ⁻	4	4
Dez	KAUZ*2//OPATA//KAUZ CRG737-1Y-010M-0Y-0IRN	2002	Spring	2 ⁻	22 ⁻	4	4
Arta	HD2206/Hork//Bul/6/CMH80A.253/2/M2A/CML//Ald/3/Ald*4/5/BH1146/H56.71//BH1146/3/CMH78.390/4/Seri/7/Hel/3*Cno79//2*Seri 82 0IRN	2005	Spring	3 ⁻	;C1 ⁻	4	4
Moghan 3	Luan/3/V763.23/V879.C8//PVN/4/Picus/5/Opata-0IRN	2005	Spring	2 ⁻	0;1	4	4
Darya	Sha4/Chil-0IRN	2005	Spring	3	1C	4	4
Bam	VEE#5/NAC//1-66-22-0K	2006	Spring	12 ⁻	1C	4	4
Akbari	1-63-31-3/12300/Tob//Cno/Sx-0IRN	2006	Spring	2 ⁻	4	4	4
Desprez Gascogne	TJB 9908 / Marengo	-	Winter	3	2 ⁻	4	4
Morocco	-	-	Spring	4	4	4	4

^a Infection types were recorded according to ; (fleck), and 0- to - 4. Symbols “-” and “+” were used to describe deviation from the pustule size of major infection types classes. C and N were used to describe extensive chlorosis and necrosis, respectively, associated with infection type and X was used for mesothetic infection types on same genotype. Recorded range of infection types were separated by comma (,).

fectiveness against races HRCTC and JTHTC was due to avirulence to *Sr31* and *Sr36*, and effectiveness against the two TTKSK collections was due to avirulence to *Sr36*.

Virulence spectra and effectiveness of *Sr*-genes

LITs of 0; to 22+ were produced by *Sr22*, *Sr24*, *Sr26+9g*, *Sr27*, *Sr29*, *Sr32*, *Sr33*, *Sr35* (*SrTm1*) and *SrNin* when tested against the nine *Pgt* collections. This indicates effectiveness of these genes against all *Pgt* collections. Since HITs of 3+ to 4 were recorded on the *Sr9g* monogenic line in Chinese Spring background for all collections tested, the LITs of 0; to 11+2 produced by Eagle against the nine collections are considered as indication of avirulence conferred by

Sr26 not *Sr9g*. In addition to HITs across all collections for *Sr6*, *Sr9a*, *Sr9d*, *Sr9g*, *Sr11* and *SrMcN* present in the North American differential lines (Table 3), HITs (susceptible) of 3 to 4 were produced by all collections when tested on *Sr7a*, *Sr8b*, *Sr9f*, *Sr12*, *Sr15*, *Sr16*, *Sr17*, *Sr18*, *Sr20*, *Sr23*, *Sr34*, *SrPL*, *SrTt3+10*, *SrWld1* and *Sr42* (Norin 40). The nine collections produced either HITs or LITs on *Sr14*, *Sr19*, *Sr21*, *Sr28*, *Sr36*, *Sr44* and *Srdp2* (Table 5).

Race TTKSK and its virulence spectrum

Race TTKSK (syn. Ug99) and related races have been extensively studied since their first detection in Uganda (Pretorius *et al.*, 2000; Wanyera *et al.*, 2006; Singh *et al.*, 2006; Jin *et al.*, 2007; 2008; 2009). TTKSK

(Ug99) was the first characterized race of *Pgt* with virulence to gene *Sr31* (Pretorius *et al.*, 2000). Using a set of 16 stem rust monogenic lines of the North American nomenclature system (Roelfs and Martens, 1988), Wanyera *et al.*, (2006) designated Ug99 as race TTKS and it was redesignated as TTKSK when *Sr31*, *Sr24*, *Sr38* and *SrMcN* were added as a fifth set to the North American differential set (Jin *et al.*, 2008).

In addition to *Sr31*, Ug99 carries a striking combination of virulence for important stem rust resistance genes from hexaploid and tetraploid origins which have been extensively used in agriculture (Singh *et al.*, 2006). Stem rust samples collected from Kenya in 2004 were found to be identical to the original Ug99 when tested for virulence phenotype using the 16-line North American differential system (Wanyera *et al.*, 2006). The original Ug99 collected from Uganda in 1988 was determined for virulence for *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9b*, *Sr9e*, *Sr9g*, *Sr11*, *Sr15*, *Sr17*, *Sr30*, *Sr31* and *Sr38* and avirulence for *Sr21*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr27*, *Sr29*, *Sr32*, *Sr33*, *Sr34*, *Sr35*, *Sr36*, *Sr39*, *Sr40*, *Sr42*, *Sr43*, *SrAgi* and *SrEm* (Pretorius *et al.*, 2000). The Kenyan Ug99 collections were virulent on a gene present in Waldorn (*SrWld-1*) (Wanyera *et al.*, 2006), an important gene for stem rust resistance in North American hard red spring wheat varieties (Leonard, 2001; Leonard and Szabo, 2005). In addition to the abovementioned genes, the Ug99 races from Kenya were also avirulent for *SrTmp*, *Sr13*, *Sr37* and *Sr44* derived from Triumph 64 (Roelfs and McVey, 1979), *T. timopheevii* (McIntosh, 1988), *T. turgidum* L. var. *dicoccum* cultivar Kaphli (Knott, 1962) and *Thinopyrum intermedium* (Friebe *et al.*, 1996), respectively. Stem rust resistance gene *Sr21* was initially reported as an effective gene against Ug99 (Pretorius *et al.*, 2000) but compatible reaction with an IT of 3 was recorded on diploid and hexaploid sources of *Sr21* (Einkorn and CnSSr21Tm) when Kenyan collections were tested for virulence (Wanyera *et al.*, 2006). Einkorn showed LITs of X⁺ and ;1= to TTKSK races from Boroujerd and Hamadan, respectively, while an IT of 3+ was observed when the same races when CnS-T. *monococcum* was used as the source of *Sr21* in the North American differential set. The virulence pattern of the Ug99 collections collected from Uganda and Kenya were identical to those of the two TTKSK races identified in the present study. Use of an expanded set of designated *Sr*-genes revealed that the two TTKSK races from southwest Iran produced HITs on Line G (*Sr7a*),

Barleta Benvenuto (*Sr8b*), ISR5SB (*Sr9f*), CH.SP.TC3B (*Sr12*), W2691SR15NK and Norka (*Sr15*), LC/Kenya Hunter (*Sr17*), LCSR18PL (*Sr18*), LCSR20MG (*Sr20*), Exchange (*Sr23*), Compair (*Sr34*), W2691SRTt2 (*Sr37*), Peliss (*SrPL*), Fed.*2/*SrTt3* (*SrTt3*+*Sr10*), Baart/Waldorn (*SrWld 1*) and Norin 40 (*Sr42*). These two races showed LITs for *Sr1RS-Am* present in TAM 107, Amigo and Fleming; *SrNin* in Ningadhu; and *SrAgi* in Taf 2.

Evaluation of seedling resistance to Ug99

Using AHC analysis, the 30 varieties (Table 6) were classified into two major resistance groups at 33.3% and 25% of Jaccard's coefficient of dissimilarity comprising 26 (87%) and four (13%) varieties, respectively (Figure 1). At 25% dissimilarity the two clusters were divided into four sub-clusters (resistance groups). The major sub-cluster comprises 19 varieties (63%) because of their LIT to the two *Sr31*-avirulent collections (*Pgt* SR10 and *Pgt* 86-20) and HIT to the two TTKSK races (*Pgt* 86-31 and *Pgt* 86-55). Known varieties for 1B.1RS translocation, Falat (Seri 82), Shiroudi (Attila 4Y) and MV17 were clustered in this group. The second sub-cluster was formed by Alamout, Tajan, Marvedasht, Toos, Darya, and Gascogne. These four varieties showed LIT to *Pgt* 86-20 while HIT was recorded against the other three *Pgt* collections including the two *Sr31* virulent races. Niknejad and Atrak, and Morocco and Akbari were classified into sub-cluster 3 and 4, respectively. The sub-cluster 4 is representative of the two cultivars without effective genes against the four *Pgt* collections, while the two cultivars in sub-cluster 3 were resistant to *Pgt* SR10 (Figure 1). The identity of resistance gene/genes in wheat varieties conferring resistance to *Pgt* SR10 and *Pgt* 86-2010 in sub-clusters 2 and 3 is unknown.

In conclusion, the TTKSK races had identical virulence phenotypes to the virulence phenotypes of the TTKSK races identified from Kenya and Uganda, indicating that the race TTKSK in Iran belongs to the same Ug99 lineage that likely migrated from Yemen to Iran in 2007 (Nazari *et al.*, 2009). Given the regular north-easterly airflows out of Yemen (Singh *et al.*, 2006; Singh *et al.*, 2008), and the relative proximity of Yemen compared to other countries where race TTKSK has been confirmed, it is possible that race TTKSK spread from Yemen to south-western Iran.

According to the predicted Ug99 migration pathway (Singh *et al.*, 2006; Singh *et al.*, 2008) based

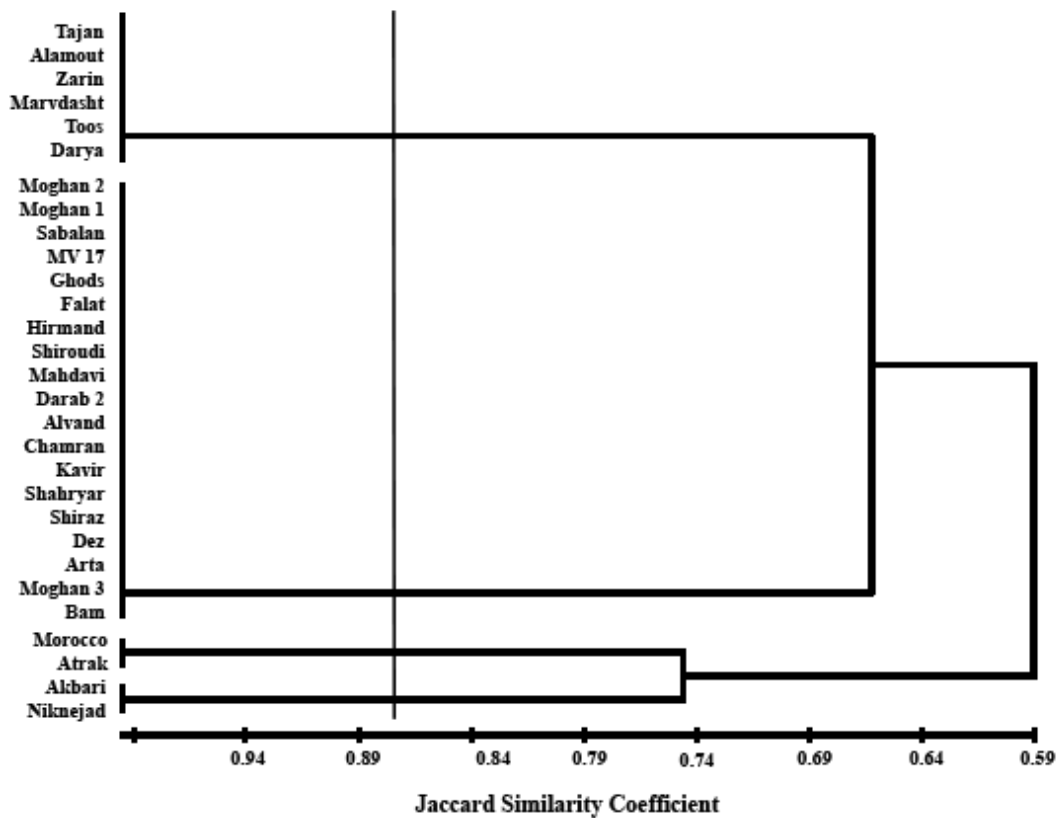


Figure 1. Dendrogram of 29 Iranian wheat varieties based on UPGMA cluster analysis and the Jaccard similarity coefficients calculated from evaluation of seedling resistance against four physiological races of *Puccinia graminis* f. sp. *tritici*

on the recorded west-east migration pathway of *Yr9*-virulent *Pst* races during the 1980s, the wheat-growing areas in southern Iran were expected to be more vulnerable to *Ug99* incursion than the south-western areas. Southern areas in Iran are drier and warmer than south-western areas, and wheat varieties with early maturity are grown to avoid terminal heat-stress late in the season. The cultivars Chamran (CIMMYT name Atilla 50Y) and Dez are the predominant wheat varieties grown in this region, and both are susceptible to *Ug99* at seedling stages (present study) and in the field (in Kenya and Yemen). Environmental conditions are favourable to stem rust disease development during December–March in Yemen, when wheat crops in southern Iran are at the heading stage. The wind trajectories during this period have shown a frequent tendency for airborne particles originating from Yemen to move towards southern Iran (Hodson *et al.*, 2005; Singh *et al.*, 2006).

It would be reasonable to conclude that the occurrence of *Ug99* in southwest Iran, as indicated in the present study, and not in southern wheat-growing areas, is due to lack of timing and monitoring of stem rust infection. It is possible that wheat in southern Iran has escaped infection or that initially undetected infections moved to south-western Iran. A case could be made for possible independent airflow originating from Yemen and directly heading to the areas where TTKSK races were detected. Facultative and winter wheat varieties are grown in Boroujerd and Hamadan, and stem rust has not been a problem in these areas during recent decades, in particular in Hamadan where winter wheat varieties are grown and winter temperatures do not allow the pathogen to grow in early growth stages. However, the most recent pathogenicity survey of *Pgt* in the southern part of Iran indicated reoccurrence of *Ug99* (TTKSK) in farmer fields (Patpour *et al.* 2011, BGRI Workshop,

St Paul, Minnesota, USA), indicating the validity of predicted pathway of Ug99 by Nazari *et al.*, (2009).

The occurrence of race TTKSK in Iran, the susceptibility of Iranian wheat cultivars, the presence of environmental conditions conducive to disease epidemics in different parts of the country, and the wide spread occurrence of the alternate host *Berberis* spp. in vast mountainous areas of Iran, indicate a serious threat by stem rust to wheat production in Iran and hence to neighbouring countries. Aecial infections on *Berberis* spp. were observed in Zagros and Alborz mountain ranges (Scharif *et al.*, 1971; Bamdadian and Torabi, 1978). Although wheat is not grown on a large scale in mountainous areas, cultivation of susceptible local varieties near *Berberis* could allow for sexual recombination to take place. The favourability of environmental conditions both for stem rust epidemics on wheat and aecial infection on *Berberis*, in combination with the susceptibility of currently grown wheat varieties in the Caspian Sea region, means that this area would be an ideal place in the Central and West Asia and North Africa regions for the development of new races and inoculum build-up. Historical stem rust epidemics have occurred near the Caspian Sea (Scharif and Bamdadian, 1974). Evaluation of Iranian variety reactions to Ug99 indicated that greater than 98% of tested materials were susceptible to stem rust race TTKSK, implying the vulnerability of wheat production to stem rust epidemics. Susceptibility of the representative set of Iranian varieties to race TTKSK in seedling (present study) and adult-plant stages (Njoro, Kenya) reinforce the serious threat of race TTKSK to wheat production in Iran.

Along with the immediate needs for strengthening breeding programs for resistance to Ug99 stem rust, given the serious implications of the airborne nature of rust pathogens and the susceptibility of wheat varieties to Ug99, there is a clear need for a regional coordinated monitoring system and tracking of the movement of Ug99 in projected 'at-risk' regions of countries in East Africa, the Middle East, Central Asia and South Asia.

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