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Races and virulence of *Puccinia graminis* f. sp. *tritici* in some regions of Iran

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Stem rust represents the major threat to wheat production in Iran. It has been present for many years and epidemics occur in different regions on both winter and spring wheat. The objective of this study was to characterize races of *Puccinia graminis* f. sp. *tritici* present in Khuzestan and Lorstan provinces of Iran in 2012. Using the international system of nomenclature for *P. graminis* f. sp. *tritici*, 20 races were identified. A total of 20 races were identified from 30 isolates, which included the most prevalent races TTKSK and TTJQC with a frequency of 36.4 and 33.3%, respectively. The second most frequent and dominant race was KTTSK with a frequency of 18.2%. The resistance genes *Sr24* and *Sr36* were found to confer resistance to most of the races prevalent from two provinces while genes *Sr5, Sr9e, Sr7b, Sr6, Sr8a, Sr9g, Sr30, Sr9a, Sr9d, Sr10* and *SrMcn* were ineffective against most of the races detected. Among these genes, resistance genes, *Sr11*, *Sr38* and *SrTmp* were found as low infection types (more effective resistance genes) against most of the races detected. The wide virulence diversity that was found in this study across regions and over time will undoubtedly render the task of breeding durably resistant materials more difficult.

Key words: Pathogen races, resistance genes, wheat stem rust.

INTRODUCTION

Stem rust caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., is a highly damaging disease of wheat that primarily occurs in warm weather and can cause great damage to susceptible wheat varieties. The rust diseases of wheat have historically been one of the major biotic production constraints both in Asia and the rest of the world. There are more than 3000 rust species in the world (Ershad, 1995; Abbasi et al., 2005), three of which are pathogenic on wheat. These include *P. graminis* f. sp. *tritici* (causal agent of stem rust), *Puccinia striiformis* f. sp. *tritici* (causal agent of stripe rust) and *Puccinia triticina* (causal agent of leaf rust). In most wheat producing areas, yield losses are caused by *P. graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. Studies on stem rust have been undertaken since the 1760s throughout the world, and there are records of stem rust in Iran dating back to 1946 (Esfandiari, 1947). Although *P. graminis* has been observed on local wheat cultivars (Sharif et al., 1970), there were no significant outbreaks of the disease since the last outbreaks in 1975-76 that occurred in Caspian

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Sea and southern parts of the country (Bamdadian and Torabi, 1978).

According to Bamdadian and Torabi (1999) stem rust was historically a major problem in Iran. More than 50 numerically catalogued resistance (*Sr*) genes confer resistance to the various races of the stem rust pathogen. However, virulence for a large proportion of these genes is now common. Although *P. graminis* has been observed occasionally on local wheat cultivars, there were no significant outbreaks of the disease since the last epidemics occurred in the southern parts of the country on Iran during 1975-78 (Bamdadian and Torabi, 1978). New stem rust pathotypes identified in Uganda in 1999 (Ug99) has altered this quiet period of stem rust. Ug99 have defeated an important number of effective resistance genes including those that were used in several breeding programs.

Since first reported in 1999 (Pretorius et al., 2000), TTKSK and its variants have been found throughout eastern and southern Africa (Jin et al., 2008; Singh et al., 2011; Visser et al., 2010; Wanyera et al., 2006; Wolday et al., 2011) and in 2007 Ug99 was detected in the southwest of Iran (Nazari et al., 2009). This is of paramount importance in developing wheat cultivars with durable stem rust resistance. In addition, virulence surveys are important for studying the evolution of new races and forecasting the virulence shifts in a population. The objective of this study, were to analyze the genetic relationship and spatial distribution of physiologic races of *P. graminis* f. sp. *tritici* in the major wheat growing areas in Khuzestan and Lorstan provinces of Iran.

MATERIALS AND METHODS

In this study, field surveys were conducted in the southeast (Ahvaz, Dezful, Behbahan, Shushtar, Shadegan, Izeh and western (Khorramabad, Aligoodarz, Dorood, Borujerd, Koohdasht) regions of Iran in 2012 cropping seasons to collect samples of wheat stem rust. Samples of infected stems were collected at 5-10 km interval from wheat fields. Seedlings of the universally rust susceptible variety “Morocco” which does not carry any known stem rust resistance genes were raised in suitable 8 cm diameter pots. Leaves of seven-day-old seedlings or seedlings with fully expanded primary leaves and second leaves, were rubbed gently with clean moistened fingers. Greenhouse inoculations were done using the methods and procedures developed by Stakman et al. (1962). Spores from the stem rust infected sample were scraped off with scalpels on to a watch glass and suspended in distilled water to make rust spore suspension, which was rubbed on the seedlings of Morocco. The plants were then moistened with fine droplets of distilled water produced with an atomizer and placed in an incubation chamber for 18 h dark at 18 to 22°C followed by exposure to light for 3 to 4 h to provide condition for infection and seedlings were allowed to dry their dew for about 1 to 2 h. The seedlings were transferred from the dew chamber to glass compartments in the greenhouse where conditions were regulated at 12 h photoperiod, at temperature of 18 to 25°C and relative humidity (RH) of 60 to 70%. The remaining rust spore samples were kept in the refrigerator at 4°C and were used for samples which failed to produce infection on the universally susceptible variety in the greenhouse. After seven to ten days, leaves containing a single fleck that produced a single pustule, were selected from the base of the leaves. The remaining seedlings within the pots were removed using scissors.

After two weeks, spores from each pustule were collected using a power-operated vacuum aspirator and stored separately in gelatine capsules. A suspension, prepared by mixing urediospores with lightweight mineral oil (Soltrol 170), was used for inoculating seven-day-old seedlings of the susceptible variety 'Morocco' for multiplication purposes. This was done for each of the single pustules on separate pots. Immediately after inoculation, the seedlings were placed in a humid chamber at 18 to 22°C for 18 h in the dark and for 3 to 4 h in the light, after which they were transferred to a greenhouse. About 14 to 15 days after inoculation, the spores of each single pustule were collected in separate test tubes and stored at 4°C until they were inoculated on the standard differential sets.

Five seeds of the twenty wheat stem rust differentials with known resistance genes (*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr31*, *Sr17*, *Sr21*, *Sr30*, *Sr36*, *Sr38*, *Sr24*, *SrTmp* and *SrMcN*) and one susceptible variety Morocco were separately grown in 3 cm diameter pots in the greenhouse (Table 1). The single pustule-derived spores were suspended in distilled water and inoculated onto seven-day-old seedlings using atomizers and/or an air pump. After inoculation, plants were moistened with fine droplets of distilled water produced with an atomizer and placed in an incubation chamber at 18 to 22°C for 18 h in the dark and 3 to 4 h in the light. The seedlings were allowed to remove their dew for about 1 to 2 h in a dew chamber. Upon removal from the dew chamber, plants were placed in separate glass compartments in a greenhouse to avoid contamination.

Stem rust infection types (ITs) were scored 14 days after inoculation using the 0 to 4 scale of Stakman et al. (1962). Infection types were grouped in to two, where, low (resistant) = (0, 0; (fleck), 1, 1+, 2 and 2+) and high (susceptible) = (3-, 3+ and 4). Race designation was done by grouping the differential hosts into five subsets in the following order: (i) *Sr5*, *Sr21*, *Sr9e* and *Sr7b*; (ii) *Sr11*, *Sr6*, *Sr8a*, and *Sr9g*; (iii) *Sr36*, *Sr9b*, *Sr30* and *Sr17*; (iv) *Sr9a*, *Sr9d*, *Sr10*, *SrTmp*; and (v) *Sr24*, *Sr31*, *Sr38* and *SrMcN* (Table 1). Each isolate was assigned a five letter race code based on its reaction on the differential hosts (Roelfs and Martens, 1988; Jin et al., 2008).

RESULTS

Virulence structure of stem rust pathogens

From 20 field samples from the two provinces of the country, 11 races were identified. The two adjacent regions, Khuzestan and Lorestan, had eleven similar races out of six and five races detected, respectively (Table 2). The highly virulent races of Ug99 (TTKSK) were the most abundant and widely distributed race across the Khuzestan region, with a frequency of 36.4%. The other abundant races countrywide included KTTSK and TTJQC with frequencies of 18.2% each. TRFSC and RRHSC made up the least dominant races in this region, with frequencies of 9.1% each. There was variation between the virulence spectra of races within the regions (Table 2). Of the 9 isolates studied in Lorestan, the highly virulent races TTJQC was the most abundant and widely distributed race across the region, with a frequency of 33.3%. Race TTKSK (Ug99) and the closely related race RRHSC were predominant, each with frequencies of

Table 1. Code for the 20 differential hosts for *Puccinia graminis* f.sp. *tritici (Pgt*) in ordered sets of five.

	Subset	Infection type produced on host lines with stem rust			
	1	Sr5	Sr21	Sr9e	Sr7b
	2	Sr11	Sr6	Sr8a	Sr9g
Pgt-Code	3	Sr36	Sr9b	Sr30	Sr17+13
	4	Sr9a	Sr9d	Sr10	SrTmp
	5	Sr24	Sr31	Sr38	SrMcn
B		Low*	Low	Low	Low
C		Low	Low	Low	High**
D		Low	Low	High	Low
F		Low	Low	High	High
G		Low	High	Low	Low
H		Low	High	Low	High
J		Low	High	High	Low
K		Low	High	High	High
L		High	Low	Low	Low
M		High	Low	Low	High
N		High	Low	High	Low
P		High	Low	High	High
Q		High	High	Low	Low
R		High	High	Low	High
S		High	High	High	Low
T		High	High	High	High

Source: Roelfs and Martens (1988); Jin et al. (2008); *Low: Infection types 0, 1 and 2 and combinations of these values. **High: Infection types 3 and 4 and a combination of these values.

Table 2. Races of *Puccinia graminis* f. sp. *tritici* identified in in Lorstan Province of Iran in 2012.

Resistance gene	Stem rust isolates collected in Lorstan Province								
	A1	A2	A3	A4	A5	A6	A7	A8	A9
5	3+	2+	3+	3+	2	2+	2+	3+	3+
21	3+	2+	3	2+	3	3+	2+	2	3
9e	3+	3+	3+	3+	3+	2-	2-	2	2
7b	33	33	33	3+	3+	2+	3+	3+	3+
11	3	2-	2;	2	3	2	1	2	2-
6	33+	33+	33	3+	3+	3+	33+	33+	3+
8a	3+	2+	3+	2+	2+	2+	2+	2	3+
9g	2	33+	3+	3+	3+	3+	3+	3+	3+
36	;C	1-	2-	1	2-	2-	;	2+	;C
9b	3+	2+	3+	2+	3+	3+	2+	2+	3+
30	33+	3+	2	33+	3+	33+	3	3	33
17	3	3+	3	3	3	3	3	3+	3+
9a	3+	3+	3+	3+	3+	3+	3+	33+	33+
9d	3+	3+	3+	3+	3+	3+	3+	3+	3+
10	33+	33+	3+	3+	3+	3+	33+	3+	3+
Tmp	2+	22+	3+	2+	2+	3+	2+	3+	2
24	2	1	2	;	2+	2+	2+	2	2
31	2	3	3+	3	3	3	3+	3	2
38	3	1-	1	2	3	2-	1	2-	3
McN	3+	3+	3	2+	3	3+	33	2	3+
Races	TTKSK	TTJQC	TRFSC	RRHSC	KTTSK	TTJQC	TTJQC	RRHSC	TTKSK

Table 3. Races of *Puccinia graminis* f. sp. *tritici* identified in in Lorstan Province of Iran in 2012.

Resistance gene	Stem rust isolates collected in Khuzestan Province										
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**
5	3+	3+	2+	3	2+	2	2+	2	3+	3+	+2
21	3+	3+	3	22+	3	3+	2+	2-	3	3+	2+
9e	3+	3+	3+	3+	33	2-	33	2+	2	33	3
7b	33	33	3+	3+	3+	2+	3+	3+	3+	3+	3
11	2+	2;	2;	2	3-	3	1	2	2-	3	1
6	33+	33+	33	3+	3+	3+	33+	33+	3+	3+	3+
8a	3+	3+	2+	3+	3+	2	2+	2+	2+	2	3+
9g	2	33+	3+	3+	3+	3+	3+	3+	3+	33+	3+
36	;	2-	2+	;1	2-	2-	;	2+	+2	1-	2+
9b	3+	3+	2+	3+	2+	3+	2+	2+	2+	2	3+
30	33+	3+	2+	33+	3+	33+	3	3	33	33+	3
17	3	3+	2	3	3	3	3	3+	3+	3+	3+
9a	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
9d	3+	3+	3+	3+	3+	3+	3+	3+	3+	3	3
10	33+	33+	3+	3+	3+	3+	33+	3+	3+	3+	3+
Tmp	2+	22+	22+	2+	3+	2+	3+	3+	2	3	22+
24	2	1	1	2	2+	2+	2	2-	2	22+	2-
31	2+	3	3+	3	3	3	3+	3	3	3	3
38	3	1-	1	2	1	2-	1	2-	3	2	2
McN	3+	3+	2-	3+	3	3+	2+	2	3+	3+	3
Races	TTKSK	TTKSK	TTJQC	TRFSC	RRHSC	KRKSC	TTJQC	TTJQC	KTTSK	TTKSK	KRKSC

22.2%, followed by races TRFSC and KTTSK with 11.2% each. From the Khuzestan region, races TTKSK (4 isolates), TTJQC (2 isolates), KTTSK (2 isolates), TRFSC (1 isolate), RRHSC (1 isolate) and KRKSC (1 isolate) were found.

Frequency in Lorestan province showed a similar trend, as TTJQC and TTKSK had the highest frequency of 33.3%. In this region, the least dominant races were TRFSC and RRHSC detected only once. The race pattern in Lorestan was different from that of the other region (Table 3). In two parts of the country, TTJQC and TTKSK were the dominant races, accounting for 33.3 and 36.4% of the total populations. Although most of the races were confined to specific locations, some had wider spatial distributions. Five races TTKSK (Ug99), TTJQC, TRFSC, RRHSC and KTTSK were present in two regions surveyed. KRKSC was present in southeast, but not in western Iran. Similarly, KRKSC was present in Khuzestan region (Table 2). In view of the results obtained (11 races from 20 samples), it is clear that race variations in stem rusts are very wide in Iran, and that with a sample size of only 20 isolates, only a minimal part of the races were actually found. In our study, more samples were collected from a lower area. This could be why lower races were identified in our study.

Virulence to Sr resistance genes

The majority of pathotypes identified during the survey were virulent on most of the wheat differentials. After analyzing 20 isolates from regions representing the major wheat-growing areas of the country, two important stem rust resistance genes, namely *Sr24* and *Sr36* were found to confer resistance to most of the races prevalent in two provinces of Iran. Three resistance genes, *Sr11*, *Sr38* and *SrTmp*, were found as low infection types (more effective resistance genes) against most of the races detected in this study (Table 4). Race TTKSK, was virulent to all except three resistance genes *Sr36, Sr24* and *Sr38*. Among these 11 races, race KTTSK thus poses a serious threat to two provinces of the country. Race KRKSC, which had a wide spectrum of distribution (Table 4) was virulent to all differentials except those carrying the Sr24 and Sr36 genes. The resistance genes *Sr5, Sr8a, Sr9e, Sr9b* and *Sr21* were found to be effective against races TTJQC and RRHSC while genes *Sr6, Sr7b, Sr9g, Sr17, Sr30, Sr9a, Sr9d, Sr10,* and *SrMcn* were ineffective for all races identified in Khuzestan region. A total of 5 different races were identified from 9 stem rust isolates in the Lorestan province.

Among these 5 races, Race TTKSK, was virulent to all except four resistance genes *Sr24, Sr36, Sr38* and *SrTmp* while resistance genes *Sr24* and *Sr36* were effective against race KTTSK (Tables 2 and 3). In general, the resistance genes *Sr11*, *S24*, *Sr36, Sr38* and *SrTmp* showed resistance or low infection types against all isolates, while genes *Sr5, Sr6, Sr9a, Sr10* and *SrMcn*

Table 4. Virulence spectrum and frequency of races of *P. graminis* f. sp. *tritici* collected from two provinces of Iran.

Race	Ineffective resistance genes[b]	Location	No. of isolates	Frequency (%)
Khuzestan				
TTKSK	*5*, *6, 7b, 8a, 9a, 9b, 9d, 9e, 10*, *11*, *17, 21, 31,38, Tmp*, *McN*	Ahvaz	4	36.4
TTJQC	*5* , *6, 7b, 9a, 9b, 9d, 9e, 9g, 10, 17, 31, Tm,*	Dezful	2	18.2
TRFSC	5 **,** *6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 30, 31*, *McN*	Behbahan	1	9.1
RRHSC	*6*, *7a, 7b,* 8a, *8b, 9g, 9a,* 9b, *9d, 9e, 10, 11, 13, 17,* 30, 31, *Tmp, McN*	Shushtar	1	9.1
KRKSC	*6, 21, 7b, 7a, 8b, 9e, 9g, 9a, 9d, 11, 13, 17, 21, McN 10,*	Shadegan	1	9.1
KTTSK	5 *, 6, 9a, 9b, 9d, , 9g, 10, 17, 31*, *38*, *McN*	Izeh	2	18.2
Total			11	100
Lorestan				
TTKSK	5*, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 31,38, McN*	Khorramabad	2	22.2
TTJQC	*6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 17, 21, 31, Tmp*, *McN*	Aligoodarz	3	33.3
TRFSC	*5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 17, 21, 30*, *31, Tmp*, *McN*	Dorood	1	11.2
RRHSC	5*, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 17, 21, 30*, *31, Tmp, McN*	Borujerd	1	22.2
KTTSK	*6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10,11, 17, 21, 30, 31, 38, Tmp, McN,*	Koohdasht	2	11.2
Total			9	100

showed high infection types against all isolates in 2012 (Table 4). Only two of the 20 differential lines carrying resistance genes, *Sr24* and *Sr36* were found to confer resistance to most of the races prevalent in two provinces of the country.

DISCUSSION

The identification of 11 races from 20 samples was a clear indication of high virulence diversity within the *Pgt* population in two provinces of country. A comparison of the races identified in the present study with earlier reports (Nazari et al., 2008 and 2009) revealed some differences.

Even though stem rust incidences were relatively high in both provinces, the percentage of plants infected with stem rust in diseased fields was low. The number of fields inspected during our study was different (Tables 2 and 3). The highest stem rust pathogens was found in a field of Khuzestan province. Although Khuzestan and Lorstan provinces have climates that favor rust development, the diseases were not apparent.

It is also important to note that the race spectrum in Iran is clearly different from that reported in other parts of the world. Surveys in the USA (Jin et al., 2007, 2008) detected fewer races, than in Iran. According to our reports, out of the 20 field samples from the two provinces of the country, 11 races were identified. Race TTKSK, which had a wide spectrum of distribution was virulent to all differentials except those carrying the *Sr24, Sr36* and *SrTmp* genes.

Since it is not known which resistance genes local landraces and commercial wheat cultivars grown in Iran contain, it is not possible to determine their effect on race composition of the *Pgt* population. The two adjacent regions, Khuzestan and Lorstan, had six similar races out of five races detected, respectively. The present study also detected the race at one additional location, indicating that the race is spreading in the region. Furthermore, the new Ug99 variant TTKSK, which is identified in this study, was also detected in other countries. Positive Iranian Ug99 isolates were collected in 2007 from two sites, Borujerd and Hamadan, in northwestern Iran, but underwent extensive testing to confirm the race (Nazari et al., 2008). Detection in Iran in 2007 was followed by drought conditions, and no reports on Ug99 were received from Iran in 2008 (Nazari et al., 2009). However, in 2012 Ug99 was found in the southern Iranian province of Khuzestan, where spring wheat is grown and growing conditions are favorable. Alternatively, Ug99 may have been introduced into Khuzestan in 2007 but remained undetected and migrated to the northwest, where facultative and winter wheats are grown and mature approximately two months later. There is no evidence that the Ug99 lineage has become well established in Iran, and no crop losses have been reported so far. Also, to date it is not known to have spread beyond Iran.

Reports of the unusual occurrence of stem rust in Pakistan in 2009 prompted some fears of a Ug99 incursion, but greenhouse phenotyping of samples on differentials and DNA analysis of dead spores indicated conclusively the absence of Ug99 and presence of another important race (RRTTF) (Mirza et al., 2010).

In general, the virulence spectrum of the pathogen in this study confirmed the presence of wider range of virulence in the study area and is in line with previous studies conducted in Iran (Patpour et al., 2011; Nazari et al., 2008). A comparison of the races identified in the present study with these earlier reports revealed differences. This could be due to variation in location and time, as the prevalence of races in a specific season and region depends on the type of wheat cultivars grown and to some extent on the predominant environmental conditions, especially temperature (Roelfs et al., 1992). It was evident that the majority of the resistance genes were ineffective against most of the isolates. Resistance genes *Sr5, Sr6, Sr7b, Sr9g, Sr17, Sr30, Sr9a, Sr9d, Sr10* and *SrMcn* were ineffective, accounting for more than 65% of the isolates tested. Nazari et al. (2009) reported similar findings. These *Sr* genes were ineffective for more than 85% of the isolates collected in 2007 from northwest regions of Iran. Earlier studies indicated that virulence to *Sr6*, *Sr8b*, *Sr9a*, *Sr9d* and *Sr11* is common worldwide (Roelfs et al., 1992). In contrast, *Sr24* was effective against most of the isolates tested. This confirms the report of Patpour et al. (2011), which stated that these genes are amongst the effective genes, which have an adequate and some immediate values against almost all races in the world, except few occasional high infection types in some countries including Iran. For instance, virulence to *Sr24* was reported in Kenya in 2006. A variant of Ug99 that added virulence on stem rust gene *Sr24* (Ug99 + *Sr24* virulence, called TTKST) has further increased the vulnerability of wheat to stem rust worldwide (Jin et al., 2008). On the other hand, Admassu et al. (2009) reported that race TTKSR was avirulent on *Sr24*. Teklay et al. (2012) reported that *Sr24* was effective against many races, however, in our study, this genotype was effective fir most isolates tested.

Nazari et al. (2009) previously detected a *Pgt* isolate virulent to *Sr31* in BorooJerd, but the lack of stem rust in Iran during 1997-2006 due to unfavorable climatical conditions hindered more investigation on virulence factors of *Pgt,* therefore statement on virulence for *Sr31* in the region during last ten years is rather difficult. However, virulence for *Sr31* should be considered as serious threat to wheat production in Iran.

The results of this study also support this fact and show that large variation was observed in terms of the determined races. In general, it appears that many resistance genes have different reactions to the races, and our results confirmed that there are many stem rust races in Iran. The geno-types possessing at least one of the resistance genes *Sr11*, *Sr24*, Sr36, *Sr38* and *SrTmp*, were resistant or found with low infection types against most of the races detected in this study (Tables 2, 3 and 4). Each of these resistance genes or a combination of them could be used in developing stem rust resistant cultivars. On the other hand, the absence of virulence towards *Sr6, Sr7b, Sr9g, Sr17, Sr9a, Sr9d, Sr10, Sr30* and *Sr31* genes in all rust races studied during the two provinces of country indicate that these genotypes could serve as a source of resistance to the prevailing rust races in Iran.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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