

Identification of physiological races of *Puccinia graminis* f.sp. *tritici* in Iraq

Emad Mahmood Al-Maarroof

College of Agricultural Sciences, Sulaimani University, KRG, Iraq

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*Corresponding author

Email: ealmaarroof@yahoo.com

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Abstract

Stem rust has become a major cause for concern with the emergence of race *Ug99* in Eastern Africa. The potential impact of the disease is particularly serious in Iraq. The current study was conducted to identify the physiological races of *Puccinia graminis* f.sp. *tritici* (Pgt), the causal agent of stem rust. Single pustules of stem rust samples collected from different wheat fields from Sulaimani, Erbil, Diala, Nineveh and Babylon were separately multiplied on the susceptible cultivar cv Morocco. Seven-day-old seedlings of the North American stem rust differential set inoculated with urediniospores of each pathogen isolate; the inoculated plants were scored two weeks after inoculation using the infection type scale described by Stakman. Race analysis indicated prevalence of five races of the pathogen. Race TKTRC was prevalent in Sulaimani; RKJTF in Erbil; TKTTC in Nineveh; PRJSC in Diala and KRKSC in Dheqar. The resistance genes *Sr24* and *Sr31* were effective against all Pgt isolates, while resistance genes *Sr7b*, *Sr9g*, *Sr9b*, *Sr30*, *Sr9a*, *Sr9d*, *SrMcN* were ineffective against all the isolates. None of the identified races of Pgt belonged to race *Ug99* or members of this race group. Real-time PCR analysis of DNA extracted from dead spores, representing each of the Pgt races, at USDA-ARS cereal disease Lab, St Paul, Minnesota confirmed these results. .

Key words: Stem rust; *Triticum aestivum*; races; fungal diseases

1. Introduction

Stem rust of wheat caused by *Puccinia graminis* f.sp. *tritici* (Pgt), was a devastating disease in many wheat-growing regions of the world. The disease has been successfully controlled by introduction of the semi-dwarf resistant wheat cultivars during the 1970s (Bayrlee, 1995). Resistance of wheat cultivars were based mainly on *Sr31* resistant gene introduced from *Secale cereale*. In addition to the high levels of resistance to stem rust, powdery mildew and stripe rust diseases, the new wheat cultivars gave significant yield advantage (Mcintosh *et al.*, 1995). Stem rust has again become a major cause for concern with the breakdown of stem rust resistance gene *Sr31* with emergence of the new Pgt race *Ug99* in Uganda in 1999 (Pretorius, 2000). The report "Sounding the

Alarm on Global Stem Rust", issued 29 May 2005 by the Expert Panel on the Stem Rust Outbreak in Eastern Africa, unequivocally declared *Ug99* to be a strategic threat to global wheat production. The potential impact of stem rust is particularly serious in Iraq and could become a serious problem to irrigated wheat production in the northern and Kurdistan regions. This disease was usually distributed in the central and southern zones of Iraq (Al. Beldawi *et al.*, 1996). High stem rust infection in some wheat fields recently in Kurdistan and other northern parts of Iraq is a threat to wheat production under the scenario of the new race *Ug99* movement ahead in the region towards Iraq along the *Yr9* virulence pathway (Al-Maarroof *et al.*, 2012; IPM/ ICARDA, 2011).

Stem rust has not been a major problem for wheat production as compared to other rust diseases in Iraq due to the late appearance of the disease in the season (Al-Hamdany and Abas, 2003). Therefore, the disease is not well studied and the information concerning physiological specialization of the pathogen is rare. Natour *et al.* (1971) identified 13 physiological races of *P. graminis* f.sp. *tritici*, while 19 physiological races of Pgt (5, 9, 11, 14, 16, 17, 19, 24, 42, 53, 117, 130, 218, 220, 245, 270, 299 and 320) were identified by Al-Beldawi *et al.* (1996). Races 24 and 42 proved to be the more frequent ones followed by 117 and 17. With the spread of the race *Ug99* from east Africa to the middle east (IPM annual report 2013; Singh *et al.*, 2008), extensive surveys were annually conducted in wheat fields of Iraq for monitoring of *Ug99* race group and other Pgt races. Virulence against *Sr31* was detected in Iran in 2007 (Nazari *et al.*, 2009). Recently ten variants of the *Ug99* race group have been reported in thirteen countries including Uganda, Kenya, Ethiopia, Egypt, Sudan, Tanzania, Eriteria, Rawana, South Africa, Zimbabwe, Mozambique, Yemen and Iran (Paptour *et al.*, 2016a, 2016b). Presence of the alternate host "Barberry" is not known in Iraq, but it is present in Iran (Tahraniyar, 2003). Diagnosis of the Pgt races on molecular basis recently provide a powerful tool for population genetic studies (Schaad and Frederick, 2002; Szabo, 2007; Newcomb *et al.* 2016). The objective of the current study was to map the distribution of Pgt race groups in different wheat growing areas of Iraq.

2. Material and methods

Rust samples were collected from the commercial wheat fields, biological wheat trap nurseries and yield trial plots. About 3-5 of infected wheat leaf and stem samples with single uredial pustules were collected. The samples were cut into 10cm pieces, the core tissue of the stems were removed to easily dry down the samples. All the important information including, geographic data "Latitude, longitude and Altitude", date of collection, cultivar, disease severity and incidence were recorded on each sample. The samples were air dried, placed in a glassine paper envelope and then kept in a refrigerator at 4°C until used for virulence analysis. Stem rust samples were collected from different wheat cultivars from 15 locations representing Sulaimani, Erbil, Diala, Nineveh and Babylon.

Urediniospore of each sample were suspended in lightweight mineral oil (Soltrol 170, ChemPoint, Limburg, Netherlands) and sprayed on 7-day old seedling of the susceptible wheat cultivars cv. Morrocco for multiplication. The inoculated seedlings were placed in dew chamber for 48h at 20°C. The plants were then placed in greenhouse at 20-25°C and each culture was maintained separately in clear plastic chamber (Pathan and Park, 2007). Sufficient amount of uredospores were produced to inoculate the North American stem rust differential sets used for race identification (Jin *et al.*, 2008). The differential wheat lines possessed resistance genes *Sr5*, *Sr6*, *Sr7a*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr13*, *Sr17*, *Sr21*, *Sr30*, *Sr36*, *Sr Tmp* and *SrMcN* (Table 1).

Table 1. A key for identifying of *Puccinia graminis* f. sp. *tritici* (Pgt) races.

Pgt-Code	Subset	Infection type produced on the four gene differential sets			
		<i>Sr5</i>	<i>Sr21</i>	<i>Sr9e</i>	<i>Sr7b</i>
	1	<i>Sr5</i>	<i>Sr21</i>	<i>Sr9e</i>	<i>Sr7b</i>
	2	<i>Sr11</i>	<i>Sr6</i>	<i>Sr8a</i>	<i>Sr9g</i>
	3	<i>Sr36</i>	<i>Sr9b</i>	<i>Sr30</i>	<i>Sr17+13</i>
	4	<i>Sr9a</i>	<i>Sr9d</i>	<i>Sr10</i>	<i>SrTmp</i>
	5	<i>Sr24</i>	<i>Sr31</i>	<i>Sr38</i>	<i>SrMcN</i>
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

Source: Jin *et al.* (2008). L: Low infection types, H: High infection types.

Seven-day-old seedlings of the differential sets were inoculated separately with each isolate of the pathogen, the inoculated seedlings were incubated in the dark for 18h at 18-20°C and high relative humidity and then transferred to a greenhouse condition with 20-25°C night/day temperature regime. The plants were assessed two weeks after inoculation according to the infection types described by Stakman *et al.* (1962). Infection types 0, 1 and 2 and combinations of these values were considered as low infection type (L).

Infection types 3 and 4 and their combinations were considered as high infection type (H). Races were identified using the international *P. graminis* f. *sp. tritici* code (Table 1) for the North American differential set (Jin *et al.*, 2008). While five single pustule samples of *P. graminis* f. *sp. tritici* collected from different wheat fields were sent to USDA-ARS Cereal Disease Laboratory, University Minnesota for *Ug99* race group analysis using real-time polymerase chain methodologies and fluorophore-labeled hydrolysis probes (Szabo, 2012). This assay consists of a suit of four different reactions targeting a different region of the Pgt genome. Only samples from Pgt *Ug99* race group will be positive (Ct value less than 40) for all four reactions.

3. Results and discussion

Host parasite interaction of Pgt isolates with stem rust differential set showed some variation in the infection types of these genotypes with Pgt isolates collected from different agro-ecological zones in Iraq at seedling stage (Table 2). Isolate *Sr-SUI* 1 was virulent against resistance genes *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr17*, *Sr21*, *Sr30*, *Sr36* and *SrTmp*, *SrMcN* and a virulent against resistance genes *Sr10*, *Sr11*, *Sr24*, *Sr31*, *Sr38*. High infection types resulted from interaction of isolate *Sr-ERI* 5 with resistance genes *Sr5*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9g*, *Sr10*, *Sr11*, *Sr21*, *Sr30*, *Sr38*, *SrTmp* and *SrMcN* and low infection type with resistance genes *Sr6*, *Sr17*, *Sr24*, *Sr31* and *Sr36*. *Sr-NII* 7 characterized with its virulence on resistance genes *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr17*, *Sr21*, *Sr30*, *Sr36*, *SrTmp* and *SrMcN* and avirulence on resistance genes *Sr11*, *Sr24*, *Sr31* and *Sr38*. *Sr-DII* 12 induced high reaction on resistance genes *Sr5*, *Sr6*, *Sr7b*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr30* and *SrMcN* and low infection type on resistance genes *Sr8a*, *Sr17*, *Sr21*,

Sr24, *Sr31*, *Sr36*, *Sr38* and *SrTmp*. Reaction of stem rust differential sets with isolate *Sr-DHI* 15 resulted from high infection type reaction with resistance genes *Sr7b*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr17*, *Sr21*, *Sr30*, *Sr36*, *SrTmp* and *SrMcN*, whereas Low infection type with resistance genes *Sr5*, *Sr8a*, *Sr24*, *Sr31*, *Sr36*, *Sr38* and *SrTmp*. The resistance genes *Sr24* and *Sr31* were effective against all Pgt isolates, while resistance genes *Sr7b*, *Sr9g*, *Sr9b*, *Sr30*, *Sr9a*, *Sr9d*, *SrMcN* were ineffective against all the isolates. Resistance genes *Sr5*, *Sr6*, *Sr9e*, *Sr10*, *Sr21* and *Sr38* were ineffective against only one isolate of Pgt. According to the North American key for identification of Pgt races (Table 1), isolate *Sr-SUI* 1 from Sulaimani is race TKTRC. Isolate *Sr-ERI* 5 from Erbil is race RKJTF, *Sr-NII* 7 from Nineveh is race TKTTC, *Sr-DII* 12 from Diala is race PRJSC and *Sr-DHI* 15 from Dheqar is race KRKSC.

Table 2. Seedling response of stem rust differentials to different isolates of *Puccinia graminis* f. *sp. tritici* and the suspected races of the pathogen in Iraq

<i>Sr</i> Resistant genes	Isolate Code and Location				
	<i>Sr-SUI</i> 1 "Sulaimani"	<i>Sr-ERI</i> 5 "Erbil"	<i>Sr-NII</i> 7 "Nineveh"	<i>Sr-DII</i> 12 "Diala"	<i>Sr-DHI</i> 15 "Dheqar"
<i>Sr5</i>	3+	4	3++	3+	2+
<i>Sr21</i>	3++	3-	3	2-	3-
<i>Sr9e</i>	3-	2-	3+	3+	3
<i>Sr7b</i>	4	3+	3+	3	3
<i>Sr11</i>	2+	3-	2-	3-	2+
<i>Sr6</i>	4	2+	3+	3+	3
<i>Sr8a</i>	3+	3-	3	2-	2+
<i>Sr9g</i>	4	3	3+	3	3+
<i>Sr36</i>	4	0;	3+	X	2+
<i>Sr9b</i>	3+	3	3-	3	3-
<i>Sr30</i>	4-	3	3+	3	3++
<i>Sr17</i>	33+	2-	3++	0;	3+
<i>Sr9a</i>	4	3+	3+	4	33+
<i>Sr9d</i>	3	3	3+	3+	3
<i>Sr10</i>	1++	3+	3	3+	3
<i>SrTmp</i>	3+	3+	3+	1+	2-
<i>Sr24</i>	2-	1+	1+	1+	2-
<i>Sr31</i>	1+	;	0;	;	;
<i>Sr38</i>	2+	3-	2-	2-	1+
<i>SrMcN</i>	4	3+	3+	3+	3
Races	TKTRC	RKJTF	TKTTC	PRJSC	KRKSC

Race analysis of 15 stem rust isolates collected from different agro-ecological zones in Iraq resulted from identification of five different races of *P. graminis* f. *sp. tritici* (Table 3), Race TKTRC was predominant in Sulaimani province, The A virulence/virulence pattern of race

TKTRC was *Sr10, Sr11, Sr24, Sr31, Sr38/ Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr17, Sr21, Sr30, Sr36, SrTmp, SrMcN*; while for race RKJTF which was predominant in Erbil was *Sr6, Sr17, Sr24, Sr31, Sr36/ Sr5, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9g, Sr10, Sr11, Sr21, Sr30, Sr38, SrTmp, SrMcN* followed by race TKTTC; Race TKTTC was only detected in Nineveh and showed virulence on resistance genes *Sr5, Sr6, Sr7b, Sr8a, Sr9a,*

Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr17, Sr21, Sr30, Sr36, SrTmp, SrMcN; Two races were detected in Diala, Race PRJSC that was virulent on *Sr5, Sr6, Sr7b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr30, SrMcN* followed by race TKTRC. The race KRKSC representing Dheqar was virulent on *Sr7b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr30, Sr36, SrTmp, SrMcN*.

Table 3. Avirulence/Virulence combination of *P. graminis* f. sp. *tritici* races and their frequency in different locations of Iraq

Race	Avirulence/ Virulence combination	No. of recovered samples	No. of recovered samples	Location
TKTRC	<i>Sr10, Sr11, Sr24, Sr31, Sr38/ Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr17, Sr21, Sr30, Sr36, SrTmp, SrMcN</i>	3	36.4	Sulaimani
RKJTF	<i>Sr6, Sr17, Sr24, Sr31, Sr36/ Sr5, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9g, Sr10, Sr11, Sr21, Sr30, Sr38, SrTmp, SrMcN</i>	2	18.8	Erbil
TKTTC	<i>Sr11, Sr24, Sr31, Sr38/ Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr17, Sr21, Sr30, Sr36, SrTmp, SrMcN</i>	1	27.3	
TKTTC	<i>Sr11, Sr24, Sr31, Sr38/ Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr17, Sr21, Sr30, Sr36, SrTmp, SrMcN</i>	2	27.3	Nineveh
PRJSC	<i>Sr8a, Sr17, Sr21, Sr24, Sr31, Sr36, Sr38, SrTmp/ Sr5, Sr6, Sr7b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr30, SrMcN</i>	1	09.1	Diala
TKTRC	<i>Sr10, Sr11, Sr24, Sr31, Sr38/ Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr17, Sr21, Sr30, Sr36, SrTmp, SrMcN</i>	1	36.4	
KRKSC	<i>Sr5, Sr8a, Sr24, Sr31, Sr36, Sr38, SrTmp/ Sr7b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr30, Sr36, SrMcN</i>	1	09.1	Dheqar

No virulence detection against resistance genes *Sr24* and *Sr36* in all Pgt races is a good evidence for absence of *Ug99* race or its group member in all wheat fields in Iraq. Confirmation of this fact is supported by Real-Time PCR analysis (Table 4). All the Iraqi tested samples showed negative response to three of the four markers in the *Ug99* stage-1 assay, while the *Ug99* control sample (o4KEN156-4, race TTKSK) was positive for all four markers. However, this test could not separate *Ug99* race group members. The SNP marker assay results (Table 5) also confirmed that all of the Iraqi samples of Pgt are not members of the *Ug99* race group. Although there are difficulties with some of the SNP probes, as they did not amplify.

That's why the assay was repeated twice with no amplification success. However, enough of the SNP markers did work on the samples (with the exception of D11Irq004) to be confident with the results. The top sets of Table 4 is the result for different isolates of the *Ug99* race group for each of these 8 SNP markers. The bottom set represents the Iraqi tested samples results. This assay system provides a rapid and accurate method for diagnosis of *Ug99* RG and will allow pathologists to monitor the movement of this economically important group of races of the wheat stem rust fungus.

Historically, presence of black stem rust in the northern parts of Iraq including Kurdistan region was rare or not known before particularly in Sulaimani province

which is a cold area since the disease is predominant in warm and humid areas (Al-Beldawi, 1993; Roelfs *et al.*, 1992). Extensive survey results during the period 2009-2011 which covered 330 wheat fields and research stations in Iraq revealed from detection of trace infections in Sulaimani in 2009, low to high stem rust incidence in 23% of wheat fields in 2010, and limited infections occurred in 1% of the fields in 2011. Disease severities reached to 80% on some genotypes in Sulaimani (IPM, 2012). Detection of high stem rust infection on some wheat genotypes in Sulaimani is concerning because the area is adjacent to Iran, and not far from the region where the race *Ug99* was earlier reported (Nazari *et al.*, 2009). Furthermore the possibility of movement of this race to our area by wind trajectory or other ways is high (Roelfs *et al.*, 1988), particularly most of the local grown wheat cultivars are susceptible to this race (IPM, 2013). Local analyses of virulence in this location indicated virulence against resistance genes *Sr6*, *Sr9e*, *Sr13*, *SrTmp*, *Sr14*, *Sr28* and *Sr36* at adult plant stag (Al-Maarof *et al.*, 2012;

Al-Hamdany and Abass, 2003). Previous results on race identification of *P. graminis* f.sp. *tritici* in Iraq referred to identification of high number of races comparing with our results (Al-Beldawi *et al.*, 1996; Natouret *et al.*, 1971). This may be due to the cultivation of diverse and indigenous wheat germplasm grown at that time. Race TKTTC representing Nineveh was formerly identified in Turkey (Mert *et al.*, 2010), Race KRKSC representing Dheqar was predominant in Lorestan, Iran (Gomari, 2014), Race RKJTF representing Erbil is similar to RKGTF that was dominant in Turkey (Mert *et al.*, 2010). Races TKTRC and PRJSC representing both Sulaimani and Diala, respectively are not known in the neighboring countries. Possibility of moving Pgt races from their origin sources in Turkey and Iran to wheat fields in Iraq by wind trajectory is very high. The occurrence of Barberry, alternate host of the pathogen in both countries enhances the genetic variation in the pathogen population and appearance of new races that may move to our area (Jahani *et al.*, 2013).

Table 4. Real-Time PCR analysis of five Iraqi Pgt isolates comparing with race TTKSK

	Reaction 1	Reaction 2	Reaction 3	Reaction 4
SR-SUI 1	Negative	30.82	Negative	Negative
SR-ERI 5	Negative	32.93	Negative	Negative
SR-NII 7	Negative	29.90	Negative	Negative
SR-DII 12	Negative	34.63	Negative	Negative
SR-DHI 15	37.93	Negative	Negative	38.08
Positive control Pgt Isolate 05 KEN156/4	23.79	24.22	25.62	26.49
Negative control	Negative	Negative	Negative	Negative

The long-distance transport of the pathogen spores depends on the urediospore resistant to atmospheric conditions and the effect of harmful rays (Roelfs *et al.*, 1992). Studying the dynamics and pathways of the wheat stem rust will be very important and essential in Iraq. This information will be helpful in the proper exploitation and management of the available sources of resistance. This study indicates the absence of the race *Ug99* of Pgt or its group members in wheat field in

Iraq. It also refers to identification of five races of Pgt, TKTRC, RKJTF, TKTTC, PRJSC and KRKSC from wheat fields belongs to Sulaimani, Erbil, Nineveh, Diala and Dheqar, respectively. It also recommends conducting similar studies to diagnose all the possible presence of Pgt races and to determine their distribution and frequencies in all wheat fields in Iraq.

Table 5. DNA analysis of *P. graminis* f. sp. *tritici* races collected from different location in Iraq compared with *Ug99*- race group members using 8 SNP markers.

	TTK SF1	TTK SF2	TTK SK1	TTK SK2	TTT SK1	TTT SK2	TTK ST1	TTK ST2	TTK SP	PTK ST
CDL_PGT_A003	CC	CC	CC	CC	CC	CC	CT	CC	CC	CT
CDL_PGT_A007	GG	GG	GG	GG	GG	GG	AG	GG	GG	GG
CDL_PGT_A010	TT	TT	TT	TT	TT	GG	TT	TT	TT	TT
CDL_PGT_A013	CC	CC	CC	CC	CC	CC	TT	TT	CC	TT
CDL_PGT_A014	AA	AG	AA	AA	AA	AA	AA	AA	AA	AA
CDL_PGT_A017	CC	CC	CC	CC	TT	CC	CC	CC	CC	CC
CDL_PGT_A021	AA	AA	TT	TT	TT	TT	TT	TT	AA	TT
CDL_PGT_A022	CT	TT	CT	CT	CT	CT	CT	CT	TT	CT

	SR-DII 12 “Diala”	SR-NII 7“Nineveh”	SR-ERI 5 “Erbil”	SR-SUI 1 “Sulaimania”	SR-DHI 15 “Deqar”
CDL_PGT_A003	Negative	CC	CC	CC	Negative
CDL_PGT_A007	GG	GG	GG	GG	Negative
CDL_PGT_A010	Negative	TT	TT	TT	TT
CDL_PGT_A013	CC	CC	Negative	CC	Negative
CDL_PGT_A014	Negative	Negative	Negative	Negative	Negative
CDL_PGT_A017	Negative	Negative	Negative	CC	Negative
CDL_PGT_A021	Negative	AA	AA	AA	AA
CDL_PGT_A022	CC	CC	CC	CC	Negative

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