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Phenotypic and molecular characterization of resistance to stem rust in wheat cultivars and advanced breeding lines from Iran and Syria

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ABSTRACT

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Seedling and adult-plant response to stem rust of 103 Iranian and Syrian bread and durum wheat genotypes was investigated using stem rust races TKTTC and TTKSK in seedling tests and TKTTC and TTKST at the adult-plant stage. The same genotypes were characterized with simple sequence repeat (SSR), sequence tagged site (STS) and cleaved amplified polymorphic sequence (CAPS) markers linked to *Sr2*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr31*, *Sr36*, *Sr39*, and *Sr46*. In seedling tests, three phenotypic groups were identified: those lacking effective resistance genes, those postulated to carry *Sr31*, and those that were resistant to TKTTC and TTKSK. Adult-plant assessment indicated the presence of adult-plant resistance (APR) to TKTTC and TTKST mainly due to *Sr2* and uncharacterized resistance gene(s). Using molecular markers, *Sr2* was confirmed as the most common resistance gene detected in Iranian genotypes. It was detected alone in 38 genotypes, in combination with *Sr31* in 11 genotypes, and combined with *Sr31*+*Sr25* in one genotype. In total, *Sr31* was postulated in 19 genotypes. Among Syrian genotypes, *Sr2* was detected alone in one and in combination with *Sr31* in two bread wheat cultivars. No diagnostic DNA fragment associated with the markers was present in the durum wheat genotypes. Haplotype analysis of 103 genotypes using *Sr2*-linked markers *csSr2* and *STS#Sr2* indicated that *csSr2* was a better predictor of the presence of *Sr2* gene in wheat.

Keywords: adult plant resistance, bread wheat, durum wheat, haplotype analysis, resistance genes

INTRODUCTION

Wheat is one of the world's most important crops and a major staple food for many people in Central, West Asia and North Africa (CWANA), including Iran and Syria. About 6.3 and 1.7 million hectares are sown to wheat in Iran and Syria, with an annual production of 12.3 and 3.9 million tons, respectively (FAO, 2012). However, wheat productivity is threatened by abiotic and biotic stresses, including the wheat rusts. Stem rust caused by *Puccinia graminis* Pers f. sp. *tritici* Eriks. & E. Henn. (*Pgt*) is the most devastating of the three rust diseases; historically, it has caused severe crop losses in many parts of the world (Singh *et al*., 2008). Occasional stem rust epidemics have been reported in CWANA countries, particularly prior to the use of stem rust resistant semi-dwarf cultivars (Shariff and Bamdadian, 1974; Mamluk and El-Naimi, 1992; Torabi *et al*., 1995). Since the mid-1950s, deployment of stem rust resistance genes in wheat cultivars has effectively controlled wheat stem rust worldwide (Singh *et al*., 2008; Yu *et al*., 2010).

Generally, resistance was based on very few resistance genes, and *Sr31* is an example of major-gene resistance that remained effective for a long time (Pretorius *et al*., 2000; Singh *et al*., 2006). The *Sr31*-virulent pathotype known as race Ug99 (or TTKSK) was first collected in Uganda in 1998. Subsequently, the presence of the same race was confirmed in Kenya in 2001 (Wanyera *et al*., 2006), Ethiopia in 2003 (Singh *et al*., 2006), Yemen and Sudan in 2006 (Jin *et al*., pers. comm., cited in Singh *et al*., 2008) and Iran in 2007 (Nazari *et al*., 2009).

In early studies, wheat genotypes with *Sr24* and *Sr36* were highly resistant to race TTKSK, but variants that were virulent to *Sr24* (TTKST) were found in Kenya in 2006 (Jin *et al*., 2008) and increased to cause epidemics on cultivar KS Mwamba, which possesses *Sr24* (Njau *et al*., 2009). A second variant (TTTSK) virulent to *Sr36* was recorded in Kenya in 2007 (Jin *et al*., 2009). Race TTKSK was reported in southwestern Iran in 2007 (Nazari *et al*., 2009) and in areas further south in 2009 and 2010 (Patpour *et al*., unpublished data). Although stem rust occurs sporadically in Syria, so far none of the Ug99 group has been identified. This early work and the realization that the Ug99 race group constituted a major threat to wheat cultivars grown worldwide led to the launching of the Borlaug Global Rust Initiative, a major international effort to contain its further spread to important wheat growing areas in a number of developing as well as developed countries.

The continuing presence of Ug99 in Iran has not only been cause for concern to farmers and agricultural authorities, but also poses a threat to neighboring countries in the event of a significant epidemic. Although more than 50 stem rust resistance genes have been identified and formally catalogued, only a few are effective against the Ug99 group. Among the seedling (or all stage) resistance genes, *Sr13*, *Sr22*, *Sr25*, *Sr26*, *Sr28*, *Sr32*, *Sr33*, *Sr35*, *Sr44*, *Sr45*, and *Sr46* are effective against Ug99. The only well documented adult plant (and potentially durable) resistance genes are *Sr2*, *Sr55*, *Sr57* and *Sr58*, but others of potential value are continuing to be reported in a range of conventional genetic and association mapping studies, but none, including the widely deployed *Sr2*, confer high levels of resistance in all genetic backgrounds when present alone (McIntosh and Pretorius, 2011).

The genetics of stem rust resistance was traditionally studied by inheritance studies, and using gene-postulation and cytogenetic techniques (Knott, 1989). These methods are time-consuming, laborious, and depend on the availability of testing facilities and a degree of expertise (Yu *et al*., 2010). Molecular markers linked to *Sr* genes may be useful in overcoming the difficulties of these approaches and are proving to be promising tools for characterizing resistance to wheat rusts (Yu *et al*., 2011; Bernardo *et al*., 2013; Haile *et al*., 2013). To date, more than 20 molecular markers linked to *Sr* genes are available (McIntosh *et al*., 2008; see http://rustopedia.get-traction.com/ traction and http://maswheat.ucdavis.edu) and some have been used in marker-assisted selection (MAS) practices for characterizing elite breeding lines, haplotyping to clarify the relationship among different sources of resistance and pyramiding rust resistance genes. Despite the successful development of high yielding wheat cultivars, there is a paucity of information on the genetic basis of stem rust resistance in wheat genotypes grown in Iran and Syria.

The purpose of this study was to determine the presence and prevalence of important stem rust resistance genes in commercial cultivars and advanced lines of bread and durum wheat that represent historical and current gene pools in breeding programs in Iran and Syria.

MATERIALS AND METHODS

Host materials

A collection of 89 Iranian bread wheat cultivars and advanced breeding lines and 14 Syrian wheat cultivars (seven bread wheat and seven durum wheat) obtained from the Seed and Plant Improvement Institute (SPII), Karaj, Iran, and ICARDA, respectively, were assessed for seedling and adult-plant resistance to stem rust (Table 1). In each seedling test, the North American stem rust differential lines (Jin *et al*., 2008) were used as controls and for confirming race identities. In addition, lines possessing *Sr2* (Inia 66), *Sr22* (Mq*6//Stewart*3/RL 5244), *Sr24* (Prelude*6//Prel/Mq*8/Agent), *Sr25* (Agatha/9*LMPG-6)), *Sr26* (Eagle), *Sr31* (Benno/6*LMPG-6), *Sr36* (W2691SrTt-1), *Sr39* (RL 5711) and *Sr46* were used as controls in marker analyses.

Pathogen isolates and seedling assessment

Pgt races TKTTC and TTKSK (Table 1), identified in Syria (K. Nazari, unpublished data) and Iran (Nazari *et al*., 2009), respectively, were used in seedling assessments of test genotypes at ICARDA Experimental Research Site in Tel-Hadya, Syria, and

Table 1. Pgt races used in seedling and adult-plant tests of Iranian and Syrian wheat cultivars and advanced lines.

Isolate Accessions	Race	Avirulence/ virulence formula†
ICARDA Pgt 10-2S1	**TKTTC**	***11, 24, 31, 38 / 5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 17, 21, 30, 36, McN, Tmp***
IR CPU Pgt 88-4	**TTKSK**	***24, 36, Tmp / 5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 31, 38, 30, McN***
Ug99+vir Sr24	**TTKST**	***36, Tmp / 5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 24, 31, 38, 30, McN***

† In avirulence /virulence formula, Sr genes are ordered according to the revised North American differential genotypes (Jin *et al*., 2007).

SPII, Karaj, Iran. Race identities were based on the North American race nomenclature system (Jin *et al*., 2008). Prior to inoculation of test genotypes, differential lines and susceptible cultivar Morocco were grown in peat moss at 20°C for 10 days.

Urediniospores of races TKTTC and TTKSK that were stored at -80°C were first heat-shocked at 42°C for 5 minutes and then urediniospore suspensions in light industrial mineral oil (Soltrol 170; Phillips Petroleum Co., the Woodlands, TX) were atomized over seedlings at first-leaf stage using a small pressure inoculator. Inoculated plants were incubated in a humid chamber at 18±2°C in darkness for 16 h followed by 8 h fluorescent light, and then maintained in a glasshouse at 18±2°C with 16 h light/ 8 h darkness. Seedling infection types (IT) were recorded 14 days post inoculation following a 0; (flecks) and 0 to 4 scale (Stakman *et al*., 1962; McIntosh *et al*., 1995). Genotypes with ITs of 0; and 0 to 2, or combinations thereof, were considered resistant, whereas genotypes with ITs 3 to 4 were considered susceptible.

Pathogen isolates and adult-plant assessment

In order to evaluate wheat genotypes' adult-plant responses to *Sr31*-avirulent *Pgt* race (TKTTC), adult-plant assessments were conducted on inoculated nurseries in a plastic house at ICARDA, Tel Hadya, Syria, and in field trials inoculated with TTKST (virulent to *Sr31* and *Sr24*) at Kenya Agricultural Research Institute (KARI), Njoro, Kenya. At ICARDA, each entry was planted as a 10-cm hill plot with 10 cm spacing between plots. At Njoro, entries were planted in two 1-m rows with 30 cm spacing between rows. Infection was ensured with the use of susceptible spreaders, including Morocco and PBW#343.

Rust responses were recorded as resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) (Roelfs *et al*., 1992), together with a percentage severity rating according to the modified Cobb scale (Peterson *et al*., 1948). Field responses were recorded three times, with the final rating taken at the soft-dough stage when disease severity on susceptible cultivars was 100%.

Molecular marker analysis

Genomic DNA extraction was carried out according to Ogbonnaya *et al*. (2001), on two-week-old seedlings using pooled leaf samples from five individual plants, that were frozen in liquid nitrogen and stored at -80ºC before DNA extraction. DNA concentration was estimated on 0.8% agarose gel.

DNA fragment analysis

All test genotypes were analyzed using published SSR, CAPS and STS markers associated with stem rust resistant genes *Sr2, Sr22, Sr24, Sr25, Sr26, Sr31, Sr36, Sr39,* and *Sr46* (Table 2). However, only *Sr2*, *Sr25*, and *Sr31* showed polymorphism among the 103 lines reported in the present study.

PCR amplifications were carried out in 10 µl volumes containing 50 ng of genomic DNA, 10 pmol each of forward and reverse primers, 1 U of *Taq*-polymerase, 1X PCR buffer, 2.5 mM $MgCl_2$, and 0.25 mM dNTPs. PCR were carried out for individual genes in a 96-well plate using an Applied Biosystem thermal cycler. PCR products for *Sr24, Sr25, Sr26, Sr39, Sr46,* and *Sr31* were separated in 1.5% agarose gels in TBE (tris-borate-EDTA) buffer at 100 V power, followed by staining in ethidium bromide. The previously described protocol (Mago *et al*., 2011) was followed for the *csSr2* CAPS marker. Once the PCR for the *csSr2* was completed, an additional 5 µl of a mix consisting of 2.5 ml of 109 NEB buffer 4 and 0.5 µl of *Pag*I (*Bsp*HI) (10 U/µl; NEB) was added and the tubes were incubated at 37°C for 1 h. The CAPS product was separated on 2.5% (w/v) agarose and the genotypes were evaluated for three Hope-type fragments (172, 112, and 53 bp).

Fragment sizes were visualized under a UV lighting system. PCR products for *Sr22* and *Sr36* were separated in 8% denaturing polyacrylamide gel electrophoresis (PAGE) followed by silver staining (Chen *et al*., 2004).

Data analysis

The 103 genotypes in the Iranian group were divided into spring/facultative and winter entries, and Syrian cultivars were separated into bread wheats and durum wheats. Within the spring/facultative category, genotypes were grouped according to their seedling infection types against TKTTC and TTKSK to form three seedling resistance groups: 1) group 1 comprising genotypes with high (seedling) infection types (HIT) to both races; 2) group 2 comprising genotypes with HIT to TKTTC and low infection type (LIT) to TTKSK; 3) group 3 comprising genotypes with LIT against TKTTC and HIT against TTKSK.

RESULTS AND DISCUSSION

Iranian genotypes

Iranian spring/facultative genotypes

According to the seedling infection types against TKTTC and TTKSK and the adult-plant responses of test genotypes to TKTTC at ICARDA and TTKST at Njoro, or a combination thereof, the Iranian spring/facultative wheat genotypes were

Table 2. Diagnostic stem rust resistance gene markers used for molecular characterization of stem rust resistance genes in selected Iranian and Syrian bre

Sr-gene	Chromosome location[†]	Marker	Forward/Reverse primers	PCR conditions	Expected fragment size (
Sr2	3BS	*STS*	(details available from Dr. Wolfgang Spielmeyer, CSIRO, Canberra, ACT - Australia)	(Details from Dr. W. Spielmeyer, CSIRO, Canberra, ACT, Australia)	800
Sr2	3B	*csSr2*	5□ CAAGGGTTGCTAGGATTGGAAAAC 5□ AGATAACTCTTATGATCTTACATTTTTCTG	95°C 2 min 1 cycle, 95°C 30 s 30 cycles, 60°C 40 s, 72°C 50 s, 72°C 5 min 1 cycle, 15°C 1 min	172, 112, and
Sr22	7AL	*cfa2019*	5□ GACGAGCTAACTGCAGACCC 5□ CTCAATCCTGATGCGGAGAT	94°C- 30sec, 60°C- 30sec, 72°C-30sec (30 cycles)	234
Sr24	3DL or 1BS	*Sr24#12*	5□ CACCCGTGACATGCTCGTA 5□ AACAGGAAATGAGCAACGATGT	94°C-30sec, 65°C-30sec, 72◦C-40sec, -1°C touchdown (7 cycles) 94°C-30sec, 58°C-30sec, 72°C-40sec, 30 cycles	500
Sr25	7DL	*Gb*	5□ CATCCTTGGGGACCTC 5□ CCAGCTCGCATACATCCA	94°C- 30sec, 60°C- 30sec, 72°C- 1min (40 cycles)	130
Sr26	6AL/6Ae	*Sr26#43*	5□ AATCGTCCACATTGGCTTCT 5□ CGCAACAAAATCATGCACTA	94°C- 30 sec, 56°C-30sec 72°C- 40 sec (30 cycles)	207
Sr31	1BL.1RS	*iag95*	5□ CTGTGGATAGTTACTTGATCGA 5□ CCTAGAACATGCATGGCTGTTACA	94°C- 30sec, 55°C- 1min, 72°C- 1.10min (30 cycles)	1100
Sr36	2BS	*WMC477*	5□ CGTCGAAAACCGTACACTCTCC 5□ GCGAAACAGAATAGCCCTGATG	94°C- 1min, 61°C- 1min, 72°C- 2min (45 cycles)	190
Sr39	2B	*Sr39-Lr35*	5□ AGAGAGAGTAGAAGAGCTGC 5□ AGAGAGAGAGCATCCACC	94°C- 1min, 60°C- 1min, 72°C- 2min (35 cycles)	900
Sr46	2DS	*csSC46*	-(Details from Dr. E. Lagudah, CSIRO, Canberra, ACT, Australia)	(details available from Dr. Evans Lagudah, CSIRO, Canberra, ACT - Australia)	600

[†]***Sr2*: (Hare and McIntosh, 1979); *Sr22*: (The, 1973); *Sr24, Sr25, Sr26* (McIntosh *et al*., 1977); *Sr31* (Friebe *et al*., 1996); *Sr36* (McIntosh and Gyarfas, 1971) Unpublished data).**

Table 3. Seedling infection types and adult-plant responses of Iranian spring/facultative bread wheat cultivars and elite lines tested with races aviru

Entry	Name	Pedigree	Seedling IT†		Adult plant responses	
			TKTTC	TTKSK	ICARDA	Keny
1	M-83-17	Alvd/Bcn	4	4	20S	100S
2	Azadi	Mexp//4820/1-32-15409	4	4	40S	90S
3	N-86-6	Vorona/CNO79//Kauz/3/Milan	4	4	80S	90S
4	Zarrin	PK15841	4	4	50S	90S
5	WS-82-9	Ww33G/Vee'S'//Mrn/4/HD2172/Bloudan //Azd/3/San/Ald'S'//Avd	4	4	60S	90S
6	Ghods	Rsh/5/Wt/4/Nor10/K54*2//Fn/3/Ptr/6/Omid//Kal/Bb	4	4	30S	90S
7	N-85-12	W462//Vee/Koel/3/Peg//Mrl/Buc	4	4	80S	90S
8	Shiraz	Gv/D630//Ald'S'/3/Azd	4	4	80S	80S
9	Sorkhtokhm	Local landrace	4	4	90S	80S
10	Pishtaz	Alvand//Aldan/Ias58	4	4	80S	80S
11	S-85-10	PRL/2*Pastor	3+4	4	50S	80S
12	Sabalan (W)	908/FnA12// 21-32-438	4	3	10S	70S
13	N-86-3	Nanjing 82149/Kauz/3/Pfau/Seri//Bow (DH)	4	4	20S	70S
14	Tajan	Bow'S'/Nkt'S'	4	4	80S	70S
15	Mahdavi	Ti/Pch/Mt48/3/Wt*//Nar59/Tota63/4/Mus	4	4	60S	70S
16	Darab#2	Maya'S'/Nac	4	4	70S	70S
17	Karaj 2	Omid//Fa/Th-Mt	4	4	70S	70S
18	Karaj 1	Rsh/Vfn	4	4	70S	70S
19	Arvand	Rsh//Mt-Ky/My48	3+	4	70S	70S
20	Chamran	Attila (CM85836-50Y)	4	4	70S	70S
21	Marvdasht	HD2172/Bloudan//Azd	4	4	70S	60S
22	Darab	Rsh/Irni49//C271/3/PK868	4	4	60S	60S
23	Sholeh	Local	4	4	30S	60S
24	Inia 66	Lr64/Sn64	3+4	4	80S	60S
25	Moghan 1	LRr/N10B//3*AnE	3+4	4	50S	50S
26	Niknejad	F13471/Crow'S'	4	4	50S	60M
27	S-85-14	Venac'S'/Florkwa'S'	4	4	40S	60M
28	Alvand	1-27-6275/CF1770	4	4	70S	50M
29	S-85-19	Inqalab 91*2/Kukuna	4	4	80S	50M
30	Alborz	Frontana/Mida//Kenya117-A/3/2*Collafen/4/Son64/Klein-REND/3/Cno67(SIB) //LR64*2/Son64	3+	4	60S	40M
31	Sistan	Bank'S'/Vee'S'	3+	4	80S	10M
32	Sirvan	PRL/2*Pastor	3+	4	50S	50M
33	Kavir	Stm/3/Kal//V534/Jit716	4	4	80S	50M
34	Akbari	1-63-31-/3/12300/Tob//Cno/Sx-0IRN	4	4	80S	30M
35	Tabasi	Local landrace	4	4	80S	30M
36	N-86-4	Milan CM75118//KA CM 75118/K1/3/Tajan (Doubled Haploid)	4	4	80S	5MR
37	Adl 2	Fr3*/MM/Mt//Rsh	4	4	80S	30R
38	Hirmand	Bty/4/Jar//Cfn/Sr70/3/Jup'S'	4	4	80S	30R
39	Parsi	Dove'S'/Buc'S'//2*Darab	3+	4	80S	10R
40	Sivand	Kauz'S'/Azd	3+4	4	80S	10R
41	Bam	Vee'S'/Nac//1-66-22	3+	33+	80S	5R

† Seedling infection types were recorded according to a 0; (flecks), and 0- to – 4 scale. Symbols "–" and "+" were used to describe deviation from the C and N were used to describe extensive chlorosis and necrosis, respectively, associated with infection type.

divided into three major groups.

Group I

The first group (Table 3) comprised 41 cultivars/lines that exhibited high seedling infection types (HIT) of 3 to 4 against TKTTC and TTKSK. The seedling HITs are interpreted as indications of the absence of effective seedling resistance genes against the two *Pgt* races. The 41 genotypes exhibited varying responses ranging from 10S to 90S when tested with race TKTTC at ICARDA. Among these genotypes, advanced lines M-83-17 and N-86-3, and cultivars Ghods, Sabalan, and Sholeh showed relatively low disease severities of 10 to 30%. The remaining genotypes displayed high disease severities ranging from 40 to 90%.

In Njoro, Kenya, entries 1 to 25 (Table 3) showed high stem rust severity responses at the adult plant stage which ranged from 50S to 100S. In this group, 15 genotypes were postulated to carry *Sr2* using both the *STS#Sr2* and *CsSr2* markers. This result is consistent with previously published studies. Very high stem rust severities in wheat genotypes possessing *Sr2* alone were previously noted by Singh *et al*. (2008).

Six genotypes (entries 26 to 31) exhibited adult plant responses ranging from 10MS to 60MS in Njoro, Kenya. Within this group, *Sr*-positive alleles for *csSr2* were postulated in five genotypes (Niknejad, S-85-14, Alvand, S-85-19 and Alborz) with the *Sr2*#STS marker. However, presence of *Sr2* was postulated in two cultivars, Alvand and Niknejad, when marker *csSr2* was used. The susceptibility of Sistan (entry 31) to race TKTTC while showing an adult response of 10MS to TTKST in Njoro, was interpreted as an indication of the presence of an uncharacterized stem rust APR gene for TTKSK in cv. Sistan.

Entries 32 to 36 (Table 3) exhibited moderate adult plant responses ranging from 5MR to 50MR. The presence of *Sr2* was postulated in cultivars Kavir, Akbari, and advanced line N-86-4 with both *Sr2*-linked markers. The adult plant resistances of cv. Sirvan and cv. Tabasi have yet to be characterized.

Among entries 37-41, Adl 2, Hirmand, Parsi, Sivand and Bam bread wheat cultivars showed high levels of adult-plant resistance ranging from 5R to 30R in Kenya, indicating the presence of effective race-specific APR gene(s) in these cultivars. *Sr2* was postulated in Hirmand, Parsi and Bam using the *Sr2* marker *STS#Sr2*; however, with *csSr2* this gene was not detected in Parsi (Table 3). Nonetheless, the high level of adult plant resistance of these three cultivars was considered as evidence for the presence of additional resistance genes and not just *Sr2* alone. The seedling and adult-plant susceptibility of the remaining 10 genotypes in this group indicated the absence of effective seedling and adult plant resistance genes in these genotypes.

In general, the disease severities of the genotypes tested against TKTTC were higher than the disease severities of those same genotypes tested against TTKST in Njoro, Kenya. This difference was most likely due to the high inoculum pressure and prolonged favorable conditions in the plastic house at ICARDA. Entries 34-41 (30MR-5R) seemed to have some race-specific APR genes, but these uncharacterized genes did not show an effect on race TKTTC in ICARDA.

Group II

Only cultivars Morvarid and Maroon (Table 4) were included in this group. Seedlings of these cultivars were susceptible to race TKTTC and resistant to TTKSK. Seedlings of both cultivars were susceptible to race TKTTC, but resistant to TTKSK. Both showed moderately resistant responses in Kenya. The temporarily designated stem rust resistance gene, *SrSha7,* postulated in Shanghai 7, a parent of Morvarid (R. Singh, pers. comm.) confers a low seedling infection type similar to that of *SrTmp* against TTKST, while the *SrTmp* control showed IT $3^{+}4$ against TKTTC. This may explain Morvarid's seedling and adult-plant susceptibility to TKTTC at ICARDA in contrast to its resistance at SPII and at Njoro. Maroon showed somewhat similar seedling and adult plant responses and may have a resistance gene in common with Morvarid. In addition, Morvarid was postulated to carry *Sr2* using the markers.

Although the responses of cultivars Morvarid and Maroon to TKTTC (virulent to *SrTmp*) and TTKSK (avirulent to *SrTmp*) may be due to *SrTmp*, there appear to be additional differences between these two pathotypes for *Sr*-genes not included in the North American differential set. Further tests are required to characterize these resistances.

Group III

This group comprised 27 genotypes (Table 5) and its distinguishing characteristics are that 26 genotypes showed almost identical low seedling responses to TKTTC, whereas one gave a slightly higher, but low, IT 2+. All were susceptible to TTKSK and showed a seedling LIT/HIT pattern against the two races that was similar to that of Benno/6*LMPG-6 with *Sr31*. *Sr31* was therefore postulated in these genotypes. This was also confirmed by the presence among the genotypes of a diagnostic DNA fragment linked to the *Sr31* gene.

Among genotypes with seedling resistance to TKTTC, 15 genotypes (entries 1 to 15) showed an

Table 4. Seedling infection type and adult-plant responses of Iranian spring/facultative bread wheat cultivars and advanced lines to r *Sr31* in resistance group II.

Entry	Name	Pedigree	Seedling IT		Adult plant response		*Sr2*
			TKTTC	TTKSK	ICARDA	Kenya	#STS
1	Morvarid	Milan/Shanghai7	3+4	;-2	50S	5MRMS	+
2	Maroon	Avd/5/Pchu/4/28mt54A/3/N10//Kt54B/Nar59/3/7c	4	1+2	30S	40MS	-

Table 5. Seedling infection types and adult plant responses of Iranian spring/facultative bread wheat cultivars and advanced lines to races resistance group III.

Entry	Name	Pedigree	Seedling IT		Adult plant response		*Sr2*
			TKTTC	TTKSK	ICARDA	Kenya	#STS
1	N-86-12	TNMU/6/CEP80111/CEP81165/5/MRNG/ 4/YKT406/3/AG/ASN//ATR	1	4	5R	10MR	+
2	Moghan 2	Choti Lerma	1	4	5R	30MR	+
3	N-86-8	Pastor/3/Vorona/CNO79//Kauz	1+	4	5R	50MR	+
4	Atrak	Kauz	11+	4	5R	50MS	-
5	N-86-7	Seri*3//RL6010/4*YR/3/Pastor/4/BAV92	1	4	5R	70MS	+
6	N-85-5	Atrak/Wangshuibai	1-	4	5R	40S	+
7	Dez	Kauz*2/Opata//Kauz	1	33+	5R	60S	-
8	Golestan	Alondra'S'	1	4	5R	70S	-
9	WS-85-8	Site/Mo/4/Nac/TH.AC//3*PVN/3/Mirlo/Buc	;1	4	5R	70S	-
10	WS-85-15	PBW343*2/Konk	;1	4	5R	80S	-
11	Bahar	Blayka	11+	4	5R	80S	+
12	Chenab	NA	1+	4	5R	90S	+
13	N-85-10	Cndo/R143//Ente/Mexi_2/3/Aegilops …	11+	4	5R	80S	+
14	M-85-7	Seri 82//Shuha'S'/4/Rbs/Anza/3/Kvz/Hys//Ymg/Tob (Accession No 1-8261)	1	4	5R	80S	-
15	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	1+	4	5R	80S	-
16	M-85-16	Pastor/3/Vorona/CNO79//Kauz	1	4	5MR	60MS	-
17	M-85-17	SITE/MO/3/Vorona/Bau//Bau	;1	4	10MR	60MS	-
18	Navid	Kirkpinar79	1+	4	10MR	10S	+
19	WS-85-16	PBW343*2/Khvaki	;1	4	10MR	80S	+
20	M-85-15	MV 22-77//Stephon/3/Mon'S'/Lmu'S'//Falke/4/Zarin	1	4	10MR	90S	-
21	Shiroudi	Attila (CM85836-4Y)	;1	4	20MR	90S	+
22	Rassool	Veery'S'≠7 = Kvz/Buho'S'//Kal/Bb	;1-	4	20MR	40S	+
23	Falat (# Seri 82)	Kvz/Buho'S'//Kal/Bb	;1	4	30MR	50MS	-
24	Zagross	Tan'S'/Vee'S'//Opata 85	1	4	30MR	90S	+
25	M-85-6	Seri 82//Shuha'S'/4/Rbs/Anza/3/Kvz/Hys/ /Ymg/Tob (Accession No 1-8260)	1	4	30MS	90S	-
26	WS-85-9	Weaver/4/NAC/TH.AC//3*PVN/3/Mirlo/BUC	1+	33+	30MS	40MRMS	-
27	N-85-14	Berkut	2+	4	50S	80S	+

adult-plant resistance response of 5R, nine genotypes were moderately resistant (MR) with disease severities of 5 to 30%, and two genotypes displayed an adult-plant response of 30MS. The genotype with an IT of 2+ (entry 27) displayed an adult-plant response of 50S to TKTTC. Adult plant responses of the 27 genotypes in Njoro ranged from 10MR to 90S.

Among the 15 genotypes resistant to TKTTC, cultivar Moghan 2 and elite lines N-86-12 and N-86-8, both from CIMMYT, showed acceptable levels of APR (30MR, 10MR, and 50MR, respectively) against TTKST, Atrak (Kauz) and elite line N-86-7 displayed MS type reactions with relatively high disease severities, and the remaining genotypes were susceptible. Among the nine genotypes moderately resistant to TKTTC, cultivar Falat (Seri 82) and elite lines M-85-16 and M-85-17 showed 50-60MS, and the other six genotypes were more susceptible when tested with TTKST. Although Navid was susceptible at Njoro, the low disease severity of this cultivar was due to its relatively late maturity and hence late disease development compared to the spring-type genotypes in this group. Except for the elite line N-85-14 (entry 27), the remaining 26 genotypes were phenotypically expected to carry *Sr31*, according to the seedling LIT and HIT profiles and resistant/susceptible responses to TKTTC and TTKST.

The seedling infection type of genotypes known to carry the 1B.1R translocation (Falat = Seri 82, Atrak = Kauz 'S', and Shiroodi = Attila 4Y) was one indication of the presence of *Sr31*. In some cases, the presence of the source of *Sr31* in the pedigrees of test genotypes explains the presence of *Sr31*. In analysis of the *Sr31* marker, of the 26 test genotypes predicted to carry *Sr31*, 17 genotypes (in group 3) contained *Sr31* based on its diagnostic DNA marker *iag95*, of which six genotypes were postulated to carry *Sr31* singly, while one (M-85-17) had *Sr2*, *Sr25* and *Sr31* combined based on the STS *Gb* marker for *Sr25/Lr19* (Liu *et al*., 2010; Mago *et al*., 2005b). Gene combination of *Sr2+Sr31* was intensified in 10 genotypes. The adult-plant resistance of cultivar Arta (entry 15) and elite lines M-85-7 and M-85-15 has yet to be characterized.

Iranian winter wheat genotypes

Based on seedling infection types, 21 winter wheat genotypes were classified into two response groups.

Group I

This group consisted of 14 genotypes that showed seedling HIT of 3-4 against both races (Table 6), indicating an absence of seedling-effective resistance genes. In adult-plant tests with TKTTC at ICARDA, cultivars Karaj 3 and Gascogne both showed adult responses of 5R, and the elite line C-84-4 displayed an adult response of 10MS. The resistance responses of these three genotypes are considered an indication of uncharacterized adult-plant resistance to TKTTC which has yet to be investigated. The remaining 11 genotypes showed susceptible reactions ranging from 30S to 85S. Cultivar Gascogne was not scored at Njoro due to its very late maturity and the other two genotypes were susceptible.

Cultivars Azar 2, Zareh, Kaveh, Soissons, Bezostaya and Gaspard were also very late and therefore their field reactions at Njoro, Kenya, was not included in Table 6. Interestingly, local cultivars Roshan, Shahpasand and Omid, which exhibited a susceptible response of 80S to TKTTC, displayed adult plant responses of 30MR, 10R, and 10R, respectively, against TTKST at Njoro, Kenya. Shahpasand and Omid were postulated to carry *Sr2* using the *Sr2* markers. The high levels of adult resistance (10R) of these cultivars indicated they possess additional race-specific APR gene(s) against TTKSK. The basis of the adult plant resistance of Roshan is unknown and needs further investigation. *Sr2* was also detected in Azer 2. According to McIntosh *et al*. (1995) and a recent publication by Mago *et al*. (2011), Bluebird (BB in CIMMYT's pedigree abbreviation system) is a source of *Sr2* in CIMMYT germplasm, and the pedigree information of Azar 2 indicates that Bluebird is the most likely origin of *Sr2* in this cultivar. The origin of *Sr2* in resistant local cultivars has yet to be characterized.

Group II

The second group of winter genotypes comprised four elite lines and Hungarian cultivar MV17 (Table 7). These genotypes had LIT of 0; 1 to 1- to TKTTC and HIT of 33^{+} and 4 against TTKSK.

In contrast to elite line C-85-3, which had an adult response of 40MS (slightly higher than the response of lines with *Sr31*), elite lines C-81-10 and C-84-8 showed resistance responses of 5R to TKTTC, while elite lines C-85-6 and MV17 displayed resistance responses of 10MR and 20MR, respectively, at ICARDA. Because of their late maturity, MV17 and C-84-8 were not scored at Njoro but the other three lines were susceptible. Based on the similar IT pattern of those genotypes with differential source of *Sr31*, the test genotypes may carry *Sr31*. However, using the *iag95* marker, *Sr31* was detected only in MV17 and C-85-6. Since MV17 appears in the pedigree of C-85-6, the presence of the same gene is likely. *Sr31/Yr9* was already reported in Hungarian cultivar MV17

Table 6. Seedling infection types and adult-plant responses of Iranian winter bread wheat cultivars and advanced lines to races avi in winter wheat resistant group I.

Entry	Name	Pedigree	Seedling IT		Adult plant response		*Sr2* #STS	S #
			TKTTC	TTKSK	ICARDA	Kenya		
1	Karaj 3	Drc/Mxp//Son64/Tzpp-Y54/3/Nai60	4	4	5R	60S	-	
2	C-84-4	MV17/Zrn (Accession No 4017)	3	4	10MS	100S	-	
3	C-81-4	Ald'S'/Snb'S'/6/T.aest/5/Ti/4/La/3/Fr/Kad//Gb	3	4	40S	80SMS	-	
4	Oroum	Alvand//NS732/Her	4	4	80S	60S	-	
5	Roshan	Local	4	4	80S	30MR	-	
6	Shahpasand	Local	4	4	80S	10R	+	
7	Omid	Local	4	4	80S	10R	+	
8	Gascogne	TJB-990-8/Marengo	3+	4	5R	-	-	
9	Azar#2	Kvz/Ym71//3/Maya'S'//Bb/Inia/4/Sefid	4	4	30S	-	+	
10	Zareh	130L1.11//F35.70/Mo73/4/Ymh/Tob//Mcd/3/Lira	4	4	85S	-	-	
11	Kaveh	Fta-P1	3+4	4	80S	-	-	
12	Soissons	Iena/3/Jena//Hybride-Naturel/HN-35	4	3	50S	-	-	
13	Bezostaya	-	4	4	50S	-	-	
14	Gaspard	Arminda/FD-71036	4	4	80S	-	-	

Table 7. Seedling infection type and adult-plant responses of Iranian winter bread wheat cultivars and advanced lines to races avir in winter wheat resistance group II.

Entry	Name	Pedigree	Seedling		Adult-plant		*Sr2* #STS	*Sr2* #cs
			TKTTC	TTKSK	ICARDA	Kenya		
1	C-81-10	1-27-6275/Cf1770/5/Quds/4/Anza/3/Pi/Nar//Hys	;1	4	5R	90S	+	-
2	C-85-6	MV17/Zrn (Accession No 4067)	;1	4	10MR	100S	+	-
3	C-85-3	Ghk'S'/Bow'S'//90 Zhong 87/3/Shiroodi	1	4	40MS	70S	-	-
4	MV17	Slaviya/3/Krasnodari 1/ Bezostaya//3Zg.4431	1-	33+	20MR	-	-	-
5	C-84-8	Bkt/90-Zhong 87	1	4	5R	-	-	-

(Purnhauser *et al*., 2011a,b). Based on pedigree information, C-85-3 was expected to carry *Sr31* from Bobwhite (Bow 'S') or Shiroudi (syn.: Attila 4Y), but this gene was not detected using markers. However, *Sr2* was postulated in C-81-10 and C-85-6 based on the *Sr2*#STS marker; the resistance of C-85-3 and C-85-8 to TKTTC is unknown and could not be explained by either of the diagnostic markers.

Syrian bread wheat cultivars

Seedling and adult plant assessments of seven Syrian cultivars (Table 8) to *Pgt* race TKTTC at ICARDA indicated that Cham 8, Cham 10, and Bohouth 8 were resistant at the seedling and adult stages due to *Sr31*. Based on the similar seedling and adult-plant responses of these cultivars, *Sr31* was present in all three. *Sr31* was also detected in Cham 8 and Cham 10 by use of *Sr31* markers, which explains the contribution of *Sr31* to the resistance responses of these two cultivars to TKTTC but the adult-plant resistance response of Bohouth 8 has yet to be investigated. Although *Sr2* was identified in Cham 8, Cham 10, and Bohouth 6, high adult-plant responses were recorded at Njoro. High disease severity of *Sr2* sources when *Sr2* is present singly or in combination with an ineffective gene (i.e., *Sr31* to Ug99) was observed in a few Iranian genotypes in the present study and also in several CIMMYT genotypes in previous studies (Singh *et al*., 2008, Yu *et al*., 2010).

Cham 4, Cham 6, Bohouth 4 and Bohouth 6 were susceptible in seedling tests with both races, but Bohouth 4 showed a resistance response to TKTTC of 5R at ICARDA and 50S at Njoro. The *Sr2* markers were identified in Cham 8 (with only *csSr2* marker), Cham 10, and Bohouth 6.

Among the seven durum lines (Table 8), Cham 1, Bohouth 7, Bohouth 9, and Bohouth 11 were resistant at the seedling and adult stages. Cham 3 showed seedling IT 0; 1= and an adult plant response of 5R to race TKTTC, but was seedling susceptible to TTKSK at SPII and moderately susceptible at the adult-plant stage at Njoro. This reaction pattern indicates the presence of effective seedling resistance gene(s) against TKTTC and a likely contribution of adult-plant resistance gene(s) against TTKST.

Marker analyses and *Sr2* haplotyping

Among the nine DNA markers linked to stem rust resistance genes *Sr2*, *-22*, *-24*, *-25*, *-26*, *-31*, *-36*, *-39*, and *-46* evaluated, only markers linked to *Sr2*, *Sr31*, and *Sr25* were identified (Fig. 1). Of 89 Iranian genotypes, 32 (36%) were seedling and adult-plant susceptible to the three races and no *Sr*-genes were detected in this group by use of markers. The frequencies of susceptible genotypes in spring/facultative (SW/FW) and winter wheat (WW) genotypes were 21% and 15%, respectively.

Sr2 was the most common stem rust resistance gene detected in Iranian wheat genotypes. It occurred singly in 43% (34 SW/FW and 4 WW), and was combined with *Sr31* in 12.3% (10 SW/FW and one WW), and in combination with *Sr25* and *Sr31* in one genotype. *Sr31* was detected singly in 8% (6 SW/FW and 1 WW). Among Syrian genotypes, *Sr2* was detected singly in only one genotype and in combination with *Sr31* in two genotypes.

Functionality and validation of the closely linked *csSr2* CAPS marker (Mago *et al*., 2011) and *STS#Sr2* for *Sr2* were assessed in 89 Iranian and 14 Syrian wheat genotypes. Among the 46 Iranian genotypes that were identified to carry *Sr2* using the STS marker, 7 failed to amplify (null allele), 6 amplified the Marquis allele lacking the *Bsp*HI restriction site and 33 (75%) amplified the Hope-type allele with the *Bsp*HI restriction site. The two Syrian genotypes with *Sr2* using the STS marker displayed Hope-type alleles. Among the non-*Sr2* genotypes identified with the STS marker, six Iranian genotypes and one Syrian genotype were positive for the Hope allele. The use of diagnostic markers in MAS is very helpful in breeding for rust resistance, but until "perfect markers" are developed and available, discrepancy among the published markers for the same gene can be expected. It has also been reported that false positives are a common problem when detecting rust resistance genes in MAS (Yu *et al*., 2010).

Seedling assessment of Iranian and Syrian genotypes suggests a narrow range of genetic diversity in stem rust resistance genes, particularly in regard to the *Sr31*-virulent TTKSK race. In the current study, two seedling phenotypic groups were identified: i) genotypes susceptible to the two races indicating an absence of effective resistance genes; and ii) a group of genotypes that showed seedling LIT/HIT patterns to TKTTC and TTKSK, respectively. Based on this phenotypic pattern, these genotypes were postulated to carry *Sr31*.

Using the diagnostic marker "*iag95*" linked to the *Sr31*, the presence of *Sr31* was confirmed in 17 spring and two winter wheat genotypes. Until the occurrence of Ug99, the frequency of the 1BL/1RS translocation was quite high in CIMMYT materials and, therefore, cultivars grown in many developing countries. Most Iranian cultivars are either direct introductions from CIMMYT germplasm, or were selected from crosses between CIMMYT germplasm

Table 8. Seedling infection types and adult plant responses of Syrian bread and durum wheat cultivars to races avirulent and v

Entry	Name	Pedigree	Seedling IT		Adult-plant response		*Sr2* #STS	*Sr2* #cs*S*
			TKTTC	TTKSK	ICARDA	Kenya		
Bread wheat								
1	Cham 8	Kauz	1	4	20MS	70S	-	+
2	Cham 10	Kauz//Kauz/Star	1	4	5R	70S	+	+
3	Bohouth 8	Kauz 'S'/Abe	1	4	5R	70S	-	-
4	Cham 4	Flk 'S'/Hork 'S'	4	4	60S	80S	-	-
5	Cham 6	W3918A/JUP	4	4	40S	70S	-	-
6	Bohouth 4	NA	3+4	4	5R	50S	-	-
7	Bohouth 6	FR316/3/MCM/KT//Y50/4/ZA75/5/BJY	3+4	4	90S	60S	+	+
Durum wheat								
1	Cham1	Pelicano/Ruff//Gaviota/Rolette	1	;2	5R	tR†	-	-
3	Bohouth 7	NA	11+	;2	5R	5R	-	-
4	Bohouth 9	NA	11+	1	5R	5R	-	-
5	Bohouth 11	NA	11+	1	5R	5R	-	-
2	Cham 3	Durum-Dwarf-S-15/Crane//Geier	;1=	33+	5R	40MS	-	-
6	Cham 7	Haurani/Jori-C-69	4	4	30S	60S	-	-
7	Cham 5	NA	3+4	4	60S	60MS	-	-
8	Morocco		3+	4	90S	100S	-	-

†- tR= trace severity (<5%) of resistance (R) response at the adult-plant stage.

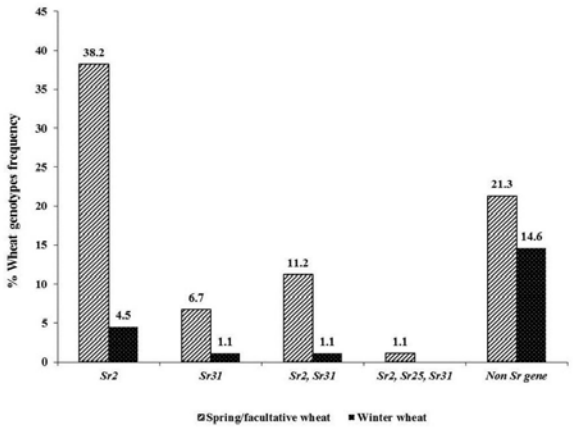

Fig. 1. Frequencies (%) of stem rust resistance genes in selected Iranian spring/facultative and winter wheat genotypes.

and local Iranian cultivars; therefore, it was expected that many Iranian genotypes would carry the 1B.1R translocation. However, *Sr31* was postulated singly or in combination with *Sr2* (and, in one case, with *Sr25* as well) in 21% of Iranian genotypes assessed in the present study.

A second gene known to occur at relatively high frequency is *Sr2*. *Sr2* was first transferred from emmer to common wheat (McFadden, 1930). *Sr2* is present in about 60% of current CIMMYT spring wheat germplasm (Singh *et al*., 2008) including some high yielding wheats that also have high levels of resistance to leaf rust and stripe rust, as well as desirable end-use quality characteristics. Similarly, Ogbonnaya *et al*. (2010) reported results of molecular characterization of more than 1000 ICARDA wheat germplasm materials, including cultivars and advanced breeding lines from the CWANA region, using linked and diagnostic markers in particular for genes effective against race TTKSK. They showed that a high proportion of ICARDA's elite wheat germplasm possesses durable stem rust resistance gene *Sr2*, with more than 50% of the germplasm showing an SSR haplotype associated with *Sr2* resistance.

The combination of *Sr2* with uncharacterized slow-rusting genes "commonly known as the *Sr2*-complex" has provided the foundation for durable resistance to stem rust in most parts of the world (McIntosh, 1988; Rajaram *et al*., 1988). *Sr2* is linked with "pseudo black chaff" (PBC), a phenotypic marker (Hare and McIntosh, 1979). So far, breeding for rust resistance in Iran and Syria has been based mainly on phenotypic selection for PBC.

Success in breeding for durable stem rust resistance will depend on comprehensive knowledge and understanding of the genetic variation available in existing wheat germplasm, a goal facilitated by the advent and availability of linked molecular markers, which are now used in characterizing elite wheat germplasm (Tsilo *et al*., 2009; Liu *et al*., 2010; Olson *et al*., 2010a,b; Yu *et al*., 2010).

It has been reported that in some genotypes, *Sr2* alone does not confer adequate resistance to stem rust (Singh *et al*., 2008). In the present study, high stem rust severities were recorded for several genotypes known to carry the *Sr2* gene or predicted to have it based on its molecular marker profile. The main reason for the high disease severities on these genotypes at ICARDA was the very high inoculum loads in the plastic house. These results, together with high disease levels recorded in Kenya on lines that likely have *Sr2*, confirm that slow-rusting adult-plant resistance genes cannot always be easily detected and therefore adult plant screening under plastic house conditions cannot be recommended in future work unless the methodology can be modified by lower inoculum loads, later inoculation dates or fewer spreaders. Considering the slow-rusting nature of *Sr2*, measuring disease progress in time intervals (i.e., Area Under the Disease Progress Curve, or AUDPC) would be a better option than terminal rust scores.

CONCLUSIONS

We have demonstrated the presence of known and uncharacterized seedling and adult plant resistances in several Iranian and Syrian cultivars. Our next priority is to undertake genetic studies to show that the genes involved are new. These new resistance genes should be used in combination with other seedling resistance genes or with *Sr2*, taking full advantage of the available genetic markers and testing facilities both locally and in Kenya. This will help bread wheat breeding programs to more effectively target the incorporation of diverse resistance genes into relevant adapted elite germplasm. With improved capacity to deploy resistance gene pyramids, the durability of resistance to Ug99 will be greatly enhanced.

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