Zagazig Journal of Agricultural Research
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WHEAT STEM RUST RACE ANALYSIS AND EFFECTIVE GENES AT DELTA EGYPT

Mostafa M. El-Shamy*, Minaas E.A. Sallam and M.H. Abd-Elkader

Wheat Dis. Res. Dept., Plant Pathology Research Institute, ARC, Egypt

ABSTRACT

A total of 21 pathotypes of *Puccinia graminis* f.sp.*tritici* were identified in 2010/2011 and 2011/2012 growing seasons at glasshouse of Gemmeiza Research Station, ARC, Egypt. Stem rust samples were collected from five Egyptian Governorates viz.Gharbia, Menufia, Behera, Demiata and Alexandria. The pathotypes BFBBB, LDCBB, TFLBC, TDHBC and SBBBB were identified in 2010/ 2011 growing season. However, 16 pathotypes were identified, in 2011/2012 growing season namely; BBBCB, BLCBB, BSGQC, CBCTC, CTJTC, FLGBB, GPGFB, KTGCB, KNCSB, PKCSB, STHGB, RTHTC, TTHSC, TTGTF, TTGBC and TPCTF. While, TTGTF, TTHSC, TPCTF and RTHTC were the most identified virulent pathotypes during the two growing seasons. The *Sr*-genes *24, 31* and *36* showed complete percentage efficacy against the identified stem rust pathotypes indicated the absence of Ug99 which known as TTKS or its variances in Egypt.

Keywords : Stem rust, *Puccinia graminis,* race analysis, pathotypes, effective genes.

INTRODUCTION

Wheat stem rust caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks.& E. Henn. is one of the most devastating diseases affecting wheat world wide.*Puccinia* spp have afflicted wheat for thousands of years, as references to rust, can be found in the literature of classical Greece and Rome and in the Bible (Chester, 1946). Stem rust is very important disease due to its affect wheat grains quantity and quality (Leonard and Szabo,2005). Breeding for stem rust resistance needs to great knowledge about its prevalent pathotypes and its virulence frequencies in the country. Unpublished data of wheat Dis.Res.Dept.in 2009/2010 growing season in Egypt revealed that among 122 stem rust isolates only 92 virulent phenotypes were identified. The most effective genes efficacy were *Sr 31* (93.44%), *Sr 26+99* (90.98%) and *Sr 29* (81.96%). Appearance of new stem rust pathotypes might be hiders the efforts of breeders and pathologists to combat rust by releasing resistant wheat cultivars. The identification of physiologic race key was based on 12 differential cultivars and differential set (Stakman *et al.*, 1962). Then, the use of single-gene in differential hosts for race identification was proposed by different systems of nomenclature (Luig and Statler, 1983; Roelfs and Martin, 1984 and Pretorius *et al.*, 2000). In 1999, stem rust has re-emerged as a threat to wheat production with the detection of new pathogen race, Ug99 in Uganda (Pretorius *et al.*, 2000) and later designated as TTKS under North American pathotype nomenclature system (Wanyera *et al.*, 2006). A new form of stem rust as TTKSK took several growing seasons from Uganda to Kenya 1999 via Ethiopia and Yemen to reach Iran by 2007. In Tanzania during August 2009, races confirmed by the USDA-ARS Cereal Disease Laboratory, USA were; TTKSK (Ug99), TTKST (*Sr24* variant of Ug99) and TTTSK (*Sr36* variant of Ug99). Races TTKSK and TTTSK were present in samples collected from the Hanang, and Ngorongoro areas, whilst race TTKST was present in the Karatu and Monduli areas (Report of BGRI, 26 April, 2011). Ug99 pathotype was found to be virulent on stem rust resistance genes *Sr5*, *Sr6*,

***Corresponding author:** , **Tel.:** 0105695654
E-mail address: dr.elshamyy@yahoo.com

Sr7b, *Sr8a*, *Sr8b*, *Sr9b*, *Sr9e*, *Sr9g*, *Sr11*, *Sr15*, *Sr17*, *Sr30*, *Sr31* and *Sr38*. Some of which have been extensively used as resistance sources in wheat varieties grown world-wide. Since then, there have been reports of this pathotype as well as a mutant derivative with virulence for *Sr24* (Stokstad, 2007 and Jin *et al.*, 2007). Some of these genes, including *Sr25*, *Sr26*, and *Sr43* from *Thinopyrum elongatum*, *Sr37* and *Sr40* from *Triticum timopheevii*, *Sr32* and *Sr39* from *Aegilops speltoides*, and *Sr44* from *T. intermedium*, have been found to be effective against Ug99 pathotype.

The main objectives of this study were, stem rust survey in North Delta region, identification the prevalent pathotypes, its frequency and virulence's on the available stem rust monogenic lines (*Sr,s*) as well as some of the new local Egyptian wheat cultivars during 2010/2011 and 2011/2012 growing seasons.

MATERIALS AND METHODS

Plant Materials

An experiment included a set of 34 isogenic stem rust wheat lines(*Sr,s)* as well as 5 commercial wheat cultivars from the national wheat program were sown at five Governorates *i.e.* Gharbia, (Gemmeiza and Tanta), Menufia (Sirs El-Lian), Behera (Etay El-Baroud), Demiata (Kafr Saad) and Alexandria (Nubaria) during 2010/2011and 2011/2012 growing seasons (Tables 1and 2). These materials were sown in rows, 3.5 m long, 40 cm apart between rows and 10 cm within plants. All of agriculture practices were carried out as recommended and left to natural field infection.

Survey of Wheat Stem Rust

Stem rust survey was started from the second half of April up-to the first half of May. A total of 25 and 72 of wheat stem rust samples were collected from the grown materials and locations during 2010/2011 and 2011/2012 growing seasons, respectively. The collected samples were dried at room temperature for 48 h and maintained in pergamin bags over a desiccators containing $CaCl_2$ and kept in a refrigerator at 2-5°C until used.

Identification of Wheat Stem Rust Pathotypes

Wheat stem rust pathotypes nomenclature were performed under controlled of glasshouse conditions of Wheat Dis. Res. Dept., Gemmeiza Res. Station, ARC. Stem rust pathogen was initially isolated from each sample and propagated on a highly susceptible wheat cultivar (Morocco) at seedling stage and repeating inoculation using single pustules technique, two–three time for each isolate. Pathotype nomenclature was detected on twenty single-gene wheat lines selected according to the new system nomenclature proposed by Pretorius *et al.* (2000). According to this system, these differential lines were divided into five groups, 4 lines for each (Table 3).

Infection type for each line\cultivar was recorded at seedling stage using the 0 - 4 scale proposed by Stakman *et al.* (1962). Infection types (ITs) lower than 3 (0, 0; , 1 and 2) were regarded as incompatible (low IT) whereas ITs 3 and 4 were regarded as compatible (high IT). According to a combination of *Sr*'s responses of each group, each pathotype has a code including five letters as shown in Table 3. Also, a virulence\ virulence formula of each pathotype was studied based on the responses of each near-isogenic stem rust line proposed by Green (1966), However, the virulence of the tested *Sr* genes was estimated according to Samborsky and Dyck (1976) as follow:

$$\text{The virulent } Sr\text{-genes \%} = \frac{\text{No. of susceptible genes}}{\text{Total No. of the tested genes}} \times 100$$

RESULTS AND DISCUSSION

Identification of Wheat Stem Rust Pathotypes

No stem rust samples were collected from the local bread wheat cultivars, it could be attributed to early mature of the wheat cultivars and the late occurrence of stem rust infection.

Out of twenty five isolates of stem rust, five pathotypes were identified from the samples collected from the near-isogenic lines in 2010/2011 growing season. These pathotypes could be ranking in descending frequency as,

Table 1. List of thirty- four *Sr*-genes used for stem rust analysis in North Delta during 2010/2011and 2011/2012 growing seasons.

No.	*Sr*-gene	Source	No.	*Sr*-gene	Source
1	*5*	Reliance	**18**	*26*	*Sr26\9*LMPG*
2	*6*	ISr6-Ra	**19**	*27*	Coroong(=Sr27Tritical)
3	*7b*	Marquis	**20**	*28*	W2691 Sr28 kt
4	*8a*	Red Egyptian	**21**	*29*	Pusa 4\Etoil de Choisy
5	*9a*	Red Egyptian	**22**	*30*	BtSr30Wst
6	*9b*	W2691Sr9b	**23**	*31*	Sr31(Benno)\6*LMPG
7	*9d*	Hope	**24**	*32*	*CnsSr32AS*
8	*9e*	Vernistein	**25**	*33*	*RL5405*
9	*9g*	Lee	**26**	*35*	Mq(2)5*G2919
10	*11*	Lee	**27**	*36*	Sr36(CI 12632)\8*LMPG
11	*13*	St464Sr13	**28**	*37*	W2691SrTt1-2
12	*14*	Line A seln	**29**	*39*	RL6082
13	*17*	Renown	**30**	*40*	RL6088
14	*21*	*Triticum monococum*	**31**	*44*	Taf-2
15	*22*	Sr22TB	**32**	*Tmp*	CnSSrTmp
16	*23*	Exchange	**33**	*McN*	Houser-McNNair 701
17	*24*	*LcSr24Ag)*	**34**	*Gt*	W2691SrGtGt

Table 2. List of the name and pedigree of 5 Egyptian bread wheat cultivars

No.	Cultivar	Pedigree
1	Gemmeiza11	BOW"S"\KVZ"S"\\7C\SER182\3\GIZA168\SAKHA61
2	Misr-1	OASIS\SKAUZ\\4*BCN\3\2*PASTOR
3	Misr-2	SKAUZ\BAV92
4	Sids-12	BUS\\7C\ALD\5\MAYA74\ON\\1160.147\3\BB\GLL\4\CHAT"S"\6\MAYA\VUL\\CMH74A.630\4*SX.,SD7096-4SD-1SD-0SD.
5	Sakha-93	SAKHA92\TR810238

Table 3. Differential set of stem rust mono-genic lines and *Puccinia graminis* f. sp. *tritici* (Pgt) code for pathotype nomenclature

Diff. Set.	*Sr*-genes			
I*	*5*	*21*	*9e*	*7b*
II	*11*	*6*	*8a*	*9g*
III	*36*	*9b*	*30*	*17*
IV	*9a*	*9d*	*10*	*Tmp*
V	*24*	*31*	*38*	*McN*
Pgt code				
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

* Cited after Pretorius *et al.* (2000).

BFBBB (32%), LDCBB (28%), TFLBC (16%), TDHBC (12%) and SBBBB (12%). While, 16 pathotypes of stem rust were identified out of 116 isolates in 2011/ 2012 growing season, *i.e.* BBBCB,BLCBB, BSGQC, CBCTC, CTJTC, FLGBB, GPGFB, KTGCB, KNCSB, PKCSB, STHGB, RTHTC, TTHSC, TTGTF, TTGBC and TPCTF. The pathotypes TTHSC, TTGTF and BLCBB showed the most prevalence since its frequency % was (17.24%) for each. However, the pathotypes GPGFB, KTGCB, PKCSB and RTHTC were the least frequent % (1.72%) for each (Table 4).

Similar pathotypes were identified in different countries, Kolmer (2001) reported that race TPMK was the most common stem rust race in the United State since the late 1950s, after the decline of race TMB (race 15B according to the Stakman differential system), which caused such devastating epidemics during 1950–1954 .Kolmer *et al.* (2007) identified six races of stem rust *i.e.* QFCS, MCCF, MCCD, TPMK, QCCN and TTTT using four groups of stem rust monogenic lines. Kokhmetova *et al.* (2011) identified 11 pathotypes of *P. graminis* in Kazakhstan from samples collected in 2008/2009 *i.e* TDT\H, TPS\H, TTH\K, TCP\H, PCP\C, PCR\Q, TCK\H, TMR\H, KTH\R, TKT\C, and TFK\R. The pathotypes TDT\H, TPS\H, TTH\K, KTH\R,TKT\C and TFK\R were highly virulent. Rouse and Jin (2011) identified stem rust races QFCSC, TTTTF, and MCCFC in USA.

Virulence of the Identified Pathotypes

Data in Table 5 reveal the virulent pathotypes based on the susceptibility percentage of the differential *Sr*-genes. Isolates of *P. graminis tritici* studied in 2011/2012 growing season were more virulent than those studied in 2010/2011 growing season. Pathotypes TFLBC and TDHBC were virulent on 15 and 13 out of 20 *Sr*-genes releasing 50.00 and 40.00% (virulent %). However, in 2011/2012 growing season, the pathotype, TTGTF was the most virulent one since showed susceptible response to 15 out of the differential set releasing 75%. While, the pathotypes TTHSC, TPCTF and RTHTC came in the second rank in their virulence's which showed 70%.Hereby, these pathotypes carrying virulent genes in their genetic makeup. The stem rust pathotypes BLCBB and BBBCB were the least virulent one since showed 10.00 and 5.00% virulence, respectively. No virulence's were detected for the resistance *Sr*-genes *24, 31* and *36* through the two growing seasons,2010/ 2011and 2011/2012 in this study.

Similar results were registered in different countries, Kolmer *et al.* (2007) found that stem rust pathotypes were virulent on stem rust monogenic lines as follow,QFCS-*5, 21, 8, 9g, 17, 9e, 9b, 10;* MCCF-*5, 7b, 9g, 17, 10, Tmp*; MCCD-*5, 7b, 9g, 17, 10*; TPMK-*5, 21, 9e, 7b, 11, 8, 9g, 36, 17, 9b, 10, Tmp*; QCCN-*5, 21, 9g, 17, 9a, 10*; TTTT-*5, 21, 9e, 7b, 11, 6, 8, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp.* Moreover. Singh *et al.* (2008) detected eight stem rust resistance genes (*Sr5, Sr8a, Sr9g, Sr12, Sr30, Sr31, Sr36* and *Sr38*) in selected wheat cultivars from UK .On the other hand, Jin *et al.* (2007) reported that *Sr24* and *Sr36*, which showed resistance to the initial forms of Ug 99 are no longer effective against some more virulent forms, so their use is no longer recommended, unless combined with other genes. Whereas, Klindworth *et al.* (2011) reported that stem rust differential tests coded the race TPPKC which was a virulent on genes *Sr6, Sr9a, Sr9b, Sr13, Sr24, Sr31*, and *Sr38*. However, the race TPMKC having added virulence on *Sr30* as well as *Sr Wld1*.

Data in Table 6 show the efficacy percentage of *25 Sr*-genes for resistance against 25 and 116 identified pathotypes of *P. graminis* f.sp. *tritici* during 2010/2011 and 2011/2012 growing seasons. Some fluctuations in *Sr*-genes efficacy percentage from the two tested growing seasons due to the appearance of more virulent pathotypes and genotype x season interaction. For example, *Sr*-genes *11, 9a,10* and *Sr Tmp* showed complete effectiveness in 2010/2011 growing season (100.00% efficacy), however, their efficacy declined to 12.06, 43.10,44.82 and 58.62%, respectively in 2011/2012 growing season.

In general, the obtained results indicated that *Sr*-genes *24, 31, 36* had the highest mean efficacy percentage against all the identified isolates during the two growing seasons releasing 100.00% efficacy. Therefore,these resistance genes could be exploited in wheat breeding programs for producing new resistant cultivars. However, the *Sr*-genes *30*, and *38* came in the second rank as effective genes (97.41 and 88.79% mean efficacy, respectively). *Sr 8a* showed the lowest mean percentage efficacy (24.10%). The other *Sr*-genes were in between, *Sr9g* (33.55% efficacy) to *SrTMP* (79.31% efficacy).

Table 4. Pathotypes of *Puccinia graminis* f.sp. *tritici* identified during 2011 – 2012 seasons and their frequencies

Identification season and pathotypes frequency %					
2010/2011			2011/2012		
Pathotype	No. of isolates	Frequency %	Pathotype	No. of isolates	Frequency %
BFBBB	8	32	BBBCB	8	6.89
LDCBB	7	28	BLCBB	20	17.24
SBBBB	3	12	BSGQC	4	3.44
TDHBC	3	12	CBCTC	4	3.44
TFLBC	4	16	CTJTC	4	3.44
			FLGBB	10	8.62
			GPGFB	2	1.72
			KTGCB	2	1.72
			KNCSB	4	3.44
			PKCSB	2	1.72
			STHGB	2	1.72
			RTHTC	2	1.72
			TTHSC	20	17.24
			TTGTF	20	17.24
			TTGBC	6	5.17
			TPCTF	6	5.17
	25			116	

Table 5. Avirulence formula of 25 and 116 *P. graminis* f. sp. *tritici* pathotypes depending on seedling reaction of 25 isogenic stem rust monogenic lines in two seasons 2011-2012

Pathotype	Avirulence\ virulence formulae	Virulent *Sr*-genes%
	2010\ 2011 season	
TFLBC	*11,6,36, 9a,9d,10,Tmp,24,31,38*	50.00
TDHBC	*11,6,9g,36,30,9a,9d,10,Tmp,24,31,38*	40.00
SBBBB	*7b,11,6,8a,9g,36,9b,30,17, 9a,9d,10,Tmp,24,31,38,McN*	15.00
LDCBB	*21,9e,7b,11,6,36,9b,30,17, 9a,9d,10,Tmp,24,31,38,McN*	15.00
BFBBB	*5,21,9e,7b,11,6,36,9b,30,17,9a,9d,10,Tmp,24,31,38,McN*	10.00
	2011\2012	
TTGTF	*36,30,17,24,31*	75.00
TTHSC	*36,30,Tmp,24,31,38*	70.00
TPCTF	*6,36,9b,30,24,31*	70.00
RTHTC	*9e,36,30,24,31,38.*	70.00
CTJTC	*Sr5,21,9e,36,17,24,31,38*	60.00
TTGBC	*36,30,17,9a,9d,10,Tmp,24,31,38*	50.00
PKCSB	*21,11,36,9b,30,Tmp,24,31,38,McN*	50.00
STHGB	*7b,36,30,9a,10,Tmp,24,31,38,McN*	50.00
KTGCB	*5,36,30,17,9a,9d,10,24,31,38,McN*	45.00
KNCSB	*5,6,9g,36,9b,30,Tmp,24,31,38,McN.*	45.00
GPGFB	*5,9e,7b,6,36,30,17,9a,9d,24,31,38,McN*	35.00
CBCTC	*Sr5,21,9e,11,6,8a,9g,36,9b,30,24,31.38*	35.00
BSGQC	*Sr5,21 ,9e,7b ,9g,36,,30,17 ,10,Tmp,24,31,38*	35.00
FLGBB	*5,21,6,8a,9g,36,30,17,9a,9d,10,Tmp,24,31,38,McN.*	20.00
BLCBB	*Sr5,21 ,9e,7b ,6,8a,9g,36,9b,30,9a,9d,10,Tmp,24,31,38,McN*	10.00
BBBCB	*Sr5,21,,9e,7b,11,6,8a,9g,36,9b,30,17,9a,9d,10,24,31,38,McN*	5.00

Table 6. Efficacy percentage of genes for resistance to the used stem rust pathotypes

No.	*Sr*-gene	Gene efficacy % in		Mean gene efficacy %
		2010/2011 i	2011/2012 season	
1	*5*	32.00	50.00	41.00
2	*21*	60.00	44.82	52.41
3	*9e*	60.00	37.93	48.96
4	*7b*	72.00	31.03	51.51
5	*11*	100.00	12.06	56.03
6	*6*	72.00	46.55	59.27
7	*8a*	12.00	36.20	24.10
8	*9g*	24.00	43.10	33.55
9	*36*	100.00	100.00	100.00
10	*9b*	72.00	37.93	54.96
11	*30*	100.00	94.82	97.41
12	*17*	72.00	46.55	59.27
13	*9a*	100.00	43.10	71.55
14	*9d*	88.00	41.37	64.68
15	*10*	100.00	44.82	72.41
16	*Tmp*	100.00	58.62	79.31
17	*24*	100.00	100.00	100.00
18	*31*	100.00	100.00	100.00
19	*38*	100.00	77.58	88.79
20	*McN*	72.00	43.10	57.55

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تحليل سلالات صدأ الساق في القمح والجينات الفعالة في دلتا مصر

مصطفى محمود الشامي – ميناس السيد علي سلام- محمد عبدالقادر حسن

قسم بحوث أمراض القمح - معهد بحوث أمراض النبات - مركز البحوث الزراعية - مصر

تم إجراء هذا العمل بالصوبة الزجاجية المكيفة للصدأ الأسود المقامة بمحطة البحوث الزراعية بالجميزة – قسم بحوث أمراض القمح – مركز البحوث الزراعية- مصر. ولقد أظهرت النتائج المتحصل عليها الآتى: تم تعريف واحد وعشرون طراز من الفطر بكسينيا جرامينيز تريتيساى المسبب لمرض صدأ الساق فى القمح على عدد عشرون سلالة من الأقماح أحادية الجين فى طور البادرة خلال عامى الدراسة ٢٠١٠/٢٠١١ – ٢٠١١/٢٠١٢م وذلك تحت ظروف الصوبة الزجاجية بعد جمع عينات قمح مصابة بصدأ الساق الأسود من خمس محافظات وهي الغربية-المنوفية – البحيرة – دمياط والإسكندرية .عرفت خمس طرز من الفطر خلال موسم ٢٠١٠/٢٠١١ وهي BFBBB, LDCBB, TFLBC, TDHBC and SBBBB في حين تم تعريف ستة عشر طرزا في موسم ٢٠١١/٢٠١٢م وهى BBBCB, BLCBB, BSGQC, CBCTC, CTJTC, FLGBB, GPGFB, KTGCB, KNCSB, PKCSB, STHGB, RTHTC, TTGTF,TTHSC, . : وكان أكثر هذة الطرز قدرة مرضية الأتى TTHSC, TTGTF, TTGBC and TPCTF. كما أظهرت سلالات القمح الأحادية الجين *اس ار ٢٤* ، *اس ار ٣١* ،*إس ار ٣٦* كفاءة TPCTF and RTHTC. ١٠٠% فى مقاومة طرز الفطر التى تم تعريفها خلال موسمى الدراسة مما يؤكد عدم وجود طرز الفطر يو جى ٩٩ أو أحد طفراتها فى مصر.