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PHYSIOLOGICAL RACES AND VIRULENCE DIVERSITY OF WHEAT STEM RUST FUNGUS *Puccinia graminis* f. sp. *tritici* IN EGYPT DURING 2012-2013

Hasan, M.A and M. Abou-Zeid
Wheat Disease Research Department, Plant Pathology Research Institute, ARC, Egypt

ABSTRACT

Stem rust collections were obtained from infected wheat stems throughout the survey of wheat fields and nurseries in (Garbia, Dakhlia, Minofia, Kafer Elshake and Sharkia governorates) during 2012/2013. The findings of this paper were based on race analysis through inoculation of stem rust populations, isolation and multiplication of single-pustule of the pathogen and race determination by inoculating on stem rust differential hosts. The phenotypic characterization of *P. graminis* f. sp. *tritici* resulted in identification of 85 isolates 40 races were identification from the collected samples were the most frequency and predominant race were BBBTC, TDBBB and BTCBB with frequency (9.44%, 7% and 7%) respectively. Race analysis from the tested samples reveled that race TDBBB was the most prevalent race with a frequency of 7% followed by TTHBB and TTHTB which gave frequency of 5.88% and 4.7% respectively at Garbia governorate. Whereas, race BTCBB was the most frequent at Dakhlia (7%) . The highest frequency during this survey was recorded with BBBJC at Minofia governorate which gave 9.41%. Cluster analysis reveled that, identified races were divided to A and B group with similarity a bout 31.10%, where A (Kafer Elshaik) and B the rest governorates. Among 20 wheat stem rust differential hosts, *Sr31* was the most effective one which exhibit 100% efficacy to all races followed by *Sr24* (89.5%). While *Sr 9g* while, *Sr5* were the least effective during this study. Thus, use of effective *Sr* stem rust resistance genes such as *Sr31* and *Sr24* in single cultivar through gene pyramiding has paramount importance as the additive effects of several genes gives the cultivar a wider base stem rust resistance along with periodic race survey.

Keywords: Wheat, stem rust, seedling resistance, adult plant resistance.

INTRODUCTION

Stem rust of wheat, caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. is the most destructive disease of wheat worldwide. Successful control of the disease over three decades through the use of genetic resistance has resulted in a sharp decline in research activity in recent years. (Wamishe and Milus 2004) reported that, many cases of genetic resistance become ineffective because the population of rust pathogen respond to selective pressure of resistant host cultivars and produced more virulent phenotypes overcome the resistant varieties.

Occurrence of new races in a geographic/ epidemiologic regions may be attributed to the migration from an outside is considered a great threat to Egyptian wheat cultivars. Detection and spread of race TTKS, in East Africa commonly known as Ug99, and its migration path i.e. to North Africa through Arabian Peninsula and then to Middle East and Asia. Identifying/developing

adapted resistant cultivars in a relatively short time and replacing the susceptible cultivars before rust migrates out of East Africa is the strategy to mitigate potential losses (Singh, *et al*.,2006). So, the breeding strategy could be implemented to incorporate diverse genetic resistance to such race into germplasme before the migration to other areas.

The value of adult plant resistance in protecting wheat cultivars against such virulent stem rust races could be achieved by combining many genes of resistance in a single cultivar that conferred high level of generalized resistance against the pathogens. In this respect, Brennan (1988) stated that a breeding program should develop rust resistant cultivars conditioned with resistance genes(both race- specific and race- non specific resistance) exist in wheat should be used. The inheritance of adult plant resistance has often been considered as a complex, but there is also an evidence that it is oligogenic (Barcellos *et al*.,2000). The identification of genes conferring stem rust adult plant resistance would be a significant step towards a good control of such disease (Manninger *et al*.,1998; Nazim *et al*.,2001; Hermas,2003; Mousa *et al*.,2004 and EL-Shamy *et al*.,2011). Stem rust of wheat, caused by *Puccinia graminis* f. sp. *tritici* is the most destructive disease of wheat worldwide, it can causes complete inhibition of wheat crops over wide areas during epidemic years. Hence, this study was carried out to detect the virulence diversity of *P. graminis* f. sp. *tritici* in five Egyptian governorates and studying the efficacy of stem rust resistance genes in greenhouse at Gemmiza Agricultural Research Station.

The present study aimed to identify and nomenclature the physiological races. and estimate the efficacy of stem rust resistance gene under green house condition in Egypt during 2012/2013.

MATERIALS AND METHODS

The collected samples from (Garbia, Dakhlia, Minofia, Kafer Elshake and Sharkia governorates) during 2012/2013 Fig (1) included wheat stems having the symptoms of stem rust disease caused by *Puccinia graminis.* f. sp. *tritici*. Were used to identify physiologic races. The collected samples (rusted stems) were kept in glassine envelopes (8 x 15 cm) and left at room temperature for 24 hours to remove the humidity in the samples. After that the samples were preserved in dissector in fridge till usage. The infected specimens were transferred to very susceptible wheat variety Morocco using the spatula method. The method of inoculation was carried out as described by Stakman *et al*. (1962). Eight days old seedlings were sprayed with an atomizer in the inoculation chambers with water then inoculated by shaking and brushing rusted materials over the plants and sprayed gently again with water in order to induce “dew” on the plant. Finally, the inoculated plants were kept in damp chambers for 24 hours to allow the rust spore to germinate and cause infection. The plants were transferred and placed on benches in the greenhouse and kept for 14 days. After developing the rust, three to five single pustules were separately isolated from each sample for spore increase on higher susceptible wheat variety Morocco seedlings to obtain enough urediospores for inoculating the differential sets.

Seedling reaction was recorded as low or high infection type depending on the infection type produced according to the method adopted by Stakman *et al*. (1962). Infection types were categorized as either being Low (incompatible or resistant; ITs of 0, 0;, 1, and 2) or High (compatible or susceptible; ITs of 3 & 4). For race identification and nomenclature Race designation was done by grouping the differentials into five subsets: (i) *Sr5, Sr21, Sr9e, Sr7b*, (ii) *Sr11, Sr6, Sr8a, Sr9g*, (iii) *Sr36, Sr9b, Sr30, Sr17*, (iv) *Sr9a, Sr9d, Sr10, SrTmp*, (v) *Sr24, Sr31, Sr38* and *SrMcN* (Roelfs and Martens, 1988; Jin *et al*., 2008). The differential host series consisted of wheat rust monogenic lines, arranged in five subsets Table (1). Races were assigned using the international Pgt-code as suggested by Roelfs and Martens (1988).

The frequency of each race was calculated as a percentage from the total number of isolates analyzed.

$$\text{Virulence (\%)} = \frac{\text{susceptible resapons}}{\text{Total number of isolates}} \times 100$$

According to the method adopted by Green (1965).

Cluster analysis were used to study the simiertry between the identified races from governrators

Table (1). A key for idefining the Pgt-code races of *Puccinia graminis* f. sp t*ritici.*

Infection types produced on host lines with *Sr*				
Diff.subset	5	21	9e	7b
	11	6	8a	9g
	36	9b	30	17
	9a	9d	10	TmP
PgtCode*	24	31	38	MCN
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

***Pgt Code consist of designation of substance followed by subset etc.**

RESULTS AND DISCUTIONS

Races identified.

Race analysis of the collected samples (Table 2) reveled that total of 85 single uredinial isolate were chara races were identified based on their reaction on 20 differential sets. Most of the identified races varied in their virulence on stem rust resistance genes; this indicated a high level of variation both in quantity and virulence spectrum. The obtained data gave evidence that, the most frequent and predominant races identified were BBBJC, TDBBB and BTCBB with a frequency of (9.41% -7% and7%). The second most frequent and dominant races were TTHBB, TTHTB,QRDTB and CFBBB with a frequency of (5.8% , 4.7%, 4.7%and 4.7%). While there were 25 races occupied the lowest virulence frequency (1.1), this mean that these races were not significant on pathogen population. Virulence The broadest virulence spectra were recorded from the race TTTTB making 16 stem rust resistance genes ineffective, meanwhile it was a virulent to Sr24,31,38 and SrMCN. On the other hand race BBBJC was the a virulent race which low infection type on most of the tested stem rust resistant genes except (Sr. 9d,10,MCN). (Youssef, I.A.M. *et al*. 2012) reported that race BBDB proved to be the most a virulent race from the identified races, on the other hand, race TTTT proved to be the most virulent to the tested differentials. (Teklay , A., *et al.* 2013) found that the most prevalent races were TTSNK, RRJJC and HRJJC with a frequency of 9.4% each and the most virulent races TTKSK and TTSSK each making 85% of Sr genes ineffective. Whereas the most important races in Ethiopia were (TTSSK, TTSNK and RRTTF).(Abebe *et al.* 2012) identified a total of 20 races from 32 isolates, which included the most prevalent races TTSNK, RRJJC and HRJJC.

(Olivera *et al.* 2012) identified JRCQC, TRTTF and TTKSK from 34 isolates, both races JRCQC and TRTTF possess virulence on stem rust resistance genes *Sr13* and *Sr9e*. In addition, race TRTTF was virulent to three stem rust resistance genes that were effective to race TTKSK, including *Sr 36*, *Sr Tmp*, and resistance conferred by the 1AL.1RS rye translocation.

Regarding the distribution of these races at the obvious governorates, data in (Table 3) and figure(1), showed that, out of the total 40 races, 25 were identified from Garbia governorate whereas the lowest number of races identified from Sharkia governorate(12 races).The highest level of race variation was detected from Garbia governorate, which contains the most virulent races, such as TTTTB,TTRTR and TTHBB. These races showed high infection type to differential sets, whereas the most a virulent races observed at Sharkia governorate.

Table (2). Virulence formula, number of isolates and frequency of stem rust races identified in Egypt during 2012/2013 growing season.

No.	Races	Virulence formulae	No. of isolates	Freq. %
1	BBBJC	9d,10,MCN/	8	9.41
2	BFBBB	8a,9g/	1	1.1
3	BFBBC	8a,9g,MCN,/	1	1.1
4	BLFTC	11,30,17,9g,9d,10,tmp,MCN/	3	3.52
5	BMRTF	11,9g,36,9b,17,9a,9d,10,tmp,38,MCN/	1	1.1
6	BPGMB	8a,9b,9a,tmp/	1	1.1
7	BTCBB	11,6,8a,9d,17/	6	7
8	CFBBB	7b,8a,9g/	4	4.7
9	CSTTF	7b,11,6,8a,36,9b,30,17,9a,9d,10,tmp,38,MCN/	1	1.1
10	FTTPN	9e,7b,11,6,8a,9g,36,9b,30,17,9a,10,tmp,24,38/	1	1.1
11	GBHTF	21,9b,17,9a,9d,10,tmp,38,MCN/	1	1.1
12	GLKTC	21,11,9b,30,17,9a,9d,10,tmp,MCN/	1	1.1
13	JLDFF	21,9e,7b,11,30,10,tmp,38,MCN/	1	1.1
14	LDCKC	5,8a,9g,1`7,9d,10,tmp,MCN/	1	1.1
15	LLGTB	5,11,9b,9d,30,17/	1	1.1
16	LPPTB	5,7b,11,8a,9g,9a,9d,10,tmp/	3	3.52
17	LRBPD	5,11,6,9g,9a,10,tmp,38/	1	1.1
18	MCCPB	5,7b,9g,17,9a,10,tmp/	1	1.1
19	PMTTD	5,9e,7b,11,36,9b,30,17,9a,9d,10,tmp,38/	1	1.1
20	QRDTB	5,21,11,6,9g,30,9a,9d,10,tmp/	4	4.7
21	RKHTB	5,21,7b,6,8a,9g,9b,17,9a,9d,10,tmp/	1	1.1
22	RKKJB	5,21,7b,6,8a,9g,9b,30,17,9d,10/	1	1.1
23	RKKSB	5,21,7b,11,36,tmp/	3	3.52
24	SCTTD	5,21,7b,9a,36,9b,30,17,38,MCN/	3	3.52
25	SCTTF	5,21,7b,9a,36,9b,30,17,9a,9d,tmp,38,MCN/	1	1.1
26	SJKKB	5,21,9e,6,8a,9b,17,30,9d,10,tmp/	1	1.1
27	SPQKL	5,21,9e,11,8a,9g,36,9b,9d,10,tmp,24/	1	1.1
28	TBMTB	5,21,9e,7b,36,17,9a,9d,10,tmp/	1	1.1
29	TBTKC	5,21,9e,7b,36,9b,30,17,9d,10,tmp,MCN/	2	2.35
30	TDBBB	5,21,9e,7b,8a/	6	7
31	TFSLM	5,21,9e,7b,8a,9G,36,9b,30,9a,24,MCN/	2	2.35
32	TGPLB	5,21,9e,7b,11,6,36,17,9a/	1	1.1
33	TTBBB	5,21,9e,7b,11,6,8a,9g/	3	3.52
34	TTBBF	5,21,7b,11,6,8a,9g,9g,38,MCN/	3	3.52
35	TTCTB	5,21,9e,7b,11,6,8a,9g,17,9a,9d,10,tmp/	1	1.1
36	TTDSD	5,21,9e,7b,11,6,8a,9g,30,9a,9d,10,38/	1	1.1
37	TTHBB	5,21,7b,11,6,8a,9g,9b,17/	5	5.88
38	TTHTB	5,21,7b, 11,6,8a,9g,9b,179a,9d/	4	4.7
39	TTRTR	5,21,9e,7b,11,6,8a,9g,36,9b,17,9g,9d,10,tmp,24,30,MCN/	2	2.35
40	TTTTB	5,21,7b, 11,6,8a,9g,9b,36,9b,30,tmp,9a,9d,10,tmp/	1	1.1
Total			85	100%

Table(3) Number of isolates of Races of *Puccinia graminis* f. sp. *tritici* collected from five governorates in Egypt in 2012/13 growing season.

No.	Races	Garbia	Dakhlia	Minofia	Kafer Elshik	Sharkia	Total of isolates
1	BBBJC	1		5	1	1	8
2	BFBBB					1	1
3	BFBBC			1			1
4	BLFTC			3			3
5	BMRTF		4				4
6	BPGMB		1				1
7	BTCBB	1	4	1			6
8	CFBBB		4				4
9	CSTTF				1		1
10	FTTPN		1				1
11	GBHTF				1		1
12	GLKTC		1				1
13	JLDFF				1		1
14	LDCKC				1		1
15	LLGTB		1				1
16	LPPTB	2		1			3
17	LRBPD				1		1
18	MCCPB			1			1
19	PMTTD					1	1
20	QRDTB		4				4
21	RKHTB		1				1
22	RKKJB					1	1
23	RKKSB				3		3
24	SCTTD					3	3
25	SCTTF				1		1
26	SJKKB				1		1
27	SPQKL				1		1
28	TBMTB	1					1
29	TBTKC				2		2
30	TDBBB	3	1			2	6
31	TFSLM				2		2
32	TGPLB	1					1
33	TTBBB	3					3
34	TTBBF					3	3
35	TTCTB				1		1
36	TTDSD	1					1
37	TTHBB	5					5
38	TTHTB	4					4
39	TTRTR	2					2
40	TTTTB	1					1
Total		25	19	13	16	12	85

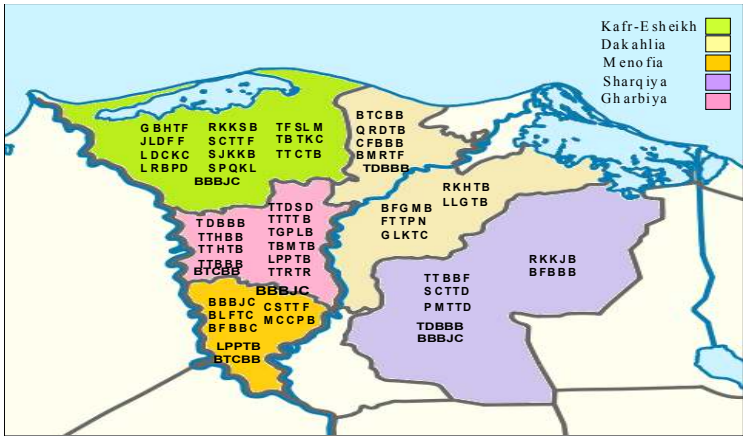

Figure (1). Map of Egypt showing wheat stem rust survey districts in five governorates.

To study the similarity between the identified races from five governorates cluster analysis were don. Data in Figure (2), reveled that, these races were divided to A and B group with similarity a bout 31.10%, where A (Kafer Elshaik) and B the rest governorates. The highest similarity were observed between the identified races from Sharkia and Menofia (about53%).

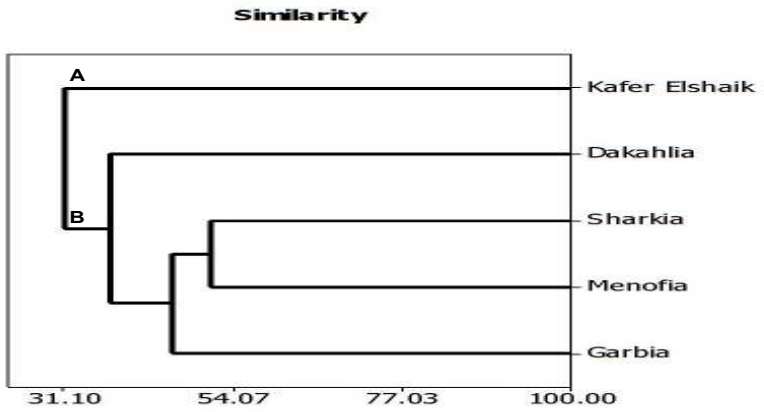

Figure (2). Dendrogarme between the identified races and the tested governorates and race groups.

Effectiveness of stem rust resistance gene(s) (*Sr,s*) at seedling stage.

It was evident that the majority of the resistance genes were found ineffective against most of the isolates tested in this study, Data in (Table 4) reveled that Sr31 is completely resistant to all races of *Puccinia graminis* f. sp. *tritici* which gave 100% efficacy followed by *Sr24* (89.5%). Meanwhile, Sr38,36 and SrMCN posses a good level of resistance their efficacy were (77.7, 75.3 and 70.6%) efficacy. On the other hand 10 stem rust resistant genes were intermediate. The lowest Sr genes at this study were Sr9g and Sr5. El-Daoudi *et al.* (1995), McVey *et al.* (1997), Singh *et al.* (2008) and Moussa et al. (2013). Concluded that there were many genes posses resistant to stem rust fungus at seedling stage and some of them resistant at adult stage whereas a little number posses resistance at the two stages.

Table (4). Efficacy (%) of 20 *Sr's* evaluated against 85 isolates of *P.g.t* in Egypt during 2012/2013

No.	Sr's	No. of		Total	Efficacy %
		Low infection type	High infection type		
1	5	30	55	85	35.3
2	21	35	50	85	41.2
3	9e	44	41	85	51.8
4	7b	36	49	85	42.4
5	11	38	46	85	55.9
6	6	46	38	85	55.3
7	8a	31	54	85	36.5
8	9g	25	60	85	29.5
9	36	64	21	85	75.3
10	9b	50	35	85	58.9
11	30	54	31	85	63.6
12	17	41	44	85	48.2
13	9a	44	41	85	51.2
14	9d	37	48	85	43.6
15	10	33	52	85	38.9
16	Tmp	45	40	85	53
17	24	76	9	85	89.5
18	31	85	0	85	١٠٠
19	38	66	19	85	77.7
20	MCN	60	25	85	70.6

Regarding these data Sr31, Sr24, Sr38,Sr36 and SrMCN proved to be the most resistant genes during this study which gave efficacy% (100,89.5,77.7,75.3 and 70.6 respectively). This confirms the report of (Roelfs *et al*., 1992), that stated this gene is amongst the effective genes, which have an adequate and some immediate values to almost all races in the world. Thus, use of effective *Sr* genes such as *Sr31* and *Sr24* in single cultivar through gene pyramiding (breeding) has paramount importance as the additive effects of several genes offer the cultivar a wider base stem rust resistance along with periodic race survey. On the other hand Sr9g, Sr5, Sr8a and Sr10 were the ineffective genes against the tested races. The rest stem rust resistant genes in between (29.5-63.6%). Youssef *et al.,* (2012) and

Abou-Zeid *et al.* (2014). Reported that Sr's i.e. *Sr31, Sr33+5, Sr29+9g and Sr35* was the most effective genes against most of stem rust races. However, the ineffective genes were *Sr's: PL, WLD, 6, DP_2, 9e, 17, 8a* and *8b*.Abebe *et al.* (2012) and Abou-Zeid *et al.* (2014).Found that most of the genes possessed by the differentials were non effective against the tested isolates except *Sr24* and *Sr Tmp* were effective in 100 and 90% respectively. In contrast, *SrMcN* and *Sr9b* were non effective to 96.9 and 93.8% of the tested isolates.

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السلالات الفسيولوجية والتنوع فى القدرة المرضيه لفطر صدا الساق فى القمح فى مصر موسم ٢٠١٢\٢٠١٣

محمد عبد القادر حسن و محمد عبد الحليم أبوزيد
قسم بحوث أمراض القمح - معهد بحوث أمراض النباتات – مركزالبحوث الزراعية - الجيزة - مصر.

اجريت هذه الدراسة بهدف تعريف السلالات الفسيولوجية لفطر صدأ الساق فى القمح ومدي التنوع في القدرة المرضية بالاضافة الي اختبار كفأة الجينات المستخدمه لمقاومة هذا المرض من خلال الحصر السنوى فى خمس محافظات وهى (الغربية والدقهليه والمنوفيه وكفرالشيخ والشرقيه) خلال موسم ٢٠١٢/٢٠١٣ وتمت هذه الدراسه بالصوبة المقامة بمحطة البحوث الزراعيه بالجميزة. تم تعريف ٨٥ سلاله من فطر صدأ الساق من العينات التى تم جمعها من المحافظات تحت الدراسه حيث اوضحت النتائج ان السلالة TDBBB كانت الاكثر تكرارا (٧%) من بين السلالات المعرفه بمحافظة الغربيه متبوعة ب TTHBB, TTHTB بنسبة تكرار(٧٢.٤% , ٨٨.٥%) علي التوالي بينما كانت السلالة BTCBB أكثر تكرار في محافظة الدقهلية (٧%) كما أوضحت النتائج أن السلاله BBBJC المعرفة من عزلات محافظة المنوفيه هي الأكثر تكرار علي مستوي السلالات المعرفه من الخمس محافظات تحت الدراسة بنسبة تكرار٤١.٩%. قسمت السلالات المعرفة الي مجموعتين A,B علي حسب درجات التشابه بنسبة ٣١.١%. كما أوضحت النتائج ان جين المقاومة *Sr 31* ١٠٠% كان الأكثر كفائة متبوعاً ب *Sr 24* ٨٩.٥% ومن ثم فإن استخدام تلك الجينات وادخالها في اصناف القمح له اهمية كبيرة في اعطائها صفة المقاومة المستديمة ضد مرض صدأ الساق في مصر.