

FREQUENCY OF VERULENCE AND VIRULENCE FORMULA OF WHEAT STEM RUST RACES IDENTIFIED IN EGYPT DURING 2008/09 - 2009/010.

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ABSTRACT

Wheat stem rust is one of the major diseases on wheat in Egypt which appears annually in virulence of different frequencies. In this investigation, stem rust samples were collected from different locations of lower Egypt during two seasons (2008/09-2009/010). Three single pustules method of isolation was followed for each sample. Rust data were recorded as infection types, and virulence frequencies were determined against 35 *Sr* genes, in monogenic lines and some Egyptian genotypes. Virulence frequencies were very high against *Sr21 +9g*, *Sr 7a*, *Sr 16*, *Sr 9e*, *Sr 6* and *Sr 17*, while the lowest frequencies were recorded against *Sr 31*, *Sr 29* and *Sr30*. The physiologic races were identified according to their reaction on the 20 *Srs* were recorded for each race. Thirteen and two physiologic races of *Puccinia garminis* were identified. Race group TT- was the most frequent (16.16 %) , race group TS- (11.76%) ,race group KT- and TK- (5.88 %) followed by race group BB-, DH, PR, and SR (2.94%). The least ones in the regard were races of group BC, BH<BK,DG,DF and DT (0.98%) keywords: Wheat stem rust race and virulence .

INTRODUCTION

Stem rust (*Puccinia garminis*) is a common disease on wheat in all areas in Egypt. Nevertheless, disease infection with stem rust usually occurs only at the late growth stages starting from flowering stage. No disease infection was recorded on seedling other under field conditions. Therefore, breeding for adult plant resistance is the most important method to control such disease under Egyptian conditions.

Stem rust can be considered as a common disease because it appears every season. It appeared in epidemic cases in 1947 and 1968 (Gomma 1968) and. More over, all wheat genotypes produced in Egypt are susceptible in Therefore, to protect the local genotypes from infection genes for resistance should be incorporated into the high yielding genotypes.

Disease resistance is controlled by major or minor genes or both together, however complementary effect between major genes may enhance the response of a variety and give higher levels of resistance (Simons *et al.*, 1978).

Many of the major genes are effective through the whole life of the plant, whereas a few are only effective at the adult stage. Resistance during later periods of plant growth is called adult plant resistance as defined by (El-Daoudi *et al.*, 1990) as the resistance that is only effective in the advanced growth stages of the plant .

Expression of resistance depends on the host-parasite interaction, environmental conditions, plant growth stage and the interaction between resistance genes in the wheat genome (Bolate, N. and A. P. Roelfs, (1991).

Therefore, the objective of the presents investigation is to study stemrust virulence against single genes for resistance and also against the local genotypes, to serve the national breeding program for resistance.

MATERIALS AND METHODS

Naturally infected leaf specimens were collected from different wheat growing areas in Egypt during 2008/09-2009/010. Samples were obtained from the commercial fields as well as from wheat stem rust trap nursery, from lines carrying known genes for stem rust resistance (*Srs*).

Each specimen was transferred onto 8-9 days – old seedlings of the highly susceptible cv. Giza 160 under greenhouse conditions.

Three single pustules were tested per each sample, and each culture was increased on seedlings cv. Giza 160 to generate sufficient inocula (spores) to inoculate the set of differentials, following the method of Stakman *et al.*, (1962).

Infection types were recorded according to scale of 0-4 grades (Stakman *et al.*, (1962).) as shown in (Table, 1). Wheat seedlings of eight days old were sprayed with tap water and the leaves were gently rubbed between moistened fingers and again sprayed with water by an atomizer in the inoculation chambers. Inoculation was carried out by shaking and brushing rust spores over the plant leaves and sprayed gently again with water in order to induce an initial thin film of water on the plant leaves. while the temperature was 20- 25 °C and relative humidity of 90 % was supplied. (Roelfs, A.P. and J.W. Mertens (1988). And Roelfs, A.P.; D.L. Long and J.J. Roberts (1993) Frequency of virulence was determined against two sets of genotypes. The first set included local wheat genotypes, whereas the second set included 36 monogenic lines carrying adult-plant resistance genes. The frequency of virulence was estimated as the percentage of virulent isolates divided by the total number of isolates for each genotype.

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

According to the method adopted by Green (1965).

Table (1): Pgt-code for the 12 Pgt differential hosts for *Puccinia graminis* f. sp. *tritici* in ordered subsets of four groups.

Pgt-code	Subset ^a	Infection type produced on host lines with Sr			
		1	5	21	9e
	2	11	6	8a	9g
	3	36	9b	30	17
	4	9a	9d	10	Tmp
	5	7a	8b	13	15
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

(C.F) The collected samples were purified and multiplied on the susceptible cv (Morocco and *Triticum spelta saharensis*) and identified on the stem rust on wheat differentials at seedling stage.

Table (2): Wheat stem rust infection types used in disease assessment for seedling stage.

Host response (class)	Infection types	Disease symptoms
Immune	0	No uredia or other macroscopic sign of infection.
Nearly immune	0;	No, uredia but hypersensitive necrotic or chlorotic flecks present.
Very resistant	1	Small uredia surrounded by necrosis.
Moderate resistant	2	Small to medium uredia surrounded by chlorosis or necrosis.
Moderate susceptible	3	Medium-sized uredia that may be associated with chlorosis.
Very susceptible	4	Large uredinia without chlorosis or necrosis or rarely necrosis.
Heterogeneous	X	Random distribution of v

RESULTS

Frequency of Virulence:

Frequency of virulence of the causal organism *Puccinia graminis* of wheat stem rust was studied using rust samples collected from lower Egypt during 2008/09- 2009/010. The obtained cultures were tested in the following seasons 2008/09-2009/010. Virulence was tested against 35 monogenic lines for stem rust resistance Occurrence of virulence was estimated as virulent isolates a divided by the total number of isolates for each wheat genotype.

Frequency of virulence to Srs genes:

1) Occurrence of virulence in 2008/09:

The results presented in Table (3), showed different frequencies of virulence to the tested lines. The lowest values were found against *Sr 31*, *Sr 6* *Sr PL* and *Sr WLD* ranging from 0% to 17.39 % in an ascending order. On the other hand, the highest occurrence of virulence was found against *Sr 21+ 9g*, *Sr 7a* and *Sr 29* ranging 69.57 to 89.13 in a descending order . whereas, the rest of lines showed moderate responses, as shown in Table (3).

Table (3): Frequency of virulence of *Puccinia graminis tritici* to 36 monogenic lines for stem rust resistance at 2008/09*.

No.	Sr's	Avir. isolates	Vir. isolates	Total	Effective gene	Vir. frequency
1	Sr5	33	13	46	71.74	28.26
2	Sr6	39	7	46	84.78	15.22
3	Sr7a	6	40	46	13.04	86.96
4	Sr7b	23	23	46	50.00	50.00
5	Sr8a	28	18	46	60.87	39.13
6	Sr8b	36	10	46	78.26	21.74
7	Sr9a	18	28	46	39.13	60.87
8	Sr9b	30	16	46	65.22	34.78
9	Sr9d	22	24	46	47.83	52.17
10	Sr9e	31	15	46	67.39	32.61
11	Sr9g	37	9	46	80.43	19.57
12	Sr10	22	24	46	47.83	52.17
13	Sr11	21	25	46	45.65	54.35
14	Sr13	29	17	46	63.04	36.96
15	Sr15	31	15	46	67.39	32.61
16	Sr16	13	33	46	28.26	71.74
17	Sr17	33	13	46	71.74	28.26
18	Sr21	30	16	46	65.22	34.78
19	Sr22	17	29	46	36.96	63.04
20	Sr23	31	15	46	67.39	32.61
21	Sr24	23	13	36	63.89	36.11
22	Sr28	10	19	29	34.48	65.52
23	Sr29	14	32	46	30.43	69.57
24	Sr30	26	20	46	56.52	43.48
25	Sr31	35	1	36	97.22	2.78
26	Sr32	16	30	46	34.78	65.22
27	Sr33+Sr5	33	13	46	71.74	28.26
28	Sr34	14	32	46	30.43	69.57
29	Sr35	14	32	46	30.43	69.57
30	Sr36	34	12	46	73.91	26.09
31	SrPL	40	6	46	86.96	13.04
32	SrWLD	38	8	46	82.61	17.39
33	SrTT3+Sr10	31	15	46	67.39	32.61
34	SrDP2	19	11	30	63.33	36.67
35	SrTMP	25	21	46	54.35	45.65
36	Sr21+9g	5	41	46	10.87	89.13

2) Occurrence of virulence in 2009/010:

Obtained results revealed that the lowest frequencies of virulence were found against *Sr WLD*, *Sr 30*, *Sr 6* and *Sr9g*. On the other hand *Sr 6*, *Sr 9e*, *Sr 17* and *Sr9a* averse arranged according to their virulence frequency 88.04, 83.87, 82.97 and 80.85 respectively .

Table (4): Frequency of virulence of *Puccinia graminis tritici* to 36 monogenic lines for stem rust resistance at 2009/010.

No.	Sr's	Avir. isolates	Vir. isolates	Total	Effective gene	Vir. frequency
1	<i>Sr5</i>	27	67	92	28.87	71.27
2	<i>Sr6</i>	11	81	92	11.95	88.04
3	<i>Sr7a</i>	30	66	96	31.25	68.75
4	<i>Sr7b</i>	28	64	92	30.43	69.56
5	<i>Sr8a</i>	31	70	91	34.06	76.92
6	<i>Sr8b</i>	21	75	96	21.87	78.12
7	<i>Sr9a</i>	18	76	94	19.14	80.85
8	<i>Sr9b</i>	21	75	96	21.87	78.12
9	<i>Sr9d</i>	33	61	94	35.10	64.89
10	<i>Sr9e</i>	15	78	93	16.12	83.87
11	<i>Sr9g</i>	19	79	98	19.38	80.61
12	<i>Sr10</i>	33	63	96	34.37	65.62
13	<i>Sr11</i>	32	58	90	35.55	64.44
14	<i>Sr13</i>	33	67	100	33.00	67.00
15	<i>Sr15</i>	27	71	98	27.55	72.44
16	<i>Sr16</i>	67	28	95	70.52	29.47
17	<i>Sr17</i>	16	78	94	17.02	82.97
18	<i>Sr21</i>	26	61	95	27.36	64.21
19	<i>Sr22</i>	40	51	91	43.95	56.04
20	<i>Sr23</i>	30	61	91	32.96	67.03
21	<i>Sr24</i>	39	52	91	42.85	57.14
22	<i>Sr28</i>	35	56	91	38.46	61.53
23	<i>Sr29</i>	82	18	100	82.00	18.00
24	<i>Sr30</i>	12	83	95	12.63	87.36
25	<i>Sr31</i>	84	7	93	90.32	7.52
26	<i>Sr32</i>	55	35	90	61.11	38.88
27	<i>Sr33+Sr5</i>	66	24	90	73.33	26.66
28	<i>Sr34</i>	47	43	90	52.22	47.77
29	<i>Sr35</i>	70	18	98	71.42	18.36
30	<i>Sr36</i>	4	90	94	4.25	95.74
31	<i>SrPL</i>	25	62	87	28.73	71.26
32	<i>SrWLD</i>	25	70	95	26.31	73.68
33	<i>SrTT3+Sr10</i>	28	64	92	30.43	69.56
34	<i>SrTMP</i>	47	49	96	48.95	51.04
35	<i>Sr21+9g</i>	74	17	91	81.31	18.68

Physiologic races identification:

a. The first season 2008/09:

Table (5): revealed the occurrence of 44 race group of stem rust (*Puccinia garminis* f. sp *tritici* West.). These race groups were i.e.

No.	Race group	No. of Isolates	Frequency (%)	No.	Race group	No. of Isolates	Frequency (%)
1	TK-	5	8.33	23	MS-	1	1.66
2	TR-	4	6.66	24	MT-	1	1.66
3	TT-	4	6.66	25	NC-	1	1.66
4	LC-	3	5.00	26	PG-	1	1.66
5	ST-	3	5.00	27	PF-	1	1.66
6	BH-	2	3.33	28	PK-	1	1.66
7	QG-	2	3.33	29	PT-	1	1.66
8	BB-	1	1.66	30	QC-	1	1.66
9	BF-	1	1.66	31	QF-	1	1.66
10	BL-	1	1.66	32	QK-	1	1.66
11	BT-	1	1.66	33	QS-	1	1.66
12	BK-	1	1.66	34	QT-	1	1.66
13	CK-	1	1.66	35	NH-	1	1.66
14	DJ-	1	1.66	36	RK-	1	1.66
15	DK-	1	1.66	37	RR-	1	1.66
16	FT-	1	1.66	38	SC-	1	1.66
17	JH-	1	1.66	39	SL-	1	1.66
18	JK-	1	1.66	40	SJ-	1	1.66
19	JR-	1	1.66	41	SK-	1	1.66
20	HR-	1	1.66	42	SR-	1	1.66
21	KT-	1	1.66	43	TS-	1	1.66
22	MQ-	1	1.66	44	TH-	1	1.66

TK, TR, TT, LC, ST, BH, QG, BB, BF, BL, BT, BK, CK, DJ, DK, FT, JH, JK, JR,HR, KT, MQ, MS, NC, PG,PF, PK, PT, QC, QF, QK, QS, QT, NH, RK, RR, SC, SL, SJ, SK, SR, TS and TH were determined on the basis of sum of high infection types for each of 20 wheat stem rust monogenic differentials.

The surveyed samples of the 2008/09 in Northern governorates of Egypt. These data revealed that race group TK was the most frequent one (8.33%) followed by race group TR- and TT which were more frequent (6.66 %) followed by race group LC- and ST (5.00%), by race group BH- and QG- (3.33 %)- least ones in this regard were race groups i.e. BB, BF, BL,BT, BK and CK (1.66%) . the rest of races recorded less than 1.66

b. The second season 2009/010:

Table (6): Physiologic races of *Puccinia graminis* f. sp. *tritici* and their frequency (%) in Egypt during 2009/010.

No.	Race group	No. of Isolates	Frequency (%)	No.	Race group	No. of Isolates	Frequency (%)
1	TT-	17	16.66	25	GH-	1	0.98
2	TS-	12	11.76	26	KJ-	1	0.98
3	KT-	6	5.88	27	KF-	1	0.98
4	TK-	6	5.88	28	CQ-	1	0.98
5	BB-	3	2.94	29	FT-	1	0.98
6	DH-	3	2.94	30	NR-	1	0.98
7	LC-	3	2.94	31	NT-	1	0.98
8	PR-	3	2.94	32	NS-	1	0.98
9	SR-	3	2.94	33	PT-	1	0.98
10	BL-	2	1.96	34	PJ-	1	0.98
11	JT-	2	1.96	35	LT-	1	0.98
12	KG-	2	1.96	36	LK-	1	0.98
13	KK-	2	1.96	37	RK-	1	0.98
14	TP-	2	1.96	38	HR-	1	0.98
15	TR-	2	1.96	39	QR-	1	0.98
16	ST-	2	1.96	40	SM-	1	0.98
17	BC-	1	0.98	41	SG-	1	0.98
18	BH-	1	0.98	42	SH-	2	1.96
19	BK-	1	0.98	43	SK-	1	0.98
20	DG-	1	0.98	44	TP-	1	0.98
21	DF-	1	0.98	45	TQ-	1	0.98
22	DT-	1	0.98	46	TH-	1	0.98
23	BS-	1	0.98	47	TB-	1	0.98
24	FH-	1	0.98				

TT, TS, KT, BB, LC, DH, PR, SR, BL, JT, KG, KK, TP, TR, ST, BC, BH, BK, DG,DF, DT, BS, FH, GH, KJ,KF, CQ, FT, NR, NT, NS, PT, PJ, LT, RK, HR, QR, SM, SG, SH, SK, TP, TQ, TH and TB were determined on the basis of sum of high infection types for each of 20 wheat stem rust monogenic differentials.

The survey samples of the 2008/09 in Northern governorates of Egypt. These data revealed that race group TT was the most frequent one (16.66%) followed by race group TT- was most frequent (16.16 %) followed by race group TS- (11.76%) followed by race group KT- and TK- (5.88 %) followed by race group BB-, DH, PR, and SR (2.94%).. The least ones in the regard were races group BC, BH, BK,DG,DF and DT (0.98%) .

DISCUSSION

Stem rust of wheat (*Triticum aestivum* L.) caused by *Puccinia graminis tritici* is considered one of the most serious diseases in Egypt. The disease became a very dangerous on most of the currently introduced varieties because of their susceptibility to the disease (El-Daoudi, et al., 1996). It usually occurs at higher level of severity on the late sowings than in

early ones when the environmental conditions became suitable for rust incidence and development (Bariana HS, Brown GN, Bansal UK, Miah H, Standen GE, Lui M, 2007).

Stem rust can be considered as a sporadic disease because it appears in some seasons but not in the others . It appeared in epidemic cases in 1947 and 1968 (Gomma 1968). Moreover, all wheat genotypes produced in Egypt were susceptible in terms of infection types. Therefore, to protect the local genotypes from infection by such disease, genes for resistance must be incorporated into the high yielding genotypes.

The annual survey of Wheat stem rust conducted through out two growing seasons of wheat crop in some governorates of Egypt gave evidence to the presence of different virulence of the causal agent(*Puccinia garminis tritici* West .). The obtained results were established on the comparisons of both visual symptoms as infection types of the uredial stage of the casual agent with those reported by Wiese (1977) and Agrios ((1979).

Isolation and identification of the prevailing races of the disease are an essential step to satisfy this work(AbuEI-Naga *et al.*,(1993). In the course of this study, virulence survey was carried out by collecting stem rust samples from different locations of Egypt.

The obtained results in seedling stage gave evidence to presence of 44 and 47 race groups of stem rust during the two successive seasons 2008/09 and 2009/010, respectively.

In general rust causal organisms are air- borne pathogens which are carried by wind from their source to the susceptible plants (Chen *et al.*, 2005). Therefore, the inoculate play an important role in the occurrence and frequency of virulence (AbuEI-Naga *et al.*,(1990)

The appearance of new virulence is due to the appearance of new mutation, hetrokaryosis and the hybridization of genetically different individuals (Stakman 1962).

Virulence occurrence against the different stem rust genes also was of different values depending on the host - race compatibility (Beteselassie, N., C. Fininsa & A. Badebo. 2007)

In the present, The least frequencies of virulence were recorded with Sr 31, SrPL, Sr6, SrWLD, Sr9g, Sr8b, Sr5 and Sr17 showing 2.78, 13.04, 15.22, 17.39, 19.57, 21.74, 28.28 and 28.28 frequencies, respectively. On the cother hand, Sr21+9g, Sr7a and Sr16 showed highest frequencies of virulence (89.13, 96.96 and 71.74), respectively. While the rest of Sr genes showed different frequencies of virulence from 28.28 to 71.74 during first season.

Frequencies of virulence occurrence with the tested Sr genes in terms of infection types showed vourialele responses .The least frequencies of virulence were recorded with Sr 31, Sr29, Sr35, Sr21+9g, 33+Sr5 Sr16 and Sr32 showing 7.52, 18.00, 18.36, 18.68, 26.66, 29.47 and 38.88 frequencies, respectively. On the other hand, Sr6, Sr9e and Sr9a showed the highest frequencies of virulence (88.04, 83.87 and 80.85%), respectively. The rest of Sr genes showed different frequencies of virulence ranged from 38.88 to 80.85 Chen T.*et al.* (1997) Sawhncy (1998), Pretorius *et. al.* (2000) and Hassain (2006)

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تكرار ظهور السلالات الممرضة ومعادلة القدرة المرضية لمرض صدأ الساق
علي القمح وتعريف السلالات الفسيولوجية للفطر في مصر خلال ٢٠٠٥/٠٦ -
٠٧ / ٢٠٠٦

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١- قسم أمراض النبات - كلية الزراعة - جامعة المنصورة.

٢- معهد أمراض النباتات - قسم أمراض القمح - مركز البحوث الزراعية - الجيزة - مصر.

يعتبر مرض صدأ علي القمح من الأمراض الرئيسية في مصر والذي يظهر سنوياً بدرجات مختلفة من التكرارات للسلالات الممرضة - وجمعت عينات الأوراق المصابة بالمرض من جهات متفرقة خلال موسمي الدراسة ٢٠٠٨/٠٩ - ٢٠٠٩/١٠. وقد تم عزل السلالات و أخذت بيانات الصدا بطريقة طرز الإصابة ضد ٣٥ سلالة قمح حاملة لجينات فرنية وقد تم تسجيل المعادلة الرياضية لكل سلالة مع تسجيل غياب وظهور السلالات الفسيولوجية والتغير في ظهور هذه السلالات ونسب عزلة. وقد سجلت اعلي نسبة للإصابة علي سلالات القمح الحاملة لجينات *Sr21 +9g, Sr 7a, Sr 16, Sr 9e, Sr 6* and *Sr17*, بينما كانت السلالات *Sr 31, Sr 29 and Sr30* اقل في نسب الإصابة. وتم تعريف ٤٤ سلالة. وكانت السلالة TT- أكثر السلالات تكرارا وقدرة علي إصابة سلالات وأصناف القمح.

قام بتحكيم البحث

كلية الزراعة - جامعة المنصورة

مركز البحوث الزراعية

أ.د / محمد الششتاوى عيد ربه

أ.د / صلاح الدين عبد الحميد أبو النجا