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Virulence Dynamics and Phenotypic Diversity of *Puccinia triticina* in Egypt During 2009/10 and 2010/11

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Wheat leaf rust is the most widespread and regularly occurring disease of wheat in Egypt and worldwide. Collections of *Puccinia triticina* were obtained from rust-infected wheat leaves (*Triticum aestivum*) surveys from wheat fields and Egyptian wheat rust trap nursery EWRTN in Damietta, Monufiya, Qalubiya, Suez and Beni-Suef governorates in order to determine the virulence dynamics of wheat leaf rust population in 2009/10 and 2010/11. The virulent phenotypes were tested on a set of 16 lines of Thatcher wheat that are near-isogenic for leaf rust resistance genes, *i.e. Lr1*, *Lr2a*, *Lr2c*, *Lr3*, *Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *Lr10*, *Lr18*, *Lr21*, and *Lr2b*. Out of 102 and 33 virulent phenotypes that were identified, TKTT (12.8%), TTTT (8.1%) and KKTT (3.4%) were the most common pathotypes in 2009/10. While MKKS (11.5%), and BBBB (8.2%), PSSS (8.2%) were the most common pathotypes in 2010/11. Furthermore, the lowest virulence frequencies were found for genes; *Lr*25 (15.08%), *Lr*19 (16.87%), *Lr*9 (22.34%), *Lr*28 (30.16%) and *Lr*29 (32.96%). While, *Lr's 2c, 3ka, 10, 18, 16,30* and 24 had the highest frequencies of virulence; 86.03, 85.47, 84.35, 83.79 , 82.68%, 82.68% and 82.12% , respectively in 2009/10. The lowest virulence frequencies for leaf rust resistance genes occurred with *Lr*19 (14.59%), *Lr*2a, *Lr*28 & *Lr*42 (each with 17.54%), *Lr*9 (20.35%), *Lr2b* (22.80%), *Lr36* (26.31%), *Lr25* (28.07%) and *Lr45* (29.82%). However, high virulence was recorded with *Lr's;* 1, 35, 10, 15 and 18 (100.0, 80.7, 78.94, 75.43 and 73.68%, respectively) during 2010/11.

Keywords: Leaf rust, pathotypes, phenotypes, *Puccinia triticina,* virulence and wheat.

Wheat (*Triticum* spp.) was one of the first domesticated food crops and for 8000 years has been the basic staple food of the major civilizations of Europe, West Asia and North Africa. Today, wheat is grown on more land area than any other commercial crop and continues to be the most important food grain source for humans. It is affected by many diseases that cause yield and quality losses. Rust diseases are the most important diseases that pose constant threat to sustainable production of wheat.

Leaf rust, caused by *Puccinia triticina* Eriks (*Puccinia recondit*a Rob. & Desm. f.sp. *tritici* Eriks & Henn.) is the most common disease of wheat (*Triticum aestivum* L.) worldwide (Roelfs *et al*., 1992), and regularly occurring disease of wheat in Egypt. Genetic resistance to leaf rust is the preferable method to control the disease.

Breeding for leaf rust resistance wheat cultivars began in the 1930s (Chester, 1946). Over 71 leaf rust resistance genes have been described and identified in wheat (Singh *et al*., 2013), expressed in the so called specific resistance (McIntosh *et al*., 1995& 2008).

Data from leaf rust surveys have been used to characterize virulence dynamics and phenotypic diversity within and between *P. triticina* populations in different wheat growing areas in response to host selection (Kolmer, 2013). To enhance the durability of host genetic resistance to wheat leaf rust, breeders attempted to incorporate more than one of these genes in the new released cultivars to face the dynamic nature of the causal agent (Roelfs, 1988). These genes gave us the ground to facilitate the development and improvement of resistant cultivars to manage leaf rust (McVey and Long, 1993).

The objectives of this study were to survey and characterize the virulence dynamics of *P. triticina* populations in Egypt during 2009/10 and 2010/11 in five Governorates. Also, to compare these results with those of the previous surveys and to determine the effective genes on isolates that basis of 40 near isogenic lines (Lr's) in addition to estimate the virulence frequency of *Puccinia triticina*.

Materials and Methods

1. Leaf rust occurrence and isolate collection:

Leaf rust samples (infected leaves) were collected from wheat fields and Egyptian wheat rust trap nursery (EWRTN) in annual surveys of five Governorates, *i.e*. Damietta [(north area (1N)], Qalubiya [middle area (2M)], Monufiya [west area (3W)], Suez [east area (4E)] and Beni-Suef [south area (5S)], during March to early May in 2009/10 and 2010/11 growing seasons. The leaves were air-dried and stored at 4^oC until spores were collected for inoculations.

2. Identification of P. triticina pathotypes:

Urediniospores from each collection were used to inoculate 7-days-old seedlings of the wheat cultivar Morocco that had been treated with a maleic hydrazide solution of approximately 5mg (dissolved in 50ml of H_2O) per pot to enhance spore production (Singh, 1991). The method of inoculation was carried out as described by Stakman *et al*. (1962), in which the seedling leaves were rubbed gently between moistened fingers with tap water, sprayed in the incubation chambers with water, then inoculated by shaking or brushing rusted materials over the plant leaves and sprayed gently again with water in order to induce initial film of free water on the plants which is essential for spore germination and the establishment of infection. The inoculated plants were placed in a dew chamber overnight at 18 ± 2^oC. Then were transferred to isolation chambers in a greenhouse where temperature varied between 18 and 28^oC daily under at least 10hr of natural light, with supplemental greenhouse lighting. After developing the pustules, 3-4 single pustules were isolated separately from each sample for rust propagation on the highly susceptible wheat variety Morocco to obtain enough urediniospores for inoculation.

3. Race identification:

The method used to identify races was adopted by Long and Kolmer (1989). According to this system, the plant reaction is determined on 16 lines divided into 4 groups of four near isogenic *Lr*-lines of Thatcher with the four lines in the first group (set) of differentials included *Lr* genes *1, 2a, 2c,* and *3*; the second group included *9, 16, 24*, and *26*, the third group included *3ka, 11, 17* and *10*, whereas the *Lr's 10*, *18*, *21*, and *2b* were in the fourth group of differentials. According to combination of response, of low infection type (L) and high infection type (H) plants each rust isolate was coded in letters. As a result each phenotype has a code including letters consents of English alphabet from B through T.

3. Virulence analysis of Puccinia triticina:

A set consisting of 40 near isogenic of Thatcher lines were used for virulence analysis. The frequency of virulence was estimated as the percentage of virulent isolates to the total number of isolates for each genotype. On the other hand to evaluate the efficacy of each leaf rust resistance gene under study the following formulae were used.

$$\text{Virulence frequency (\%)} = \frac{\text{No. of virulent isolates}}{\text{Total number of isolates}} \times 100$$

$$\text{Gene efficacy (\%)} = \frac{\text{No. of avirulent isolates}}{\text{Total number of isolates}} \times 100$$

4. Cluster analysis:

Pathotype and virulence frequencies were determined for collections from five geographic areas as abovementioned. A modified version of Nei's genetic distance between isolates in areas 1, 2, 3, 4 and 5 calculated with NTSYS-pc v2.1 (Exeter Software, Seatauket, NY) in which the frequency of isolates with virulence to a leaf rust resistance gene was used in place of allele frequency. The distance matrix of Nei's genetic distance between the areas was plotted with UPGMA clustering in NTSYS-pc v2.1. (Rohlf, 2000).

Results

During the two growing seasons 2009/10 and 2010/11, the highest collected samples and succeeded isolates were in Qalubiya (32 and 76), followed by Beni-Suef and Suez, while Monufiya was the lowest. Moreover, the highest number of collected samples and succeeded isolates were higher in season 2009/10 than 2010/11. Accordingly 102 and 33 pathotypes were identified during the two seasons, respectively, (Table 1).

Distribution of identified pathotypes:

One hundred and two pathotypes were identified during 2009/10.The most common pathotypes were TKTT (12.75%), TTTT (8.05%) and KKTT (3.36%). These phenotypes were found in all areas except KKTT in area 3 (5.6%), area 4

Table 1. Number of samples, succeeded isolates and races of wheat leaf rust collected and identified from the five governorates during 2009/2010 and 2010/2011 growing seasons

Area No.	Governorate	No. of samples		No. of isolates		No. of races [a]		Total		
		2009/10	2010/11	2009/10	2010/11	2009/10	2010/11	Samples	Isolates	Pathotypes*
1 (N)	Damietta	6	4	7	9	6	4	10	16	10
2 (W)	Monufiya	5	3	9	8	7	4	8	17	11
3 (M)	Qalubiya	23	9	54	22	41	14	32	76	55
4 (E)	Suez	9	5	34	8	30	4	14	42	34
5 (S)	Beni-Suef	16	9	45	14	36	12	25	59	48
Total		59	30	149	61	102	33	89	210	135

* Number of races.

(2.9%) and area 5 (2.2%). In area 1 (Damietta), the pathotype TTTT (28.6%) was the most common. In the second area (Monufiya) the most frequent pathotype was TKTT (33.3%). Meanwhile in area 3 (Qalubiya) phenotypes, TKTT& TTTT (each with frequency of 7.4%) followed by FKTT & KKTT (each with 5.6%) were the most common in this area. In area 4 (Suez) and area 5 (Beni-Suef), the most common pathotypes were (TKTT and TTT) followed by TKTT, TTTT and KJTT, respectively, (Table 2).

Thirty three pathotypes were identified during 2010/11 growing season. The most frequent pathotypes were MKKS (11.5%), BBBB & PSSS (each with 8.2%) and LCCG, LTTS, MKTS, TTJT & TTTT (each with 4.9%). In area 1 (Damietta), the pathotype PSSS (44.4%) was the most common pathotype in this area. Area 2 (Monufiya), the pathotype MKKS (50%) was the most frequent phenotypes. While the most frequent pathotypes in area 3 (Qalubiya) were BBBB (18.2%), MKTS and TTTT (each with 13.6%), but in area 4 (Suez) and area 5 (Beni-Suef), the most common pathotypes were LCCG (37.5%), MKKS and TTJT (each with 13.6%), respectively, (Table 3).

Gene efficacy:

Frequencies of virulence to *Lr* gene differ among the populations of *P. triticina* in Delta Egypt during 2009/10. Data presented in Table (4) showed different frequencies of virulence to the total Lr’s genes in terms of infection types. The least frequencies of virulence were found for *Lr25, Lr19, Lr9, Lr28* and *Lr29* being 15.08%, 16.87%, 22.34%, 30.16% and 32.96%, respectively. While, *Lr2* (86.03%), *Lr3ka* (85.47%), *Lr10* (84.35%), Lr16 (83.79%) and *Lr16* (82.68%) had the highest frequencies of virulence.

Also as regard to gene efficacy and virulence frequency during 2010/11, data in Table (4) revealed that five genes had the lowest virulence frequencies (%), *i.e.* *Lr*19 (14.59%) *Lr*2a, *Lr*28 & *Lr*42 (each with 17.54%) and *Lr*9 (20.35%). While *Lr’s*; *1, 35, 10, 15* and *18* had the highest virulence frequencies (100.0, 80.7, 78.94, 75.43 and 73.68 %, respectively).

Table 2. Number and frequency (%) of virulent pathotypes of *P. triticina* in Egypt during 2009/10 identified by virulence to 16[a] lines of wheat with single genes for leaf rust resistance

No.	Pathotype	Virulence frequency (%)	Area 1		Area 2		Area 3		Area 4		Area 5		Total	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	BBBB	0.0	0	0	0	0	1	1.9	0	0	0	0	1	0.67
2	BDBG	12.5	0	0	0	0	1	1.9	0	0	0	0	1	0.67
3	CCPR	50.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
4	DKTT	75.0	0	0	0	0	1	1.9	0	0	0	0	1	0.67
5	FBCQ	31.25	0	0	0	0	0	0	1	2.9	0	0	1	0.67
6	FDJL	37.5	0	0	0	0	0	0	1	2.9	0	0	1	0.67
7	FFLF	21.8	1	14.3	0	0	0	0	0	0	0	0	1	0.67
8	FHTR	34.3	0	0	0	0	0	0	1	2.9	0	0	1	0.67
9	FKKF	62.5	0	0	0	0	0	0	1	2.9	0	0	1	0.67
10	FKKS	34.3	0	0	0	0	2	3.7	0	0	0	0	2	1.34
11	FKMT	34.3	0	0	1	11.1	0	0	0	0	0	0	1	0.67
12	FKST	75.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
13	FKTJ	68.75	0	0	0	0	1	1.9	0	0	0	0	1	0.67
14	FKTQ	34.3	0	0	0	0	0	0	1	2.9	0	0	1	0.67
15	FKTR	75.0	0	0	0	0	0	0	1	2.9	0	0	1	0.67
16	FKTS	75.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
17	FKTT	81.0	0	0	0	0	3	5.6	0	0	1	2.2	4	2.68
18	FTSL	62.5	0	0	0	0	1	1.9	0	0	0	0	1	0.67
19	FTTR	81.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
20	JTTT	87.5	0	0	0	0	0	0	1	2.9	0	0	1	0.67
21	KFTT	81.25	1	14.3	0	0	0	0	0	0	0	0	1	0.67
22	KJTT	81.25	0	0	0	0	0	0	0	0	2	4.4	2	1.34
23	KKKT	81.25	0	0	0	0	0	0	1	2.9	0	0	1	0.67
24	KKPT	81.25	0	0	0	0	1	1.9	0	0	0	0	1	0.67
25	KKST	81.25	0	0	0	0	0	0	0	0	1	2.2	1	0.67
26	KKTR	81.25	0	0	0	0	0	0	0	0	1	2.2	1	0.67
27	KKTT	87.5	0	0	0	0	3	5.6	1	2.9	1	2.2	5	3.36
28	MFTL	56.25	0	0	1	11.1	0	0	0	0	0	0	1	0.67
29	MJTR	68.75	0	0	0	0	0	0	0	0	1	2.2	1	0.67
30	MKTP	75.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
31	MKTR	75.0	0	0	0	0	1	1.9	0	0	0	0	1	0.67
32	MMTR	68.75	0	0	0	0	0	0	0	0	1	2.2	1	0.67

Table 2. Continued

33	MMTT	75.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
34	MTTT	81.25	0	0	0	0	1	1.9	0	0	0	0	1	0.67
35	NDTR	62.5	0	0	0	0	0	0	0	0	1	2.2	1	0.67
36	NFLQ	21.8	0	0	0	0	0	0	1	2.9	0	0	1	0.67
37	NJTR	34.3	0	0	0	0	0	0	1	2.9	0	0	1	0.67
38	PBMR	50.0	0	0	0	0	0	0	1	2.9	0	0	1	0.67
39	PBTQ	56.25	0	0	0	0	0	0	0	0	1	2.2	1	0.67
40	PCRQ	56.25	0	0	0	0	0	0	1	2.9	0	0	1	0.67
41	PCTQ	62.5	0	0	0	0	0	0	1	2.9	0	0	1	0.67
42	PDTR	68.75	0	0	0	0	1	1.9	0	0	0	0	1	0.67
43	PDTT	75.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
44	PFRR	68.75	0	0	0	0	1	1.9	0	0	0	0	1	0.67
45	PGSR	62.5	0	0	0	0	0	0	0	0	1	2.2	1	0.67
46	PGTQ	62.5	0	0	0	0	1	1.9	0	0	0	0	1	0.67
47	PGTS	68.75	0	0	0	0	1	1.9	0	0	0	0	1	0.67
48	PHPC	56.25	0	0	0	0	0	0	0	0	1	2.2	1	0.67
49	PHTR	75.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
50	PHTT	81.25	0	0	0	0	0	0	0	0	1	2.2	1	0.67
51	PJKT	75.0	0	0	0	0	1	1.9	0	0	0	0	1	0.67
52	PJTL	62.5	0	0	0	0	0	0	0	0	1	2.2	1	0.67
53	PJTT	81.25	0	0	1	11.1	1	1.9	0	0	1	2.2	3	2.01
54	PKBT	62.5	0	0	1	11.1	0	0	0	0	0	0	1	0.67
55	PKGF	56.25	0	0	0	0	1	1.9	0	0	0	0	1	0.67
56	PKGS	62.5	0	0	0	0	1	1.9	0	0	0	0	1	0.67
57	PKJT	75.0	0	0	0	0	0	0	1	2.9	0	0	1	0.67
58	PKLS	62.5	0	0	0	0	0	0	1	2.9	0	0	1	0.67
59	PKPT	81.25	0	0	0	0	0	0	0	0	1	2.2	1	0.67
60	PKQC	56.25	0	0	0	0	0	0	1	2.9	0	0	1	0.67
61	PKQT	75.0	0	0	0	0	0	0	1	2.9	0	0	1	0.67
62	PKRS	75.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
63	PKSP	75.0	0	0	0	0	1	1.9	0	0	0	0	1	0.67
64	PKSR	75.0	0	0	0	0	0	0	1	2.9	0	0	1	0.67
65	PKST	87.5	0	0	0	0	0	0	1	2.9	0	0	1	0.67
66	PKTH	75.0	0	0	0	0	1	1.9	0	0	0	0	1	0.67
67	PKTQ	75.0	0	0	0	0	1	1.9	0	0	0	0	1	0.67
68	PKTR	81.25	0	0	0	0	0	0	0	0	1	2.2	1	0.67
69	PKTS	81.25	0	0	0	0	2	3.7	0	0	1	2.2	3	2.01

Table 2. Continued

70	PKTT	93.75	0	0	0	0	1	1.9	1	2.9	1	2.2	3	2.01
71	PLTH	62.5	0	0	0	0	0	0	0	0	1	2.2	1	0.67
72	PLTR	75.0	0	0	0	0	0	0	1	2.9	0	0	1	0.67
73	PQBT	56.25	0	0	0	0	0	0	1	2.9	0	0	1	0.67
74	PSRT	81.25	0	0	0	0	0	0	1	2.9	0	0	1	0.67
75	PSTJ	75.0	0	0	0	0	1	1.9	0	0	0	0	1	0.67
76	PTJR	68.75	0	0	0	0	0	0	0	0	1	2.2	1	0.67
77	PTTM	81.25	0	0	0	0	1	1.9	0	0	0	0	1	0.67
78	PTTS	87.5	0	0	0	0	1	1.9	0	0	0	0	1	0.67
79	PTTT	93.75	0	0	0	0	1	1.9	0	0	0	0	1	0.67
80	RKQD	56.25	0	0	0	0	1	1.9	0	0	0	0	1	0.67
81	RKTT	81.25	0	0	0	0	0	0	0	0	1	2.2	1	0.67
82	RRTQ	68.75	0	0	0	0	0	0	1	2.9	0	0	1	0.67
83	TDRR	68.75	0	0	0	0	1	1.9	0	0	0	0	1	0.67
84	TDTR	75.0	0	0	0	0	0	0	1	2.9	0	0	1	0.67
85	TFTT	87.5	0	0	0	0	0	0	0	0	1	2.2	1	0.67
86	THTT	87.5	0	0	0	0	0	0	1	2.9	0	0	1	0.67
87	TJTT	93.75	0	0	0	0	1	1.9	0	0	0	0	1	0.67
88	TKGN	62.5	0	0	0	0	1	1.9	0	0	0	0	1	0.67
89	TKLQ	62.5	0	0	0	0	1	1.9	0	0	0	0	1	0.67
90	TKPT	87.5	1	14.3	0	0	0	0	0	0	0	0	1	0.67
91	TKSS	81.25	0	0	0	0	1	1.9	0	0	0	0	1	0.67
92	TKTK	87.5	0	0	0	0	0	0	0	0	1	2.2	1	0.67
93	TKTR	87.5	0	0	0	0	1	1.9	0	0	0	0	1	0.67
94	TKTS	87.5	0	0	1	11.1	1	1.9	0	0	0	0	2	1.34
95	TKTT	93.7	1	14.3	3	33.3	4	7.4	4	11.8	7	15.6	19	12.75
96	TSJS	75.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
97	TSTT	93.7	1	14.3	0	0	2	3.7	0	0	0	0	3	2.01
98	TTCM	34.3	0	0	0	0	0	0	1	2.9	0	0	1	0.67
99	TTFR	81.25	0	0	0	0	1	1.9	0	0	0	0	1	0.67
100	TTFS	81.0	0	0	0	0	0	0	0	0	1	2.2	1	0.67
101	TTSM	81.0	0	0	0	0	1	1.9	0	0	0	0	1	0.67
102	TTTT	100.0	2	28.6	1	11.1	4	7.4	2	5.9	3	6.7	12	8.05
Total			7		9		54		34		45		149	
Frequency (%)			4.7		6.0		36.2		22.8		30.2			

[a] Thatcher lines with leaf rust resistance genes, *Lr1*, *Lr2a*, *Lr2c*, *Lr31*,*Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *LrB*, *Lr10*, *Lr14a* and Lr18.
Area1= Damietta, Area2= Monufiya, Area 3= Qalubiya, Area 4= Suez and Area 5= Beni-Suef.

Table 3. Number and frequency (%) of virulent pathotypes of *P. triticina* in Egypt during 2010/11, identified by virulence to 16[a] lines of wheat with single genes for leaf rust resistance

No.	Pathotype	Virulence frequency (%)	Area 1		Area 2		Area 3		Area 4		Area 5		Total	
			N0.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	BBBB	0	0	0	0	0	4	18.2	1	12.5	0	0	5	8.2
2	BBJS	12.5	0	0	0	0	1	4.5	0	0	0	0	1	1.6
3	BBQM	75.0	0	0	0	0	0	0	0	0	1	7.1	1	1.6
4	BGLQ	75.0	0	0	0	0	1	4.5	0	0	0	0	1	1.6
5	BGQN	81.0	0	0	0	0	1	4.5	0	0	0	0	1	1.6
6	BGTB	62.5	0	0	0	0	0	0	0	0	1	7.1	1	1.6
7	BLBB	81.0	1	11.1	0	0	0	0	0	0	0	0	1	1.6
8	BNML	87.5	0	0	0	0	0	0	0	0	1	7.1	1	1.6
9	DBSC	81.25	0	0	0	0	0	0	0	0	1	7.1	1	1.6
10	DBJB	81.25	1	11.1	0	0	0	0	0	0	0	0	1	1.6
11	HFTS	81.25	0	0	0	0	1	4.5	0	0	0	0	1	1.6
12	LCCG	81.25	0	0	0	0	0	0	3	37.5	0	0	3	4.9
13	LKBL	81.25	0	0	0	0	0	0	0	0	1	7.1	1	1.6
14	LTTS	81.25	3	33.3	0	0	0	0	0	0	0	0	3	4.9
15	MCDS	87.5	0	0	0	0	1	4.5	0	0	0	0	1	1.6
16	MJRQ	56.25	0	0	0	0	1	4.5	0	0	1	7.1	2	3.3
17	MJSN	68.75	0	0	0	0	0	0	0	0	1	7.1	1	1.6
18	MKKS	75.0	0	0	4	50.0	1	4.5	2	25.0	0	0	7	11.5
19	MKTN	75.0	0	0	0	0	2	9.1	0	0	0	0	2	3.3
20	MKTS	68.75	0	0	0	0	3	13.6	0	0	0	0	3	4.9
21	MLKT	75.0	0	0	0	0	0	0	0	0	1	7.1	1	1.6
22	MSFS	81.25	0	0	0	0	0	0	0	0	1	7.1	1	1.6
23	PFKS	68.75	0	0	0	0	1	4.5	0	0	0	0	1	1.6
24	PKSQ	75.0	0	0	0	0	0	0	0	0	1	7.1	1	1.6
25	PMTS	56.25	0	0	1	12.5	0	0	0	0	0	0	1	1.6
26	PSSS	75.0	4	44.4	0	0	0	0	0	0	1	7.1	5	8.2
27	PTKT	81.25	0	0	0	0	1	4.5	0	0	0	0	1	1.6
28	PTTS	87.5	0	0	0	0	0	0	0	0	2	14.3	2	3.3
29	RJGS	56.25	0	0	0	0	0	0	0	0	1	7.1	1	1.6
30	RKTS	81.25	0	0	1	12.5	0	0	0	0	0	0	1	1.6
31	TTJT	81.0	0	0	0	0	1		2	25.0	0	0	3	4.9
32	TTTS	81.0	0	0	2	25.0	0	0	0	0	0	0	2	3.3
33	TTTT	100.0	0	0	0	0	3	13.6	0	0	0	0	3	4.9
Total			9		8		22		8		14		61	
Frequency (%)			15.0		13.3		36.7		13.3		21.7			

[a] As described in footnote of Table (2).

Table 4. Virulence Frequency (%) and gene efficacy (%) of 40 wheat leaf rust resistance genes *(Lr's)* against *Puccinia triticina* isolates during 2009/10 and 2010/11

No	*Lr's*	Season 2009/2010		Season 2010/2011	
		Virulence frequency (%)	Gene efficacy (%)	Virulence frequency (%)	Gene efficacy (%)
1	*Lr1*	78.21	21.79	100.00	0.0
2	*Lr2a*	39.10	60.90	17.54	82.46
3	*Lr2b*	73.18	26.82	22.80	77.20
4	*Lr2c*	86.03	13.97	31.57	68.43
5	*Lr3bg*	46.92	53.08	31.57	68.43
6	*Lr3ka*	85.47	14.53	49.12	50.88
7	*Lr9*	22.34	77.66	20.35	79.65
8	*Lr10*	84.35	15.65	78.94	21.06
9	*Lr12*	56.98	43.02	61.40	38.60
10	*Lr13*	58.65	41.35	71.92	28.08
11	*Lr14a*	59.77	40.23	70.17	29.83
12	*Lr15*	73.74	26.26	75.43	24.57
13	*Lr16*	82.68	17.32	66.66	33.34
14	*Lr18*	83.79	16.21	73.68	26.32
15	*Lr19*	16.87	83.13	14.59	85.41
16	*Lr21*	68.71	31.29	68.42	31.58
17	*Lr22a*	61.45	38.55	61.40	38.60
18	*Lr22b*	61.45	38.55	64.91	35.09
19	*Lr23*	54.74	45.26	71.92	28.08
20	*Lr24*	82.12	17.88	68.42	31.58
21	*Lr25*	15.08	84.92	28.07	71.93
22	*Lr26*	78.21	21.79	57.89	42.11
23	*Lr28*	30.16	69.84	17.54	82.46
24	*Lr29*	32.96	67.04	43.85	56.15
25	*Lr30*	82.68	17.32	56.14	43.86
26	*Lr31*	55.30	44.70	66.66	33.34
27	*Lr32*	57.54	42.46	42.10	57.90
28	*Lr33*	53.63	46.37	47.36	52.64
29	*Lr34*	64.24	35.76	45.16	54.39
30	*Lr35*	77.09	22.91	80.70	19.30
31	*Lr36*	56.98	43.02	26.31	73.69
32	*Lr38*	48.60	51.40	64.91	35.09
33	*Lr40*	51.39	48.61	57.89	42.11
34	*Lr41*	55.30	44.70	36.84	63.16
35	*Lr42*	75.97	24.03	17.54	82.46
36	*Lr43*	33.51	66.49	43.85	56.15
37	*Lr44*	54.74	45.26	49.12	50.88
38	*Lr45*	44.13	55.87	29.82	70.18
39	*Lr46*	57.54	42.46	40.35	59.65
40	*Lr47*	55.86	44.14	35.08	64.92

Virulence frequency of P. triticina isolates in 2009/10:

Frequencies of virulence differed among population of *P. triticina* in 2009/10 (Table 5). Virulence to *Lr* 2a was 85.7% of the isolates in area 1, while virulence to *Lr2a* in areas 2, 3, 4 and 5 ranged from 38.2 to 55.5%. Virulence to *Lr2c* and *Lr3* showed the same trend in all areas.

Virulence frequency to *Lr9* ranged from 22.2 to 57.15% in areas 1, 3, 4 and 5, but it was less than 11.1% in area 2. Virulence frequencies to *Lr's*; 2a, 2c, 3, 9, 3ka, 10, 18 and 2b were highest frequencies in area1, while *Lr's*; 16, 24, 26, 30 and 21, showed the highest frequencies of virulence at the area 2. Only virulence to *Lr1* and *Lr* 5 were the lowest frequencies at area1. Whereas, virulence to *Lr's* 2a, 3, 3ka, 10 and 18 were lowest at area 3. The same trend was showed for *Lr's;* 9, 11, 17 & 2b at area 2 and *Lr's*; 16, 24, 30 and 21 at area 4. Only *Lr2c* showed the lowest frequency at area 5.

Table 5. Number and frequency (%) of isolates of *Puccinia triticina* in five Egyptian governorates in 2009/10 to 16 lines of Thatcher wheat near-isogenic for leaf rust resistance

Gene	Area 1		Area 2		Area 3		Area 4		Area 5		Total	
	N0.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Lr1	3	42.9	7	77.7	33	61.1	24	70.5	36	80.0	103	69.1
Lr2a	6	85.7	5	55.5	24	44.4	13	38.2	20	44.4	68	45.6
Lr2c	7	100.0	8	88.8	45	83.3	32	94.1	36	80.0	128	85.6
Lr3	7	100.0	9	100	48	88.8	32	94.1	42	93.3	138	92.6
Lr9	3	42.9	1	11.1	11	20.4	6	17.6	8	17.8	37	22.0
Lr16	5	71.4	8	88.8	48	88.8	16	47.0	37	82.2	114	76.5
Lr24	6	85.7	9	100	44	81.4	24	70.5	34	75.5	117	78.5
Lr26	5	71.4	8	88.8	40	74.0	26	76.4	34	75.5	113	75.8
Lr3ka	7	100	8	88.8	44	81.4	32	94.1	41	91.1	132	88.5
Lr11	4	57.1	4	44.4	40	74.0	24	70.5	33	73.3	105	70.4
Lr17	6	85.7	7	77.7	44	81.4	24	70.5	41	91.1	122	81.8
Lr30	6	85.7	8	88.8	41	75.9	24	70.5	40	88.8	119	79.8
Lr10	7	100	9	100	44	81.4	33	97.0	43	95.5	136	91.2
Lr18	7	100	8	88.8	44	81.4	30	88.2	37	82.2	126	84.5
Lr21	6	85.7	8	88.8	39	72.2	17	50.0	32	71.1	102	68.4
Lr2b	6	85.7	7	77.7	32	59.2	26	76.4	38	84.4	109	73.2
Total	7	---	9	---	54	---	34	---	45	---	149	---

Area1 = Damietta, Area2 = Monufiya, Area 3 = Qalubiya, Area 4 = Suez and Area 5 = Beni-Suef.

Virulence frequency of P. triticina isolates in 2010/11:

Frequencies of virulence differed among population of *P. triticina* in 2010/11 (Table 6). Virulence frequency to *Lr2a* was 21.4% in area 5, while virulence to *Lr2a* in areas *1, 2, 3* and *4* ranged from 31.8 to 52.0%. Virulence to *Lr2c* was at highly percentages associated with virulence to Lr2a. Ten *Lr's 1, 3, 16, 24, 26, 11, 17, 30, 18* and *21* scored were highest virulence frequencies at area 2, while only Lr10 expiated highest frequency at area5. Six *Lr's 2c, 3, 26, 30, 10* and *2b* were scored lowest virulence frequencies at area1, followed by five Lr's 16, 24, 3ka, 11 and 21 at area 4.

Table 6. Number and frequency (%) of isolates of *Puccinia triticina* in the five Egyptian governorates in 2010/11 to 16 lines of Thatcher wheat near-isogenic for leaf rust resistance

Gene	Area 1		Area 2		Area 3		Area 4		Area 5		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Lr1	7	77.7	8	100	14	63.6	7	87.5	10	71.4	49	80.3
Lr2a	4	44.4	4	50.0	7	31.8	2	52.0	3	21.4	20	32.8
Lr2c	1	11.1	2	25.0	4	18.1	2	52.0	3	21.4	12	19.7
Lr3	4	44.4	8	100	16	72.7	4	50.0	9	64.2	41	67.2
Lr9	2	22.2	2	25.0	3	13.6	2	25.0	3	21.4	24	21.4
Lr16	7	77.7	7	87.5	13	59.0	4	50.0	9	64.2	40	65.6
Lr24	7	77.7	7	87.5	14	63.6	4	50.0	10	71.4	42	68.9
Lr26	3	33.3	8	100	15	86.1	7	87.5	5	35.7	38	62.3
Lr3ka	7	77.7	4	50.0	17	77.2	4	50.0	10	71.4	42	68.9
Lr11	8	88.8	8	100	11	50.0	4	50.0	10	71.4	41	67.2
Lr17	8	88.8	8	100	15	86.1	7	87.5	9	64.2	47	77.0
Lr30	3	33.3	8	100	17	77.2	5	62.5	6	42.8	39	63.9
Lr10	3	33.3	4	50.0	17	77.2	2	52.0	11	78.5	37	60.6
Lr18	7	77.7	8	100	14	63.6	7	87.5	7	50.0	43	70.4
Lr21	7	77.7	8	100	16	72.7	4	50.0	7	50.0	42	68.9
Lr2b	0	0	0	0	7	31.8	2	25.0	3	12.4	12	19.7
Total	9	---	8	---	22	---	8	---	14	---	61	---

Area1 = Damietta, Area2 = Monufiya, Area 3 = Qalubiya, Area 4 = Suez and Area 5 = Beni-Suef.

Cluster analysis of the reaction of 16 monogenic lines (*Lr's)* against leaf rust isolates in 2009/10, showed that two main groups of *Lr's* could be distinguished (Distance 25). Group 1 consisted of four subgroups (a, b, c &d); subgroup 1-a consisted of *Lr2c, Lr18, Lr3ka, Lr3* and *Lr10* which having similar distance; 1.Whiles subgroup 1-b consisted of *Lr16* and *Lr21* at the same distance; 3.5, also subgroup 1-c consisted from *Lr24, Lr26, Lr17, Lr30* and *Lr2b* having similar distance; 2, subgroup 1-d consisted from *Lr1* and *Lr10* have been 4 distance. Second group consisted of *Lr2a* and *Lr9* having distance9 (Fig. 1).

The average of UPGMA distance for variance between isolates in governorates Qalubiya, Beni-Suef, Monufiya and Suez with isolates in Damietta was 25 distance. Isolates in Qalubiya and Beni-Suef were nearly similar (distance, 1), while isolates within Monufiya and Suez had an average distance of 7.5 and 14, respectively during 2009/10 season (Fig. 2).

Cluster analysis of 16 monogenic lines, *i.e. Lr's* against leaf rust isolates in 2010/11, showed that two main groups of *Lr's* could be distinguished reaction (distance 25). Group 1 consisted of three subgroups (a, b & c); subgroup 1-a consisted of *Lr16, Lr24, Lr11, Lr1* and *Lr14* have been similar distance (distance 1) and *Lr21* and *Lr17* have been similar distance, 2. Subgroup 1-b consisted of *Lr26* and *Lr2130* having the same distance 2 and Lr3 at distance 4, while subgroup 1-c consisted of *Lr3ka* and *Lr10* having the distances 10 and 5, respectively. Second group was divided into two subgroups distance 14, 2-a consisted of *Lr2c* and of *Lr2a* having the distance 2.5, Subgroup2-b consisted of *Lr2a* and *Lr9* at distance 6 (Fig. 3).

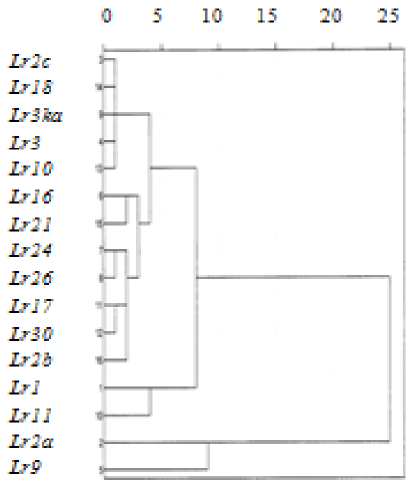

Fig. 1. UPGMA dendrogram for virulence frequency of *P. triticina* isolates on 16 monogenic lines in 2009/10

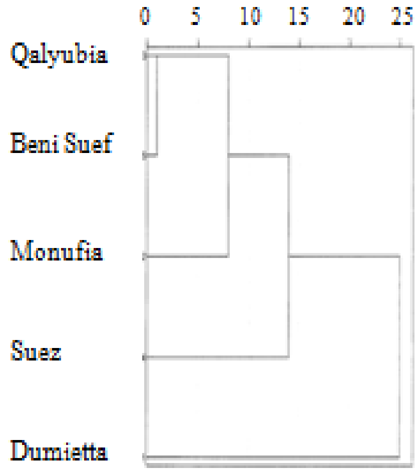

Fig. 2. UPGMA dendrogram for virulence of *P. triticina* isolates in five governorates in 2009/10

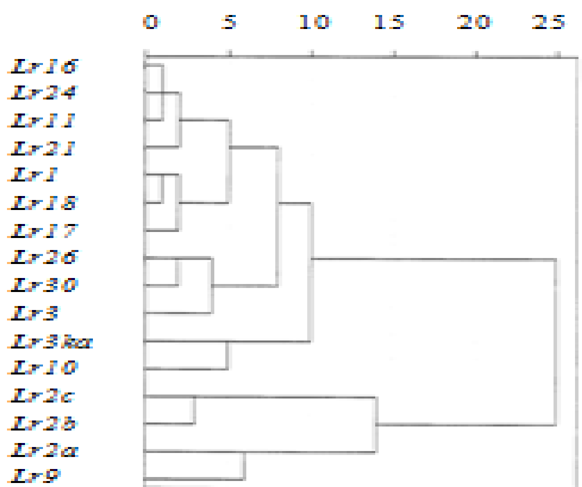

Fig. 3. UPGMA dendrogram for virulence frequency of *P. triticina* isolates on 16 monogenic lines in 2010/11.

The average of UPGMA distance for monogenic lines virulence in Qalubiya, Beni-Suef, Monufiya and Suez with isolates in Damietta was 25. Isolates in Qalubiya and Beni-Suef were nearly similar (distance 1), while isolates within Suez and Damietta had an average UPGMA distance of 7.5 and 16, respectively, during 2010/11 (Fig. 4).

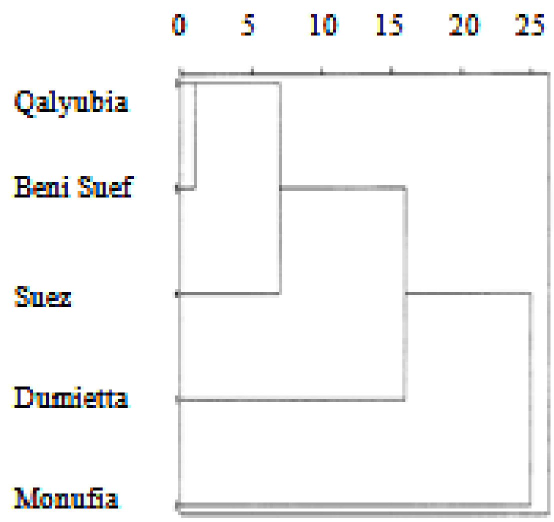

Fig. 4. UPGMA dendrogram for virulence of *P. triticina* isolates in five Governorates in 2010/11.

Discussion

The annual survey conducted in Egypt during two seasons, *i.e.* 2009/10 and 2010/11 through five governorates indicated that the incidence of leaf rust disease in the first season was more severe and earlier than that in the second one.

Data obtained revealed the existence of 102 & 33 pathotypes of *P. triticina* during 2009/10 & 2010/11 seasons, respectively. Pathotype TKTT occupied the first rank, since it recorded the highest frequency (12.75%) from the total, followed by pathotype TTTT (8.08%) and pathotype KKTT (3.36%) in 2009/10. While pathotype MKKS exhibited the highest frequency (11.5%) followed by BBBB& PSSS (each with 8.2%) in 2010/11. The frequency of the rest of the tested pathotypes ranged from 0.67% to 2.68% in 2009/10 and from 1.6 to 4.9% in 2010/11. Similar results were reported by Sherif (2002), Najeeb *et al*. (2003), Najeeb *et al*. (2005), Kolmer *et al*. (2007), Ali (2012) and Soliman *et al*. (2012). While, Nazim *et al*. (1976) found that one race 77 showed the highest frequency during 1971-1975, 1996- 1998 and 2001, respectively.

Regarding the geographical distribution of the identified races within the Egyptian governorates, the obtained results indicated that, the two races TKTT& TTTT were the most predominant ones (100%) overall the five governorates under study. On the other hand, Qalubiya comprised the highest number of races, followed by Beni-Suef, Suez and Monufiya. These results may be due to three reasons. The first is the effect of climatic changes *i.e.*, temperature, wind direction and rainfall on races migration in the different governorates which are necessary for the disease occurrence (Manninger, 2001 and Kolmer, 2013). Whereas the second reason is the ability of *P. triticina* to form new virulent races that can attack resistant varieties and their potential to develop and increase rapidly under optimal environmental conditions and cause serious losses (El-Daoudi *et al*., 1994). This observation has an indication relevant to the affinity of the cultivated susceptible varieties, in such locations. (3) These governorates are located as a front toward the winds blown from North or East and South, which are bearing with a considerable quantity of primary inocula; rust urediniospores (Abdel-Hak *et al*., 1974). The occurrence of single race in certain location is relevant to the availability of the distribution of the simultaneous cultivation of certain cultivar (s) in such location (s). So, this phenomenon must be noticed in subsequent growing seasons. Similar results were reported by Nazim *et al*. (1976 and 1983) and Sherif *et al*. (1996). Also, the population of wheat leaf rust in Egypt is made up of a great diversity of races since the inoculum arrives to Egypt from different external countries (McVey *et al*., 2004).

It could be concluded that, the high frequency of phenotypes clarified that the level of virulence in first season was higher than the second season. Similar results were reported by Long *et al*. (1992), Bartos *et al*. (2001) and McCallum and Seto-Goh, (2006).

As for the gene and virulence efficacy frequency (%) of the tested *Lr's*, the obtained results indicated the presence of high virulence between them, with the exception of *Lr's*: 25, 19, 9, 28 and 29 in 2009/10, and *Lr’s*: 19, 2a, 28, 42 and 9 in 2010/11. Since, these *Lr’s* displayed the highest level of gene efficacy (%). Similar results were reported by Bartos *et al*. (2001), Najeeb *et al*. (2005), McCallum and Seto-Goh (2006) and Zarandi *et al*. (2011), who indicated that *Lr's* 9, 21 and 3ka, recorded high efficacy.

Cluster analysis of virulence frequency *P. triticina* against leaf rust on 16 monogenic lines in five Governorates in two seasons revealed that *Lr2a* and *Lr9* were closely similar and different than all other *Lr’s*.

The population of *P. triticina* in Egypt is highly diverse for virulence phenotypes, which will continue to present a challenge for the development of wheat cultivars with effective durable resistance. Occurrence of new virulent pathotypes may require cultivation of new resistant varieties.

These results are limited by the number of isolates and available tester lines having single known *Lr’s* gene (McVey and Leonard, 1990). However, this would be an effective tool in the disease breeding program for leaf rust resistance.

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التغييرات الديناميكية للقدرة المرضية لسلالات بكسينيا تريتيسينا بجمهورية مصر العربية موسمي ٢٠٠٩/١٠ و٢٠١٠/١١

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يُعتبر مرض صدأ أوراق القمح من أكثر أمراض القمح انتشاراً وبصفة منتظمة سنوياً في مصر والعالم. تم جمع عينات من الفطر المسبب لمرض صدأ أوراق القمح (بكسينيا تريتيسنا) من حقول المزارعين وتجربة مصايد أصداء القمح النباتية المصرية، من خمس محافظات (دمياط، المنوفية، القليوبية، السويس، بني سويف).

تم تعريف السلالات الفسيولوجية لفطر صدأ أوراق القمح وتقدير القدرة المرضية لسلالات الفطر المسبب خلال موسمي ٢٠٠٩/١٠، ٢٠١٠/١١م. تم اختبار القدرة المرضية للسلالات الفسيولوجية على مجموعة من سلالات القمح الحاملة لعامل وراثي واحد مقاوم لفطر صدأ أوراق القمح (١، ٢a، c٢، ٣، ٩، ١٦، ٢٤، ٢٦، 3ka ، ١١، ١٧، ٣٠، ١٠، ١٨، ٢١، b٢). تم تعريف ١٠٢، ٣٣ سلالة فسيولوجية خلال الموسمين٢٠٠٩/١٠، ٢٠١٠/١١م علي التوالي، وكانت أكثر السلالات تكراراً داخل العشيرة الفطرية خلال الموسم الأول ٢٠٠٩/١٠م هي: TKTT (١٢.٨%)،TTTT (٨.١%)، KKTT (٣.٤%). بينما كانت نسبةالسلالات الأكثر تكراراً في الموسم التالي ٢٠١٠/١١م هي:MKKS(١١.٥%)، وكل من BBBB ، PSSS (٨.٢%).

بالاضافة إلى ماسبق، كانت أقل العوامل الوراثية إصابة بسلالات فطر صدأ الأوراق وبالتالي أعلاها كفاءة خلال موسم ٢٠٠٩/١٠م هي ٢٥، (١٥.٠٨%)، ١٩ (١٦.٨٧%)، ٩ (٢٢.٣٤%)، ٢٨ (٣٠.١٦%)، ٢٩ (٣٢.٩٦%). وعلى العكس من ذلك، كانت أكثر العوامل الوراثية قابلية للإصابة بالفطروبالتالي اقلها كفاءه هي: ٢C، ka٣ ، ١٠، ١٨، ١٦ بنسب إصابة ٨٦.٠٣، ٨٥.٤٧، ٨٤.٣٥%، ٨٣.٧٩، ٨٢.٧٩% على التوالي. وقد أوضحت النتائج المتحصل عليها خلال موسم ٢٠١٠/١١م أن العوامل الوراثية الأكثر تكراراً للإصابة هي : ١، ٣٥، ١٠، ١٥، ١٨ حيث كانت النسب المؤية للأصابة هي : (١٠٠.٠، ٨٠.٧، ٧٨.٩٤، ٧٥.٤٣، ٧٣.٦٨%). وكانت أقل العوامل الوراثة عرضة للإصابة بسلالات الفطر هي: ١٩، (a٢، ٢٨)، ٤٢، ٩ بنسب مئوية للإصابة ١٤.٥٩، ١٧.٥٤، ٢٠.٣٥% على التوالي.