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Monitoring of *Puccinia triticina* Erikss. physiologic races and effectiveness of *Lr*-genes in Egyptian wheat during 2014-2016 growing seasons

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Abstract Infected wheat leaves with leaf rust fungus, (*Puccinia triticina*), were obtained from Egyptian wheat rust trap nursery (EWRTN) located at Kafr El-Sheikh, Sharqia and Beni-Suef Governorates during 2014-15 and 2015-16 growing seasons. Leaves were used to identify virulence phenotypes prevalent in the selected Governorates. Virulence was tested with 16 lines of Thatcher wheat that differed for single leaf rust resistance (*Lr*) genes. The single pustule method was applied for isolation of each sample. A total of 37 and 90 virulence phenotypes were respectively described in the three Governorates during 2014-15 and 2015-16 growing seasons. The two most common virulence phenotypes across three areas were BBBB and BBBT that were high frequencies throughout the tow growing seasons. While, the other races were rare, which they represented by only one or two isolates in the tested pathogen populations. Frequency of race groups based on infection types (IT, s) of the first 8 differential host lines were also detected. The most common race group was DK-- (13.51%) which virulent to *Lr 2c, 16, 24* and *26,* followed by race group TT-- (10.81%) which virulent to all 8 *Lr genes* in 2014-15. On the other hand, race group BB-- (23.33%) was avirulent to all 8 *Lr genes* in 2015-16. Virulence frequency was very high against *Lr 1, 2c, 10, 11, 16, 17, 21, 24 and 26.* In contrast, virulence occurred at relatively low frequency against *Lr 2a, 2b, 3, 3ka, 9, 18 and 30.* Thus, these genes considered to be the most effective resistance genes against a large number of the pathogen isolates which were detected in the two successive growing seasons 2014-2015 and 2015-2016.

Keywords: Wheat, *Puccinia triticina*, race-specific resistance, virulence, monogenic lines (*Lr* genes), cluster analysis

Introduction

Leaf rust (caused by *Puccinia triticina*) is one of the most important fungal disease of wheat in Egypt (Nazim *et al*., 1983). It is annually occurring

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on the most commercial wheat cultivars. The widespread occurrence of leaf rust pathogen mainly attributes to, its broad climatic adaptation to the wide range of diverse environmental conditions. The nature of urediniospores enables them to migrate by air for thousands of kilometers, which causes the spread of new virulent pathotypes throughout the world (Burdon and Silk, 1997; Kolmer, 2005). Generally, leaf rust is a polycyclic fungal pathogen with a capability to produce new virulent phenotypes (races), faster than the release of new wheat varieties (McDonald and Linde, 2002; Kolmer, 2013). Thus, wheat varieties cannot extend their field resistance life (Khan, 1987).

In Egypt, leaf rust is more regular and more dominant compared to the other rust diseases. The source of primary inoculum is exogenous yearly coming from neighbor countries as airborne, because the urediniospores cannot over-summering due the high temperature and the absence of alternate hosts in Egypt (McVey *et al.,* 2004; Nazim *et al.,* 2010). The exogenous inoculums have different virulence and aggressiveness; it usually causes the disposal of recent cultivars after a very short time of their releases**.** Moreover, losses in grain yield, up to 10% and can reach to nearly 50% depending on the growth stage of wheat plants when the initial infection occurs, and the relative resistance or susceptibility of the host cultivar (Ordonez *et al.,* 2010; Ola. Mabrouk, 2012; Thabet and Khadegah Najeeb, 2017).

Identification of virulent phenotypes in wheat rust populations is critical for development of resistant cultivars. Many of the designated *Lr* genes originally from common wheat and various wild relatives of wheat. Physiological specialization of the fungus must be determining annually because of the dynamic state of *Puccinia triticina* that makes the life span of any variety is very short. Virulence surveys of the wheat leaf rust fungus have been conducted by Wheat Diseases Research Division, since 1954 to detect new virulence phenotypes and to monitor shifts of virulence phenotypes in the major wheat growing regions of Egypt (Hassan *et al*., 2012). Using resistant genotypes are the most economic and effective method to control plant diseases in general and particularly obligate parasite including leaf rust of wheat (Elyasi-Gomari and Lesovaya, 2009; Ahmad *et al*., 2010).

The objectives of this study were to characterize the virulence of *P. triticina* populations in Egypt and their geographical distribution in three Governorates, in addition to determine the relative effectiveness of wheat leaf rust resistance (*Lr*) genes to serve the national breeding program for wheat rust resistance.

Materials and Methods

Leaf Rust Trap Nurseries included (*Lr* genes) entries (monogenic lines carrying different *Lr* genes) were planted in different agro-climatic zones at locations where leaf rust disease is known to occur naturally each year (Table, 1). The main objectives of Egyptian Rust Trap Nursery were (1): to determine disease incidence with different agro-climatic zones. (2): to identify prevalent leaf rust pathotypes in wheat grown areas and (3): to assess the effectiveness of known resistance genes. Rust differential cultivars were evaluated for the specific rust (leaf rust). For monitoring of wheat leaf rust virulence/a virulence pattern, each entry was planted as a single row. Each row with 1 m long and 30 cm apart was about 3 gm of seed, with one employed row between entries.

Table 1. Wheat monogenic lines (*Lr* genes) used in this study

N0.*	*Lr* genes	Accession number[1]	Pedigree[2]
1	***Lr1***	GSTR 402	Thatcher*6/Centenario
2	***Lr2a***	GSTR 403	Thatcher*6/Webster
3	***Lr2c***	GSTR 405	Thatcher*6/Brevit
4	***Lr3***	GSTR 406	Thatcher*6/Democrat
5	***Lr9***	GSTR 409	Thatcher*6/Aegilops umbellulata
6	***Lr16***	GSTR 417	Thatcher*6/Exchange
7	***Lr24***	GSTR 425	Thatcher*6/Agropyron elongatum
8	***Lr26***	GSTR 427	Thatcher*6/Imperial (rye)
9	***Lr3ka***	GSTR 408	Thatcher*6/Klein Aniversario
10	***Lr11***	GSTR 411	Thatcher*6/Hussar
11	***Lr17***	GSTR 418	Thatcher*6/Klein Lucero
12	***Lr30***	GSTR 430	Thatcher*6/Terenzio
13	***Lr10***	GSTR 410	Thatcher*6/Lee
14	***Lr18***	GSTR 419	Thatcher*6/Africa 43
15	***Lr21***	GSTR 422	Thatcher*6/Aegilops tauschii
16	***Lr2b***	GSTR 404	Thatcher*6/Agent

1. Accession numbers in accordance with Research Service of Germplasm Resource Information Network (GRIN).
2. Pedigree in accordance with Genetic Resources Information System for Wheat and Triticale (GRIS).

Virulence frequency of P. triticina population

Samples of wheat leaves bearing the uredinia of leaf rust, were collected from the experimental plots of rust trap nurseries, throughout 3 Governorates in Egypt, during 2014-15 and 2015-16 growing seasons. Each sample (2-4 infected leaves) was kept overnight at room temperature (18-25^{0}c), to be dried off. The samples were then kept in glycine envelops and stored in

the refrigerator at 2-5^{0}c. Uridiniospores maintained their viability under these conditions for using up to 180 days (Stakman *et al*., 1962).

Isolation and Purification

The urediospores of each dried sample (infected leaf) were isolated by transferring the inoculum to the seedling's leaves of the highly susceptible wheat variety (Morocco), using the spatula method. The inoculated plants were placed overnight in humid chamber (100% RH and 18-20^{0}c) to allow the rust spores to germinate and cause infection. The plants were then moved to the greenhouse benches where daily temperature variety between 20-25^{0}c (Kolmer and Ordoñez, 2007; Wang *et al*., 2010). After, rust full developed (approximately 12-15 days) five single uredinia were separately isolated from each sample to inoculate the seedlings of the highly susceptible wheat variety (Morocco) to obtain enough urediniospores before testing for virulence on leaf rust differential lines.

Race identification

A series of 16 monogenic lines of 'Thatcher' wheat were used for race identification and nomenclature, which have different single gene for rust resistance to *P. triticina* (Table, 1). Each set contained four genotypes. The differential sets were grown in plastic pots (6 cm diameter) then inoculated according to the methods adopted by Stakman *et al.* (1962). Inoculated plants were subsequently transferred to a greenhouse bench and kept at 20 ± 2°C with relative humidity 40–60% and illuminated by about 15000 lux for 12 h each day. After 14 days, infection types were classified on a 0 to 4 scale, as described by Kolmer, (1991): 0 = immunity, no hypersensitive flecks or uredinia; **0;** = faint hypersensitive flecks; **1** = small uredinia surrounded by distinct necrosis; **2** = small uredinia surrounded by distinct chlorosis; **3** = moderate size uredinia without chlorosis; and **4** = very large uredinia lacking chlorosis. Infection types from 0 to 2+ were considered 'low' infection types (L), while those of 3 to 4 were considered 'high' infection types (H) (Stakman *et al*., 1962; Long and Kolmer, 1989; Chu *et al*., 2009).

The North American race nomenclature system for *P. triticina* was carried out to design the leaf rust races in a letter code (Pt. code) as described by Long and Kolmer, (1989) and McVey *et al*. (2004). Races were assigned four-letter codes based on their infection type on the four sets of near isogenic lines (Table, 2)

Table 2. Code for the five Egyptian differentials for *Puccinia triticina*

Infection type produced on monogenic lines:				
Host set 1	1	2a	2c	3
Host set 2	9	16	24	26
Host set 3	3ka	11	17	30
Host set 4	10	18	21	2b
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

Pt- code consists of the description for set 1 followed by that for set 2, etc. for example, race MGBL; set 1 (M)- virulent to Lr 1, 3; set 2 (G)- virulent to Lr 16; set 3 (B)- avirulent ; set 4 (L) virulent to 10, L = low infection type (a virulent race), H = high infection type (virulent race).

Virulence frequency and gene efficacy

The frequency of virulence was estimated as the percentage of virulent isolates to the total number of isolates tested for each genotype. Also, leaf rust resistance genes (Gene efficacy %) was evaluated according to the following equations adopted by Green (1965) as follows:

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

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

Virulence and distribution frequencies of the *P. triticina* races (phenotype)

Phenotype of the *P. triticina* races were determined for the three agro-ecological geographic Governorates surveyed in Egypt as illustrated in Fig. (1). Pearson correlation coefficient ® was calculated for each pair of race gene efficacy and Governorates to display relationship between virulence diversity of leaf rust races in three Governorates and leaf rust resistance gene efficacy.

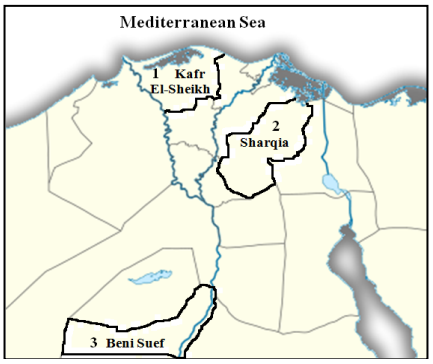

Figure 1. A map of Egypt showing the three agro-ecological areas where wheat fields were surveyed for leaf rust samples in 2014-15 and 2015-16. (1) Northern (Kafr El-Sheikh Gov.); (2) Eastern (Sharqia Gov.) and (3) Southern (Beni Suef Gov.)

Cluster analysis

A similarity matrix of virulence phenotypes of the areas 1, 2 and 3 based on the simple matching coefficient was used to construct a dendogram using the unweighted pair group method with arithmetic means clustering method (UPGMA) in numerical taxonomy system (NTSYS-pc version 2.1) according to Rohlf, (2000). Cluster analysis was performed using SPSS 6.0 software package.

Results

Race identification and geographical distribution

Infected wheat leaves with *Puccinia triticina* were collected from Wheat Rust Trap Nursery which cultivated at the three locations. Races were isolated and identified in the two successive growing seasons 2014-2015 and 2015-2016. Three single uredinal isolates were taken from each infected sample and tested using 16 wheat monogenic lines at seedling stage in the greenhouse. Thirty-seven and 90 different virulence formula were obtained in the first and second seasons, respectively. The obtained results revealed that, 37 virulent wheat leaf rust races were identified in 2014-2015 from 37 single-uredinial isolates that were tested on the differential tester monogenic lines (*Lr genes*) (Table, 3). We identified 37 physiologic races of *P. triticina* in Egypt using the North American system of nomenclature during 2014/15 season (Table, 3). These could be divided into eleven groups according to 'Unified System' i.e. **(B)** races BBBB, BBGK, BCBB, BDBB, BFJN, BFBB, BFBL,

BFDF, BKSB, BKDB and BKSB; **(C)** CBJS; (**D**) DKJB, DKKL, DKMB, DKNN and DKTD; (**J**) JKJJ; **(L)** LBHQ, LBSB, LJCB, LIHS and LTJB; (**M**) MTJT; (**N**) NHSL, NKCL, NKDK and NKJL; **(P)** PKGL and PTKF; (**Q**) QDSC and QKLD; **(S)** SKBP; **(T)** THFL, TPQJ, TSPJ and TTQT.

Table 3. Number and frequency (%) of *Puccinia triticina* virulence phenotypes in the three Governorates during 2014-15 growing season

No.	Pheno -type	Virulence formula	Virulence frequency%	Area 1		Area 2		Area 3		Total	
				No	%	No	%	No	%	No	%
1	BBBB	0.	0	0	0	1	2.70	0	0	1	2.70
2	BBGK	11,18,21,2b.	25.0	0	0	0	0	1	2.70	1	2.70
3	BCBB	26.	6.25	1	2.70	0	0	0	0	1	2.70
4	BDBB	24.	6.25	0	0	1	2.70	0	0	1	2.70
5	BFJN	24,26,11,17,10,21.	37.5	1	2.70	0	0	0	0	1	2.70
6	BFBB	24,26.	12.50	1	2.70	0	0	0	0	1	2.70
7	BFBL	24,26,10.	18.75	0	0	0	0	1	2.70	1	2.70
8	BFDF	24,26,17,21,2b.	31.25	0	0	0	0	1	2.70	1	2.70
9	BKSB	16,24,26,17.	25.00	0	0	0	0	1	2.70	1	2.70
10	BKDB	16, 24, 26, 3ka, 11, 17.	37.50	1	2.70	0	0	0	0	1	2.70
11	BKSB	3,11,17,18,21,2b.	37.50	1	2.70	0	0	0	0	1	2.70
12	CBJS	2c, 16, 24,26,11,17.	37.50	1	2.70	0	0	0	0	1	2.70
13	DKJB	2c, 16,24,26,10.	31.25	1	2.70	0	0	0	0	1	2.70
14	DKKL	2c, 16, 24, 26,3ka.	31.25	0	0	0	0	1	2.70	1	2.70
15	DKMB	2c, 16, 24, 26, 3ka, 17, 10, 21.	50.00	0	0	0	0	1	2.70	1	2.70
16	DKNN	2c, 16, 24, 26, 3ka, 11,17,30,21.	56.25	1	2.70	0	0	0	0	1	2.70
17	DKTD	1,11,30,10,18.	31.25	0	0	0	0	1	2.70	1	2.70
18	JKJJ	1, 2c, 3, 16, 24,26,11,10.	50.00	0	0	0	0	1	2.70	1	2.70
19	LBHQ	1, 3ka, 11, 17.	25.00	1	2.70	0	0	0	0	1	2.70
20	LBSB	1,16,24,30.	25.00	1	2.70	0	0	0	0	1	2.70
21	LJCB	1,9,11,30,10,18,21.	43.75	1	2.70	0	0	0	0	1	2.70
22	LLHS	1,9,16,24,26,11,17.	43.75	0	0	0	0	1	2.70	1	2.70
23	LTJB	1, 9, 16, 24,26,11,17.	43.75	0	0	1	2.70	0	0	1	2.70
24	MTJT	1, 2c, 16, 26, 3ka, 11, 17, 10.	50.00	0	0	1	2.70	0	0	1	2.70
25	NHSL	1, 2c, 16, 24,26,30,10.	43.75	1	2.70	0	0	0	0	1	2.70
26	NKCL	1, 2c, 16, 24, 26, 17, 18, 21,2b.	56.25	1	2.70	0	0	0	0	1	2.70
27	NKDK	1, 2c, 16,24,26,11,17,30,10.	56.25	1	2.70	0	0	0	0	1	2.70
28	NKJL	2a, 2c, 16,24,26,11,17,18,21.	56.25	0	0	0	0	1	2.70	1	2.70
29	PKGL	1, 2c, 3, 16, 24,26,11,10.	50.00	0	0	1	2.70	0	0	1	2.70
30	PTKF	1, 2a, 24, 3ka, 11, 17,2b.	43.75	0	0	1	2.70	0	0	1	2.70
31	QDSC	1, 2a, 16, 24, 26, 3ka, 21.	43.75	0	0	0	0	1	2.70	1	2.70
32	QKLD	1, 2a, 2c, 16, 24, 26, 10, 21,2b.	56.25	0	0	0	0	1	2.70	1	2.70
33	SKBP	1, 2a, 2c, 3, 16, 26,17,30,10.	56.25	0	0	1	2.70	0	0	1	2.70
34	THFL	1, 2a, 2c, 3, 16, 26,17,30,10.	56.25	0	0	1	2.70	0	0	1	2.70
35	TPQJ	1, 2a, 2c 3, 9, 24, 3ka,17,30,18, 21.	68.75	0	0	1	2.70	0	0	1	2.70
36	TSPJ	1,2a,2c,3,9,16,24,3ka,17,30,18, 21.	75.00	0	0	1	2.70	0	0	1	2.70
37	TTQT	1,2a,2c,3,9,16,24,26,3k,11,10, 18,21,2b.	87.50	0	0	1	2.70	0	0	1	2.70
Total				14		11		12		37	
Frequency %					37.8		29.7		32.4		100

Area 1= Kafr El -sheikh, Area 2= Sharqia, Area 3= Beni Suef.

Data present in Table (3) revealed that during 2014-2015 growing season race TTQT was the most frequent (87.50%) followed by TSPJ (75.00%) and TPQJ (68.75%). Race TTQT was virulent to fourteen *Lr* genes*, i.e.* (*1, 2a, 2c, 3, 9, 16, 24, 26, 3ka, 11, 10, 18, 21* and *2b*) while TSPJ was virulent to twelve *Lr* genes *i.e.* (*1, 2a, 2c, 3, 9, 16, 24, 3ka, 17, 30, 18* and *21*) and TPQJ to eleven *Lr* genes *i.e.* (*1, 2a, 2c, 3, 9, 24, 26, 3ka, 11, 18* and *21*). On the other hand, race BBBB was avirulent to all resistance *Lr* genes (0.00% frequency of virulence). The rest of races were ranging between (6.25% - 56.25%).

In season 2015-16 ninety physiologic races of *P. triticina* were identified (Table, 4). These could be divided into twelve groups according to 'North American System'. Data in Table (4) revealed that, 90 virulent races of wheat leaf rust were identified from 106 single-uredinial isolates that were tested on the Thatcher lines. Race TTTQ and PTTT were the most frequent (93.75%) followed by TTKS and TTQT (87.50%). All Races was virulent to fourteen *Lr* genes*, i.e.* (*1, 2a, 2c, 3, 9, 16, 24, 26, 3ka, 11, 10, 18, 21* and *2b*) while, race BBBB was found in all areas and was avirulent to all resistance *Lr* genes (0.00% frequency of virulence). The rest of races were ranging between (6.25% - 75.0%). Races varied in their found location, distribution and frequency. BBBB was the weakest race, but was the normal frequent, making up 2.7% in 2014/15 (Table, 3) and medium frequent 3.76% in 2015-16 respectively (Table 4). Race BBBT were highest frequent 5.76% in 2015-16 which also were less virulent.

Table 4. Number and frequency (%) of *Puccinia triticina* virulence phenotypes in the three Governorates during 2015-16 growing season

No.	Pheno-type	Virulence formula	Virulence frequency%	Area 1		Area 2		Area 3		Total	
				No	%	No	%	No	%	No	%
1	BBBB	0.	0	2	6.06	1	3.84	1	2.32	4	3.84
2	BBBC	2b.	6.25	0	0	0	0	1	2.32	1	0.96
3	BBBD	21.	6.25	1	3.03	0	0	0	0	1	0.96
4	BBBL	10.	6.25	1	3.03	0	0	0	0	1	0.96
5	BBBN	10,21.	12.5	0	0	1	3.84	1	2.32	2	1.92
6	BBBR	10,18,2b.	18.75	0	0	1	3.84	1	2.32	2	1.92
7	BBBP	10,21,2b.	18.75	2	6.06	0	0	0	0	2	1.92
8	BBBT	10,18,21,2b.	25.0	0	0	0	0	6	13.9	6	5.76
9	BBDB	24.	6.25	0	0	0	0	1	2.32	1	0.96
10	BBGK	11,18,21,2b.	25.0	0	0	0	0	1	2.32	1	0.96
11	BCHB	26,11,30.	18.75	0	0	1	3.84	0	0	1	0.96
12	BDBB	24.	6.25	0	0	1	3.84	0	0	1	0.96
13	BDCB	24,30	12.5	1	3.03	0	0	0	0	1	0.96

14	BDGB	24,26	12.5	1	3.03	0	0	0	0	1	0.96
No.	Pheno-type	Virulence formula	Virulence frequency%	Area 1		Area 2		Area 3		Total	
				No	%	No	%	No	%	No	%
15	BFBB	24,26.	12.5	1	3.03	1	3.84	0	0	2	1.92
16	BFGB	24,26,11.	18.75	0	0	0	0	1	0	1	0.96
17	BGGL	16,11,10.	18.75	0	0	0	0	1	2.32	1	0.96
18	BJDD	16,24,17,21.	25.0	0	0	1	3.84	0	0	1	0.96
19	BKGL	16,24,26,11,10.	31.25	0	0	0	0	1	2.32	1	0.96
20	BKKT	16,24,26,11,17,30,10,18,21,2b	62.5	0	0	1	3.84	0	0	1	0.96
21	BKTB	16,24,26,3ka,11,17,30	43.75	1	3.03	0	0	0	0	1	0.96
22	BKTT	16,24,26,3ka,11,17,30,10,18,21,2b.	68.75	1	3.03	0	0	1	2.32	2	1.92
23	BTBT	9,16,24,26,10,18,21,2b.	50.0	1	3.03	0	0	0	0	1	0.96
24	BTCT	9,16,24,26,30,10,18,21,2b.	56.25	0	0	0	0	1	2.32	1	0.96
25	CDGT	3,24,11,10,18,21,2b.	56.25	0	0	1	3.84	0	0	1	0.96
26	DBDD	2c, 17, 21.	43.75	0	0	1	3.84	0	0	1	0.96
27	DFRG	2c, 24, 26, 3ka, 11, 30,18.	18.75	1	3.03	0	0	0	0	1	0.96
28	DKBB	2c, 16, 24, 26.	43.75	1	3.03	0	0	0	0	1	0.96
29	DSKJ	2c, 9, 6,24,11,17,30,18,21.	25.0	1	3.03	0	0	0	0	1	0.96
30	GTTP	2c,9,16,24,26,3ka,17,11,30,10,21,2b.	56.25	1	3.03	0	0	0	0	1	0.96
31	KDTS	16,24,26,17,3ka,	31.25	0	0	0	0	1	2.32	1	0.96
32	LBHB	1,11,30.	18.75	0	0	0	0	1	2.32	1	0.96
33	LCBB	1,26.	12.50	1	3.03	0	0	0	0	1	0.96
34	LDBC	1,24,2b.	18.75	0	0	1	3.84	0	0	1	0.96
35	LFQQ	1, 24, 26, 3ka, 11, 10, 18.	43.75	1	3.03	0	0	0	0	1	0.96
36	LFTH	1, 24, 26, 3ka, 11, 17, 30, 18,2b.	43.75	1	3.03	0	0	0	0	1	0.96
37	LHJJ	1,16,26,11,17,18,21.	56.25	1	3.03	0	0	0	0	1	0.96
38	LGDB	1,16,17.	18.75	1	3.03	0	0	0	0	1	0.96
39	LGMJ	1, 16, 3ka, 30, 18, 21.	37.50	0	0	0	0	1	2.32	1	0.96
40	LJTC	1, 16, 24, 3ka, 11, 17, 30,2b.	50.0	2	6.06	1	3.84	1	2.32	1	0.96
41	LKCG	1,16,24,26,30,18	37.5	1	3.03	0	0	0	0	1	0.96
42	LKCL	1,16,24,26,30,10	37.5	0	0	0	0	1	2.32	1	0.96
43	LKHL	1,16,24,11,30,10.	50.0	0	0	1	3.84	0	0	1	0.96
44	LKJP	1,16,24,11,17,10,21,2b.	37.5	0	0	0	0	1	2.32	1	0.96
45	LKLQ	1, 16, 24, 3ka, 10, 18.	37.5	0	0	0	0	1	0	1	0.96
46	LKPQ	1, 16, 24, 26, 3ka,17,30,10,18	56.25	1	3.03	0	0	1	2.32	2	1.92
47	LKQQ	1, 16,24,26,3ka, 11,10,18	50.0	0	0	1	3.84	0	0	1	0.96
48	LTGD	1, 9,16,24,26, 11, 21.	43.75	0	0	1	3.84	0	0	1	0.96
49	LTJB	1,9,16,24,26,11,17.	43.75	0	0	1	3.84	0	0	1	0.96
50	MBNQ	1, 3, 3ka, 17, 10, 18.	37.5	0	0	0	0	1	2.32	1	0.96

51	MJTT	1,3,16, 26, 3ka, 11, 17, 30, 10, 18, 21,2b.	75.0	1	3.03	0	0	0	0	1	0.96
No.	Pheno-type	Virulence formula	Virulence frequency%	Area 1		Area 2		Area 3		Total	
				No	%	No	%	No	%	No	%
52	MKSS	1 ,3,6,24,26,3ka,11,17,10,18, 21.	68.75	0	0	0	0	1	0	1	0.96
53	MKTD	1,3,6,24,26,3ka,11,17,30,2 1	62. 5	1	3.03	0	0	0	0	1	0.96
54	MTKG	1,3,9,16,24,26,3ka,11,17,3 0, 18.	68.75	0	0	0	0	1	2.32	1	0.96
55	NDSJ	1, 2c, 24, 3ka, 11, 17, 18,21	50.0	0	0	0	0	1	2.32	1	0.96
56	NGJB	1, 2c, 16, 24, 3ka, 11,17,30, 10,18,2b.	68.75	1	3.03	0	0	0	0	1	0.96
57	NJTR	1,2c, 16,24,26,11,10.	43.75	0	0	0	0	1	2.32	1	0.96
58	NKGD	1, 2c,16,24,26,11,10.	43.75	0	0	0	0	1	2.32	1	0.96
59	NKGL	1, 2c, 16,24,26,11,10.	43.75	0	0	1	3.84	0	0	1	0.96
60	NKPQ	1,2c,16,24,26,3ka,11,17,30 , 10,18	68.75	0	0	1	3.84	0	0	1	0.96
61	NKQL	1,2a,16,24,26,3ka,11,10.	50.0	0	0	0	0	1	2.32	1	0.96
62	NKST	1,2c,16,24,26,3ka,11,17,10 , 18,21,2b.	75.0	0	0	0	0	1	2.32	1	0.96
63	NKTL	1,2c,16,24,26,3ka,11,17,30 ,10.	62.5	0	0	1	3.84	0	0	1	0.96
64	PCFJ	1, 2c,3,26,17,30, 18,21.	50.0	0	0	0	0	1	2.32	1	0.96
65	PGDQ	1, 2c,3, 16, 17, 10,18	43.75	0	0	1	3.84	0	0	1	0.96
66	PHTK	1,2c,3,16,26,3ka,11,17,30, 10,18,21,2b.	81.25	1	3.03	0	0	0	0	1	0.96
67	PKDC	1, 2c,3, 16,24,26,17,2b.	50.0	0	0	1	3.84	0	0	1	0.96
68	PKTF	1, 2c,3,16,24,26,3ka,11,17, 30,21,2b.	75.0	1	3.03	0	0	0	0	1	0.96
69	PKTJ	1, 2c,3,16,24,26,3ka,11,17, 30, 18,21.	75.0	1	3.03	0	0	0	0	1	0.96
70	PRFC	1, 2c,3,9,16, 26, 17,30,2b.	56.25	0	0	0	0	1	2.32	1	0.96
71	PTKF	1, 2c,3,9,16,24,26,11,17,30, 21,2b.	75.0	0	0	1	3.84	0	0	1	0.96
72	PTTT	1,2c,3,9,16,24,26,3ka,11,1 7,30,10,18,21,2b	93.75	0	0	0	0	1	2.32	1	0.96
73	QDGB	1,2a, 24,11.	25.0	1	3.03	0	0	0	0	1	0.96
74	QHFN	1,2a,1626, 17,30,10,21.	50.0	0	0	0	0	1	2.32	1	0.96
75	QKHS	1,2a16,24,26,11,30,10,18,2 1	62.5	0	0	0	0	1	2.32	1	0.96
76	QKKS	1,2a,16,24,26,11,17,30,10, 18, 21.	68.75	2	6.06	0	0	0	0	2	1.92
77	QTJS	1,2a,9,16,24,26,3ka,11,17, 10, 18,21.	75.0	0	0	1	3.84	0	0	1	0.96

78	SFTB	1,2a,2c,24,26,3ka,11,17,30	56.25	0	0	1	3.84	0	0	1	0.96
79	SHRG	1,2a,2c, 16,26,3ka,11,30,18	56.25	0	0	0	0	1	2.32	1	0.96
80	SKBB	1,2a,2c,16,24,26.	37.5	0	0	1	3.84	0	0	1	0.96
No.	Pheno-type	Virulence formula	Virulence frequency%	Area 1 No	%	Area 2 No	%	Area 3 No	%	Total No	%
81	SKGB	1,2a,2c, 16,24,2611.	43.75	0	0	1	3.84	0	0	1	0.96
82	SKTG	1,2a,2c,16,24,26,3ka,11,17 ,30, 10,18	75.0	0	0	0	0	1	2.32	1	0.96
83	SKTL	1,2a,2c,16,24,26,3ka,11,17 ,30, 10.	68.75	0	0	0	0	1	2.32	1	0.96
84	TGGP	1,2a,2c,3, 16,11,17,30,10,21, 2b.	68.75	0	0	0	0	1	2.32	1	0.96
85	TJRT	1,2a,2c,3, 16,24,3ka,11,30,10, 18,21,2b.	81.25	0	0	0	0	1	2.32	1	0.96
86	TPQJ	1,2a,2c,3,9,16,24,26,3ka,1 1,18,21.	75.0	0	0	1	3.84	0	0	1	0.96
87	TSRJ	1,2a,2c,3,9,16,24, 11,17,30,18, 21.	75.0	0	0	1	3.84	0	0	1	0.96
88	TTKS	1,2a,2c,3,9,16,24,26,11,17, 30,10,18,21.	87.5	0	0	1	3.84	0	0	1	0.96
89	TTQT	1,2a,2c,3,9,16,24,26,3ka,1 1,10,18,21,2b	87.5	0	0	1	3.84	0	0	1	0.96
90	TTTQ	1,2a,2c,3,9,16,24,26,3ka,1 1,17,30,10,18	93.75	1	3.03	1	3.84	0	0	2	1.92
Total				33		30		43		106	
Frequency %					31.1		28.3		40.5		100

Area 1= Kafr El sheikh, Area 2= Sharqia, Area 3= Beni Suef.

Number and frequency of P. triticina race groups collected from Egyptian wheat during 2014- 2016 growing seasons

Physiologic races of *Puccinia triticina* were identified as a major race groups by the two letter codes for the first two sets of 8 monogenic differential wheat lines (*Lr* genes) at seedling stage in the greenhouse condition during 2014-2016 growing seasons.

Data arranged in Tables (3 and 4) revealed the number of isolates and percentage of frequency for each race group studied. Seven race groups i.e. BB-, BF-, BK-, DK-, LK-, SK- and TT- were common, which found them in both seasons, but with different frequencies. As regard to number of race groups and frequency, data in Table (5) revealed that in 2014-2015 growing season race group DK-- was the most frequency (13.51%) followed by TT-- (10.81%). In contrast, the two race groups LK-- and SK- had the lowest frequencies. Their frequencies were lesser than 2.70%. However, other race groups ranged from 5.40% to 8.10%. The three race groups NK--, PK-- and

QK—were disappeared in the first season. On the other hand, during 2015-2016 growing season, the two race groups DK-- and TT-- had lowest frequency (1.11%). While the race group BB-- had the highest frequency (23.33%) and other race groups ranged from 2.20% to 4.44%. However, race group TT-- showed the most virulent frequency (100%) which, was virulent to eight *Lr genes (1, 2a, 2c, 3, 9, 16, 24 and 26)* followed by SK-- and PK-- (75.00%) were virulent to six *Lr genes* (*1, 2a, 2c, 16, 24 and 26*) and (*1, 2c, 3, 16, 24 and 26*) respectively. It was also showed that, race groups NK- and QK- (62.5%) were virulent to five *Lr genes* (*1, 2c, 16, 24, and 26*) and (*1, 2a, 16, 24 and 26*) respectively. On the other hand, race group BB-- was avirulent to all resistance *Lr* genes (0.00%). The rest of races were ranging between 25.0% - 50%.

Table 5. Virulence formula and frequency (%) of *Puccinia triticina* race groups in Egypt during 2014-2016

No	Race group	Virulence formula	Virulence frequency%	First season 2014-15		Second season 2015-16	
				No. of isolates	Frequency%	No. of isolates	Frequency%
1	BB--	0.	0	2	5.40	21	23.33
2	BF--	24,26.	25.0	3	8.10	2	2.20
3	BK--	16,24,26.	37.5	3	8.10	4	4.44
4	DK--	2c, 16, 24, 26.	50.0	5	13.51	1	1.11
5	LK--	1,16,24,26.	50.0	1	2.70	8	8.90
6	NK--	1, 2c, 16, 24, 26.	62.5	-	0	6	6.70
7	PK--	1, 2c, 3,16,24,26.	75.0	-	0	3	3.33
8	QK--	1, 2a, 16, 24, 26.	62.5	-	0	3	3.33
9	SK--	1, 2a, 2c, 16, 24, 26.	75.0	1	2.70	4	4.44
10	TT--	1, 2a, 2c, 3, 9,16, 24,26.	100	4	10.81	1	1.11
11	*Other (less than 3 isolates)			18	48.64	37	41.11
Total				37	100%	90	100%

*others: race groups recorded frequency less than 3 isolates were excluded from the table.

Similarity of identified race groups at different geographical areas

Cluster similarity matrix analysis (Fig. 2) indicated that, races of *P. triticina* in the geographical areas within Egypt through 2014-2016 growing seasons are divided into two main groups depending on their distribution. In 2014-15 the first group includes races found in the 2 (11 race) and 3 (12 race) areas (Eastern and Southern). Another group includes the area 1 (14 race) sampled from Northern area (Fig 2). While, in 2015-16 the first group includes races found in the 1 (33 race) and 3 (43 race) areas (Northern and Southern).

Another group include the area 2 (30 race) sampled from Eastern area (Fig 3). Area 1 is characterized by exposure to winds from Europe and Turkey. While, area 2 is affected by virulence *P. triticina* originating from the Eastern and area 3 from Northern and Eastern via the typical movement of winds in Egypt.

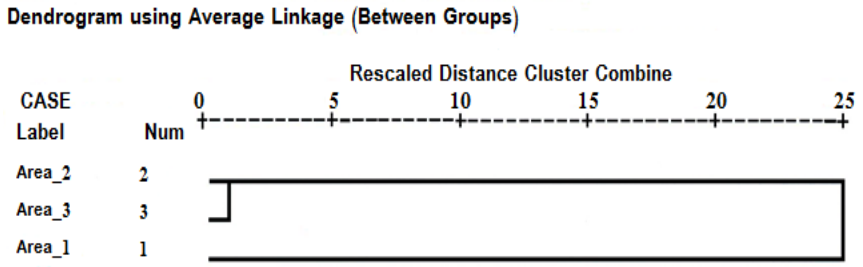

Figure 2. Dendrogram of similarity for virulent and distribution of *Puccinia triticina* race groups at three Governorates of Egypt in 2014-15, Area 1= Kafr El sheikh, Area 2= Sharqia, Area 3= Beni Suef

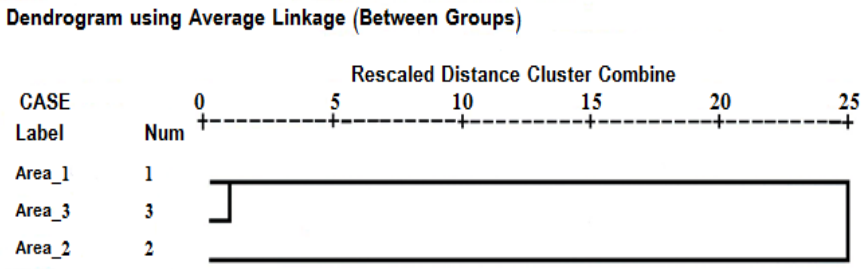

Figure 3. Dendrogram of similarity for virulent and distribution of *Puccinia triticina* race groups at three Governorates of Egypt in 2015-16, Area 1= Kafr El sheikh, Area 2= Sharqia, Area 3= Beni Suef

Virulence frequencies and gene efficacy%

The present work was concerned with the diversity in population of the *Puccinia triticina* and the frequency of the different virulence of the pathogen races against a set of 16 monogenic lines in the two growing seasons. Evaluation of the effectiveness *Lr* genes were included to serve the national breading programs for leaf rust resistance. Different virulence frequencies to the tested monogenic lines and the effectiveness of the leaf rust resistance (*Lr*) genes were presented in Table (6).

Data in Table, (6) and Fig. (4) revealed that, no major changes in the efficacy of the important leaf rust resistance (*Lr*) genes during the whole period of study. Apparently, similar trend was found in the two growing seasons. The

most effective genes i.e. *Lr 3, 9, 2a* and *2b*, were almost stable, showing little or no changes in their levels of efficacy against all the tested isolates. The lowest frequency of virulence (did not exceeded up to 30%) were found against *Lr 3, 9, 30,* 2a, *18, 26, 3ka* respectively. Inversely, the highest occurrence of virulence frequencies (ranging from 51.35% to 72.97%) was occurred against *Lr* 1*, 11, 17, 16, 26 and Lr 24* with an ascending order. Whereas, other lines under study displayed the relatively moderate responses, ranging from 37.85% to 45.93%. The lines only proved to have the highest and best efficacy (more than 71%) against the tested isolated i.e. *Lr9* (81& 82%)*, Lr3* (81& 76%), *Lr2a* (78& 78%), *Lr 2b* (75& 71%), *Lr 30* (81& 64%) and *Lr 3ka* (70 & 61%) respectively, while, moderately and relatively low levels of gene efficacy (ranged from 27% to 64.44%) were recorded with the other leaf rust resistance genes under study.

Table 6. Virulence Frequencies (%) of *Puccinia triticina and* Gene efficacy (%) for leaf rust resistance genes in Egypt during 2014 – 2016

N0 *	*Lr's*	Accession number[1]	Pedigree[2]	First season 2014-15		Second season 2015-16	
				Virulence frequency %	Gene efficacy %	Virulence frequency %	Gene efficacy %
1	***Lr1***	GSTR 402	Thatcher*6/Centenario	51.35	48.64	65.55	34.45
2	***Lr2a***	GSTR 403	Thatcher*6/Webster	21.62	78.38	21.11	78.89
3	***Lr2c***	GSTR 405	Thatcher*6/Brevit	45.94	54.06	38.88	61.11
4	***Lr3***	GSTR 406	Thatcher*6/Democrat	18.91	81.09	23.33	76.66
5	***Lr9***	GSTR 409	Thatcher*6/Aegilops umbellulata	18.91	81.09	17.77	82.22
6	***Lr16***	GSTR 417	Thatcher*6/Exchange	56.75	43.25	68.55	31.45
7	***Lr24***	GSTR 425	Thatcher*6/Agropyron elongatum	72.97	27.03	70.00	30.00
8	***Lr26***	GSTR 427	Thatcher*6/Imperial (rye)	67.55	32.45	60.00	40.00
9	***Lr3ka***	GSTR 408	Thatcher*6/Klein Aniversario	29.72	70.28	38.88	61.11
10	***Lr11***	GSTR 411	Thatcher*6/Hussar	51.35	48.64	62.22	37.77
11	***Lr17***	GSTR 418	Thatcher*6/Klein Lucero	51.35	48.64	47.77	52.22
12	***Lr30***	GSTR 430	Thatcher*6/Terenzio	18.91	81.09	35.55	64.44
13	***Lr10***	GSTR 410	Thatcher*6/Lee	37.83	62.17	48.86	51.12
14	***Lr18***	GSTR 419	Thatcher*6/Africa 43	24.32	75.68	46.66	53.34
15	***Lr21***	GSTR 422	Thatcher*6/Aegilops tauschii	37.83	62.17	42.22	57.78
16	***Lr2b***	GSTR 404	Thatcher*6/Agent	24.32	75.68	28.88	71.12

1. Accession numbers in accordance with Research Service of Germplasm Resource Information Network (GRIN).
2. Pedigree in accordance with Genetic Resources Information System for Wheat and Triticale (GRIS).

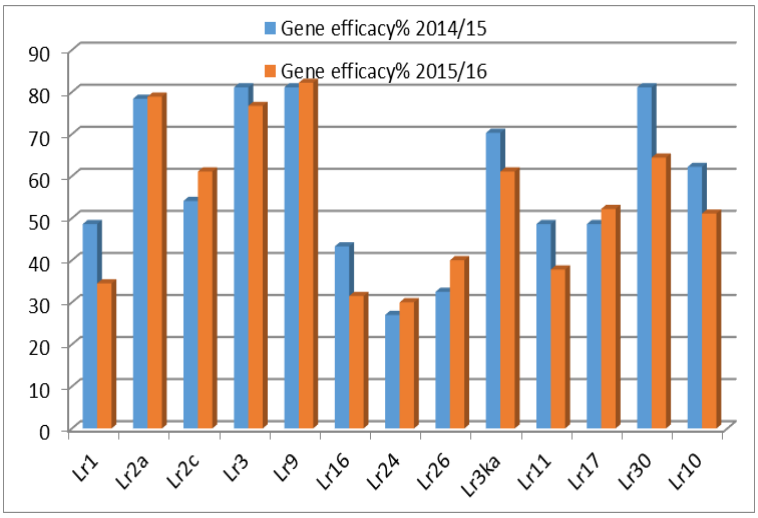

Figure 5. Gene efficacy% for leaf rust resistance genes in Egypt during 2014-2016

Discussion

Successful control of wheat leaf rust disease with race-specific gene resistance requires understanding the pathogenic races which present in the pathogen population. The impact of using the resistant cultivars on the frequencies of *P. triticina* races in Egypt dose not survive the summers in Egypt. Moreover, field observations showed that, the absence of alternate host, and the initial inoculum for each epidemic must come as windborne urediniospores from the neighboring countries (Abdel-Hak *et al.,* 1974; Nazim *et al.*, 2003; 2010). Thus, it is important to consider the amount of diversity for virulence within the pathogen population and the sources of primary inoculum. Clearly, if we could be identified the composition of pathogenic races in the external source that would be very importance to planning the use of genes for race-specific resistance to wheat leaf rust in Egypt (Mcvey *et al.,* 2004; Negm *et al.,* 2013). Therefore, the present work was concerned on the diversity of the causal organism and the frequency of the different virulence and occurrence with in different geographical areas in the country as important step for expanding knowledge of the epidemiology and population structure of wheat leaf rust in Egypt.

Obtained data during the tow growing seasons 2014/2016 clearly indicated that, a total of 127 physiologic races of *P. triticina* were identified among collected isolates. Also, survey of the virulence pathotypes in some

governorates in Egypt showed that race TTQT was the most frequent followed by TSPJ and TPQJ in 2014-2015 growing season. In 2015/2016, race TTTQ and PTTT were the most frequent followed by TTKS and TTQT. Similar results were reported by Najeeb *et al.* (2005); Kolmer *et al.* (2012); Soliman *et al*. (2012); Negm *et al*. (2013); Mohamed, *et al*. (2016). These previous findings suggest that, population of wheat leaf rust in Egypt is made up of a great diversity of races indicated an external source of primary inoculum for the fall-sown wheat crop each year. Also, this showed that, the source of airborne urediniospores is consistent from year to year.

Races varied in their found location, distribution and frequency. BBBB was the weakest race, but was the normal frequent, making up 2.7% in 2014/15 (Table, 3) and medium frequent 3.76% in 2015-16 respectively (Table 4). Race BBBT were highest frequent 5.76% in 2015-16 which also were less virulent. Race BBBT is widespread and was found in each growing season as reported previously for Lebanon and Turkey (Kassem, 2010), Egypt (McVey *et al.,* 2004) and Syria (Kassem *et al.,* 2015). Our study confirms that, Egypt has a rich supply of physiologic races of *P. triticina*. This is likely due to its geographic location, and throughout the growing season its exposure to winds originating from multiple countries of origin urediniospores. In addition, the spread of the alternate host (*Thalictrum* spp.) in neighboring countries from North such as European countries and from the North Eastern i.e. Turkey, Iraq, Lebanon, Syria and Iran, makes this region exposed to the emergence of new races (Kassem *et al.*, 2015).

Frequencies of race groups based on IT's of the first two sets of leaf rust North American race nomenclature system of wheat leaf rust differential monogenic lines were compared in three governorates in Egypt. Seven race groups i.e. BB-, BF-, BK-, DK-, LK-, SK- and TT- were common, which were found at all seasons, but with different frequencies. Data in Table (5) revealed that in 2014-2015 growing season race group DK-- was the most frequency followed by TT--. In contrast, the two race groups LK-- and SK- had the lowest frequencies. The three race groups NK--, PK-- and QK—were disappeared in the first season. On the other hand, during 2015-2016 growing season, the two race groups DK-- and TT-- had lowest frequency. While, the race group BB-- had the highest frequency. These results run in the same trend with those of (Nazim *et al.,* 2010; Khadegah Najeeb, 2013; Walid *et al*., 2015). The occurrence of races in a specific season and region depends on the type of wheat cultivars grown and the major environmental conditions, especially temperature (Roelfs *et al.,* 1992). Also, due to the long-distance dispersal of leaf rust races (Kolmer, 2005; Hanzalova and Bartos, 2014).

Similarity between leaf rust populations in different locations under study presented that, races of *P. triticina* in the geographical areas within Egypt through 2014-2016 growing seasons are divided into two main groups depending on their distribution. Area 1 is characterized by exposure to winds from Europe and Turkey. While, area 2 is affected by virulence *P. triticina* originating from the Eastern and area 3 from Northern and Eastern via the typical movement of winds in Egypt. Similar findings were also reported by Negm *et al*. (2013).

Virulence against leaf rust resistance genes showed that *Lr 1, Lr 11, Lr 17, Lr 16, Lr 24* and *Lr 26* genes were susceptible. While, *Lr 9, Lr 3, Lr 2a, Lr 2b, Lr 30*, and *Lr 3ka* proved to have the highest and best efficacy against the tested race groups. Apparently, similar trend was found in the two growing seasons. The most effective genes i.e. *Lr 3, 9, 2a* and *2b*, were almost stable, showing little or no changes in their levels of efficacy against all the tested isolates. Similar results were described by Khadegah Najeeb (2013); Omara (2013); Mohammed *et al.* (2014) and Esmail *et al.* (2015).

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