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Diversity and Virulence Dynamics within *Puccinia triticina* Populations in Egypt

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ABSTRACT

Leaf rust caused by *Puccinia triticina* Eriks., is one of the most common diseases of wheat (*Triticum aestivum* L.) in Egypt and worldwide. To investigate the population genetic structure of the causal pathogen, 468 single isolates were obtained from 193 samples collected from 12 Egyptian governorates and analyzed during 2016/17 and 2017/18 growing seasons. A total of 125 and 101 leaf rust pathotypes were designated during 2016/17 and 2017/18 growing seasons. The most frequent pathotype was STTTK (9.81% frequency), followed by race PTTNS (2.80% frequency) during 2016/17. While, pathotype PTTTT was occurred at a high frequency (12.16% frequency), followed by the four pathotypes; TTTBT, PTTGS, TTTTT and PTTCT (5.88, 5.10, 5.10 and 4.31%, respectively), during 2017/18. Pathotype STTTK is the most geographically distributed as it was detected in nine governorates in 2016/17, but in 2017/18, PTTTT is the most geographically distributed pathotype, where it was found in seven governorates. High similarity was found between leaf rust populations in the five locations, *i.e.* Domiatta, Sohag, Alexandria, Kafr El-Sheikh and Bani Sweif, during 2016/17 growing season. Also, high similarity was found between leaf rust pathogen populations of Kafr-Elsheikh, Sohag, Beheira, Dakahlia, Sharqiya, Domiatta, Gharbiya, Fayoum and Minufiy, during 2017/18 growing season. The phenotypic diversity within different populations under study was characterized using the three indexes; Shannon, Gleason and Simpson. Shannon index proved to be more suitable to accurately measure the phenotypic diversity between the tested populations of the causal pathogen, as it was sensitive to sample size, number of isolates, number of races and standard deviation of race frequency than the others.

Keywords: Wheat, leaf rust, *Puccinia triticina*, virulence dynamics, phenotypic diversity

INTRODUCTION

Wheat leaf rust, caused by *Puccinia triticina* Eriks., is one of the most common diseases in Egypt and worldwide. It is widespread occurrence in most commercial wheat cultivars in approximately all governorates nationwide. Grain Losses in grain yield due to severe leaf rust epidemics can reach to nearly 50 %, depending on the growth stage of wheat plants when the initial infection occurs, also in response to the relative resistance or susceptibility of the grown host cultivar (German *et al*., 2007).

Growing resistant cultivars is still an economical, environmentally safe, and the most effective method to successfully control wheat rusts in general, especially leaf rust. Up to date, 75 leaf rust resistance genes have been described and designated (McIntosh *et al*., 2013). Most of these genes confer race-specific resistance in a gene-for-gene relationship. While, others conditioning race non-specific type of resistance (Kolmer *et al*., 2005). As a result of race specificity of resistance genes and due to the high evolutionary potentiality of pathogen populations, most of the commercial wheat cultivars subjected to rapidly loss of their effective resistance, soon after it's new release and wide cultivation in farmer fields. The frequency (%) of *P. triticina* races with virulence to a specific resistance gene can gradually increase in it's population from less than 5% to over 60%, during a few years (Kolmer *et al*., 2005). Selection pressure imposed by a widely grown of the highly resistant cultivars for virulence against *P. triticina* races, as an evolutionary force, alters the genetic structure of pathogen population. In addition, the migration or long-distance dispersal (LDD) of airborne urediniospores of rust fungiform it's source area (neighboring countries) to the target, as well as the sexual recombination and besides of mutation events, all of these evolutionary forces can also affect pathogen diversity within it's populations (Kolmer & Ordoñez, 2007 and Kosman *et al*., 2014). The previous reports in Egypt suggested that, wheat leaf rust pathogen does not survive summer in the country, and the alternate host did not found or detect, hence sexual reproduction does not occur in the country. Therefore, the primary inoculum must come from external sources each year (Abdel-Hak *et al*., 1974; Nazim *et al*., 1976; McVey et *al*., 2004 and Negm *et al*., 2013). Due to the high diversity of regional wheat leaf rust pathogen populations, besides to a widely use of resistant cultivars in Egypt, a selection pressure considered to be a main evolutionary force, which consequently increase an emergence of new races with virulence to specific leaf rust resistance genes (McIntosh *et al*., 2013).

A total of 149 leaf rust pathotypes were detected and identified in Egypt during 2011/12 to 2013/14 growing seasons (El-Orabey *et al*., 2015). The high level of racial diversity in regional wheat leaf rust populations has made an effective and long lasting resistance in wheat genotypes very difficult to achieve. However, phenotypic diversity refers in general to a rate of temporal and spatial changes in different pathogen populations. A pathogen population considered to be more diverse and/or with high evolutionary potential if it consists of a large number of pathotypes for a given number of isolates (Groth and Roelfs, 1987). It can be characterized by even distribution of pathotypes in which case a small number of pathotypes dominant, and when differences between pathotypes in genetic and other virulence attributes are large (Kosman, 1996). There are three indexes previously adapted to measure phenotypic diversity, and widely used to characterize different pathogen populations. The first one is Shannon index that based on relative frequency (%) of different races. The ratio of pathotypes (races) to isolates sampled depends upon the sample size, and a larger sample size is required to detect rare pathotypes. The second index is Gleason index which is considered less sensitive to the sample size, because the increase in sample size is correspondingly diminished in it's logarithmic form and simple to calculate. Whereas, Simpson index is the third index used to estimate phenotypic diversity for different plant pathogen populations, to determine the number of pathotypes and evenness of their distribution (Groth & Roelfs, 1987).

The annual survey of wheat rust races, especially leaf rust races, aimed to detect and monitor shifts of

virulence pathotypes that have been introduced to a region. Thus, provides essential information to determine the direction of the national breeding program for rust resistance.

The main objectives of this study were: 1) to characterize virulence frequency of *P. triticina* pathotypes in Egypt during 2016/17 and 2017/18 growing seasons; 2) to study the geographical distribution of the identified pathotypes in 12 Egyptian governorates; 3) to estimate the diversity dynamics between wheat leaf rust pathogen populations in different locations in Egypt.

MATERIALS AND METHODS

Field survey and samples collection:

Wheat leaves infected with leaf rust urediniospores were collected during the annual survey, during 2015/2016 and 2016/2017 growing seasons, from both commercial fields and Egyptian wheat rust trap nurseries (EWRTN) grown in 12 governorates, *i.e.* Alexandria, Beheira, Kafr-Elsheikh, Domiatta, Dakahlia, Sharqiya, Gharbiya, Minufiya, Qalyubia, Fayoum, Bani Sweif and Sohag (Table 1). Samples were air-dried by keeping at room temperature (18 to 24°C) overnight in order to dry it's moisture content. Samples were kept in paper pages (8 x 15 cm) and kept in the refrigerator at 2 to 5°C, until using in isolation process.

Isolation, purification and urediniospores multiplication:

Susceptible wheat cultivar; Morocco was planted as ten seeds per 10 cm diameter plastic pots in the greenhouse of Wheat Diseases Research Department, Plant Pathology Research Institute, ARC, Egypt. When the first leaf fully expanded in seven days old seedlings, leaves were rubbed gently between moist fingers with tap water. Then infected samples were scraped using sterile spatula and transferred to these seedlings and sprayed gently again with water in order to form a film of free water, which is essential to initiate spore germination and establishment of infection. Finally, the inoculated seedlings were incubated in moist chambers for 24 h at 18 to 20°C and 100% RH, then moved to greenhouse benches and kept for 14 days at approximately 20 ± 2°C. After pustule's rupture, three single pustules were isolated separately from each specimen for spores multiplication on the highly susceptible variety; Morocco to obtain enough urediniospores for race identification as described by Kolmer *et al*. (2005).

Race designation and nomenclature:

The North American race nomenclature system was used to identify leaf rust races in a letter code, as previously adopted by Long and Kolmer (1989) and McVey *et al*. (2004). This key includes five sets with 20 differentials of near-isogenic lines of Thatcher wheat, each with a single gene for leaf rust resistance. These differential lines were *Lr*1, *Lr*2a, *Lr*2c, and *Lr*3a as the four lines in the first set of differentials; lines with genes *Lr*9, *Lr*16, *Lr*24, and *Lr*26 were the second set; lines with genes *Lr*3ka, *Lr*11, *Lr*17, and *Lr*30 were the third set; and lines with genes *Lr*10, *Lr*18, *Lr*21, and *Lr*2b were the fourth set; lines with genes *Lr*14b, *Lr*15, *Lr*36, and *Lr*42 were the fifth set (Long and Kolmer, 1989). The fifth or last set of differentials (Egyptian set) was added for the first time in 1998 by McVey *et al*. (2004). The differential lines were grown in plastic pots (6 X 6 cm), each with seeds of four lines, planted in the corners of each pot in a clockwise order (five to seven plants per line). After seven-days, seedlings were inoculated with the previously isolated single pustule isolates of *P. triticina*, by shaking. The inoculated seedlings were incubated in the humid chamber overnight (100% RH), as described above. The inoculated seedlings were transferred also, onto the greenhouse benches. After 10-12 days, infection types (IT's) were recorded for all monogenic lines using standard disease scoring scale 0-4 (Kolmer *et al*., 2005). Entries which showed low infection types (L), *i.e.* scores = 0, 0; , 1 and 2 were considered host resistant and avirulent isolates, while those showed high infection types (H), *i.e.* scores = 3 and 4 were recorded as the susceptible lines and virulent isolates. Each single isolate was assigned five letters based on high or low infection types to the differential lines (Long and Kolmer, 1989 and McVey *et al*., 2004).

Virulence frequency (%):

Percentage of virulence frequency was calculated for each of an identified race in the study as a number of virulent isolates to the total number of the tested isolates, according to the following equation:

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

Race diversity measurements:

The number and frequency of races within populations of *P. triticina* collected from 12 governorates in Egypt were compared and used to measure the diversity of each population within the different regions. To assess diversity of *P. triticina* races within each population (region), three indexes, *i.e.* Shannon index (H_{SH}), Gleason index (H_G) and Simpson index (H_S) (Groth and Roelfs, 1987) were estimated as follows:

Shannon index: was used to determine the similarities of the frequencies of the different pathotypes in a set of isolates by the following formula:

$$H_{SH} = -\Sigma (P_i \ln P_i)$$

Where: P_j **= the frequency of the** $_i$**th pathotype in the set of isolates.**

Gleason index: was used to detect the number of distinct pathotypes present, indicating the richness aspect of diversity and calculated by the following formula:

$$H_G = (n-1)/\ln(N)$$

Where: n is the number of pathotypes and N is the number of isolates in the sample population.

Simpson index: was another popular diversity index for plant pathogens to determine the number of pathotypes and evenness of their distribution. It was calculated by the following formula:

$$H_S = 1 - \Sigma [n_i(n_i - 1)/N(N-1)]$$

Where: n_i **= the number of isolates of the** $_i$**th pathotype and N = the sample size.**

Cluster analysis:

A similarity matrix of all races identified in the governorates under study was used to construct a dendogram, using the unweight pair group method with arithmetic means clustering method in numerical taxonomy system (NTSYS-pc version 2.1) and based on the simple matching coefficient according to Rohlf (2000).

Statistical analysis:

Correlation and regression coefficient "SPSS Regression Modeling" was used to determine the relationship between each of the four components of

diversity, *i.e.* sample size collected for each region, number of isolates, number of races and standard deviation of race frequency and the three diversity indexes, *i.e.* Shannon, Gleason and Simpson over the two growing seasons of the study.

RESULTS

During the wheat leaf rust annual survey in the two growing seasons 2015/16 and 2016/17, a total of 193 samples were collected from 12 Egyptian governorates. These samples produced in a total of 468 single isolates of the causal pathogen (Table 1).

In 2015/16 growing season, 95 samples were collected from the different locations under study. These samples produced 213 single isolates. The highest number of the collected samples was obtained from Qalyubia and Fayoum (each with 13 samples), while the lowest number of these samples were obtained from Domiatta and Sohag (each with only 5 samples). The highest number of single isolates of leaf rust pathogen was obtained from the samples collected from Beheira and Fayoum which produced 32 and 31 pure isolates, respectively. While, the lowest number of these isolates was obtained from Domiatta, Alexandria and Kafr-Elsheikh, which produced 7, 9 and 9 isolates, respectively (Table 1).

In 2016/17 growing season, a total of 98 samples were collected from 12 Egyptian governorates. These samples produced 255 single isolates. The highest numbers of the collected samples were obtained from Bani Sweif and Beheira, *i.e.* 19 and 18 samples, respectively, while the lowest numbers of these samples were obtained from Alexandria and Qalyubia (each with only 3 samples). Moreover, samples collected from Bani Sweif and Beheira produced the highest number of single isolates, *i.e.* 58 and 51, respectively (Table 1).

During the annual survey in the two growing season of the study, the highest number of the collected samples were obtained from Beheira and Bani Sweif (27 and 25 samples, respectively), while the lowest number of the collected samples were obtained from the three governorates; Alexandria, Domiatta and Sharqiya (each with only 10 samples). On the other hand, the highest number of leaf rust isolates was isolated from the samples of Beheira and Bani Sweif, which produced 83 and 68 pure isolates, respectively. In contrast, the lowest number of these isolates was obtained from the samples of Alexandria, as it could be isolated only 13 pure isolates of leaf rust pathogen during the two growing seasons of the study (Table 1)

Table 1. Number of leaf rust samples and isolates obtained from different wheat growing areas in Egypt during 2015/16 and 2016/17 growing seasons.

Governorate	Season / Number of samples and isolates					
	2015/16		2016/17		Total	
	No of samples	No. of isolates	No of samples	No. of isolates	No of samples	No. of isolates
Alexandria	7	9	3	4	10	13
Beheira	9	32	18	51	27	83
Kafr-Elsheikh	6	9	10	22	16	31
Domiatta	5	7	5	15	10	22
Dakahlia	7	22	4	10	11	32
Sharqiya	6	12	4	9	10	21
Gharbiya	8	15	5	12	13	27
Minufiya	10	29	6	16	16	45
Qalyubia	13	27	3	6	16	33
Fayoum	13	31	4	14	17	45
Bani Sweif	6	10	19	58	25	68
Sohag	5	10	17	38	22	48
Total	95	213	98	255	193	468

Race identification and their frequency (%) in pathogen population:

A total of 125 virulent pathotypes of *Puccinia triticina* were identified from 213 pure isolates in Egypt during 2016/17 growing season (Table 2). As indicated in this Table, race STTTK is the most common pathotype (9.81 % frequency), followed by race PTTNS (2.80 % frequency), and the two races FTSSS and PKTPT (each with 2.34 % frequency). While, the seven leaf rust races, *i.e.* KTSPT, PHTTT, PKTST, PPQST, PTTPT, PTTSS and TTTST showed relatively low frequency in pathogen population (each with only 1.87 % frequency). Most of the identified pathotypes were rare as they showed low frequency within it's population. However, 95 out of 125 identified pathotypes were represented by an only single isolate (each showed 0.47 % frequency). On the other hand, the frequencies of the other tested pathotypes (19 pathotypes) ranged from 0.93% to 1.40% (Table 2).

In 2017/18, a total of 101 *P. triticina* pathotypes were obtained and designated from 255 isolates. Pathotype PTTTT occurred at a high frequency in it's population; where, it was found at 12.16% frequency, followed by the four pathotypes TTTBT, PTTGS, TTTTT and PTTCT as they showed by 5.88%, 5.10%, 5.10% and 4.31% frequencies, respectively. Most of the identified pathotypes (76 ones) found at the lowest frequency in the pathogen population (each with only 0.39 % frequency), where each of these pathotypes represented by a single isolate. While, the frequencies of the other tested pathotypes (20 pathotypes) ranged from 0.78% to 3.92% (Table 2).

Moreover, only the two pathotypes, *i.e.* STTTK and PTTST were common and have been detected in the two growing seasons of the study. Pathotype STTTK occurred at a high frequency; 9.81% in 2016/17 and 1.18% in 2017/18. While, pathotype PTTST showed 1.40 % frequency in 2016/17 and 1.96% frequency in 2017/18. Meanwhile, some of leaf rust pathotypes under study were detected in only first growing season, and not found in the second one, but others have been detected for the first time in the second growing season (Table 2).

Table 2. Number of isolates and frequency (%) of *Puccinia triticina* races in Egypt during 2016/17 and 2017/18 growing seasons.

[a]Race	No. of isolates & frequency (%)				Race	No. of isolates & frequency (%)			
	2016/17		2017/18			2016/17		2017/18	
	No. of isolates	Frequency (%)	No. of isolates	Frequency (%)		No. of isolates	Frequency (%)	No. of isolates	Frequency (%)
BDGGK	1	0.47	-	-	NRTJT	-	-	2	0.78
BGJFL	1	0.47	-	-	NRTKT	-	-	1	0.39
BNQSF	1	0.47	-	-	NRTSS	-	-	1	0.39
BPKST	1	0.47	-	-	NSPDT	-	-	1	0.39
BPLSN	1	0.47	-	-	NSTGN	-	-	1	0.39
BTBBB	-	-	1	0.39	NTFTT	-	-	3	1.18
BTTFT	-	-	1	0.39	NTKTS	3	1.40	-	-
BTTTK	-	-	1	0.39	NTRTR	1	0.47	-	-
CPPTB	1	0.47	-	-	NTSDT	-	-	1	0.39
CTTTT	3	1.4	-	-	NTTBK	-	-	1	0.39
DBBDF	1	0.47	-	-	NTTBT	-	-	1	0.39
DFTPS	1	0.47	-	-	NTTDS	-	-	1	0.39
DHTTT	1	0.47	-	-	NTTJT	-	-	10	3.92
DSTJS	-	-	1	0.39	NTTKT	-	-	8	3.14
DSTTS	-	-	1	0.39	NTTPN	1	0.47	-	-
DTFJM	-	-	1	0.39	NTTPT	3	1.4	-	-
DTKBB	-	-	1	0.39	NTTSR	1	0.47	-	-
FFTPS	1	0.47	-	-	NTTST	-	-	7	2.75
FFTSS	1	0.47	-	-	NTTTS	-	-	4	1.57
FJQRS	1	0.47	-	-	NTTTT	-	-	9	3.53
FKRNK	1	0.47	-	-	PDTPS	1	0.47	-	-
FKRPD	1	0.47	-	-	PFGLS	1	0.47	-	-
FRTDT	1	0.47	-	-	PFKNT	1	0.47	-	-
FRTPJ	1	0.47	-	-	PFKTT	-	-	1	0.39
FTKTS	1	0.47	-	-	PFPNT	1	0.47	-	-
FTSNS	1	0.47	-	-	PFTNS	1	0.47	-	-
FTSSS	5	2.34	-	-	PFTNT	1	0.47	-	-
FTTKT	-	-	1	0.39	PFTST	1	0.47	-	-
FTTNS	1	0.47	-	-	PGTJC	-	-	1	0.39
FTTSS	1	0.47	-	-	PHTKT	-	-	1	0.39
GBHLD	1	0.47	-	-	PHTTT	4	1.87	-	-
GBTMT	1	0.47	-	-	PJSFT	1	0.47	-	-
GJBDJ	-	-	1	0.39	PKFNT	1	0.47	-	-
GTCBK	-	-	1	0.39	PKJTT	1	0.47	-	-
HPFDT	-	-	1	0.39	PKKTT	2	0.93	-	-
HSTPT	1	0.47	-	-	PKSSB	-	-	1	0.39
JKKNJ	1	0.47	-	-	PKTJT	-	-	3	1.18
JSLTT	1	0.47	-	-	PKTKS	1	0.47	-	-
JTTJS	-	-	1	0.39	PKTPQ	1	0.47	-	-
JTTKT	-	-	3	1.18	PKTPR	1	0.47	-	-
KMTTT	1	0.47	-	-	PKTPT	5	2.34	-	-
KTSPT	4	1.87	-	-	PKTSS	-	-	1	0.39
KTTJT	-	-	1	0.39	PKTST	4	1.87	-	-
LDDLD	1	0.47	-	-	PKTTF	1	0.47	-	-
LFCTF	1	0.47	-	-	PKTTR	3	1.40	-	-
LFGLL	1	0.47	-	-	PKTTT	1	0.47	-	-
LJJNT	1	0.47	-	-	PMKGC	-	-	1	0.39
LJSLN	1	0.47	-	-	PMTTS	1	0.47	-	-
LPTGT	-	-	1	0.39	PNGNJ	1	0.47	-	-
LSCBT	-	-	1	0.39	PPKNT	1	0.47	-	-
LSLTB	-	-	1	0.39	PPQST	4	1.87	-	-
LTJTS	-	-	1	0.39	PPTBT	-	-	2	0.78
LTKTP	-	-	1	0.39	PPTNT	1	0.47	-	-
LTPCS	-	-	1	0.39	PPTPR	1	0.47	-	-
LTSKT	-	-	1	0.39	PPTPT	1	0.47	-	-
LTTTT	-	-	1	0.39	PQTBT	-	-	1	0.39
MKMFT	-	-	1	0.39	PRHNK	1	0.47	-	-
MTKJT	-	-	1	0.39	PRSJT	-	-	1	0.39
MTKSS	1	0.47	-	-	PRSTT	3	1.40	-	-
MTTBT	-	-	1	0.39	PRTKF	-	-	1	0.39
MTTGT	-	-	1	0.39	PRTNT	2	0.93	-	-
MTTKT	-	-	1	0.39	PRTST	-	-	1	0.39
MTTPS	1	0.47	-	-	PSTLN	1	0.47	-	-
MTTTT	-	-	3	1.18	PSTPR	2	0.93	-	-
NBFDJ	-	-	1	0.39	PSTST	-	-	1	0.39
NDTNS	1	0.47	-	-	PSTTT	-	-	3	1.18
NFSST	-	-	1	0.39	PTFBT	-	-	1	0.39
NHTPT	1	0.47	-	-	PTJNP	1	0.47	-	-
NJTPK	1	0.47	-	-	PTJPT	1	0.47	-	-
NKKJS	-	-	1	0.39	PTKDQ	-	-	1	0.39
NKMPM	1	0.47	-	-	PTKGT	-	-	1	0.39
NKSPT	1	0.47	-	-	PTKTH	1	0.47	-	-
NKTKS	-	-	3	1.18	PTKTS	1	0.47	-	-
NKTSS	2	0.93	-	-	PTMBT	-	-	1	0.39
NNHJP	-	-	1	0.39	PTPTN	1	0.47	-	-
NNKSS	1	0.47	-	-	PTPTT	1	0.47	-	-
NPTNK	2	0.93	-	-	PTQST	1	0.47	-	-
NRKDS	-	-	1	0.39	PTRPT	1	0.47	-	-
NRRLS	1	0.47	-	-	PTSDS	-	-	1	0.39
NRSJT	-	-	1	0.39	PTSJT	-	-	1	0.39

Table 2. Cont.

Race	No. of isolates & frequency (%)				Race	No. of isolates & frequency (%)			
	2016/17		2017/18			2016/17		2017/18	
	No. of isolates	Frequency (%)	No. of isolates	Frequency (%)		No. of isolates	Frequency (%)	No. of isolates	Frequency (%)
PTSLS	1	0.47	-	-	STRTQ	1	0.47	-	-
PTSNS	3	1.40	-	-	STTLT	-	-	1	0.39
PTSTS	1	0.47	-	-	STTST	-	-	2	0.78
PTTCT	-	-	11	4.31	STTTK	21	9.81	3	1.18
PTTGS	-	-	13	5.10	TDFBQ	-	-	1	0.39
PTTJT	-	-	6	2.35	TFKTR	2	0.93	-	-
PTTNJ	1	0.47	-	-	TFSTR	1	0.47	-	-
PTTNS	6	2.80	-	-	TJQTL	1	0.47	-	-
PTTNT	1	0.47	-	-	TKTNK	1	0.47	-	-
PTTPM	3	1.40	-	-	TKTPR	1	0.47	-	-
PTTPQ	2	0.93	-	-	TLSPQ	1	0.47	-	-
PTTPR	1	0.47	-	-	TPKPS	1	0.47	-	-
PTTPS	3	1.40	-	-	TPPSS	1	0.47	-	-
PTTPT	4	1.87	-	-	TPSNT	1	0.47	-	-
PTTSP	1	0.47	-	-	TPTMP	1	0.47	-	-
PTTSS	4	1.87	-	-	TQTTQ	1	0.47	-	-
PTTST	3	1.40	5	1.96	TSPSF	-	-	1	0.39
PTTTF	1	0.47	-	-	TSSPM	1	0.47	-	-
PTTTS	1	0.47	-	-	TTBFQ	-	-	1	0.39
PTTTT	-	-	31	12.16	TTCJT	-	-	2	0.78
QHTQT	-	-	1	0.39	TTKCT	-	-	1	0.39
QRTQT	-	-	1	0.39	TTKGS	-	-	1	0.39
RDTDT	-	-	1	0.39	TTKJS	-	-	1	0.39
SBFST	-	-	1	0.39	TTKTS	3	1.40	-	-
SGTJS	-	-	1	0.39	TTSTQ	-	-	1	0.39
SKTSS	-	-	1	0.39	TTTBB	-	-	11	4.31
SQTJD	-	-	1	0.39	TTTBT	-	-	15	5.88
SRTKC	-	-	1	0.39	TTTKT	-	-	7	2.75
SSQJQ	-	-	1	0.39	TTTMS	3	1.40	-	-
STBBR	-	-	1	0.39	TTTNK	3	1.40	-	-
STBPK	1	0.47	-	-	TTTST	4	1.87	-	-
STJDD	-	-	1	0.39	TTTTT	-	-	13	5.10
Total					[b]224	213	100	255	100

[a]**Races are identified by five letters code for the five sets of monogenic differential wheat lines (*Lr*'s) (McVey *et al*., 2004).**

[b]**Total number of identified pathotypes during the two growing seasons of the study = 226 (125 pathotypes during 2016/17 and 101 pathotypes during 2017/18) and the number her is 224 due to two pathotypes were detected in the two seasons of the study.**

Geographical distribution:

Leaf rust population structure:

In 2016/17, a total of 26 pathotypes were identified in Fayoum, thus it considered to be the largest population size, as it represented by 20.80% frequency of the whole population. Followed by the three populations; Minufiya, Beheira and Qalyubia, which showed 19.20, 19.20 and 18.14% frequency of the whole population, respectively. While, each of the three governorates Alexandria, Domiatta and Sohag showed a small population size (each with 3.20% frequency). Pathotype STTTK is the most common and widespread leaf rust pathotype nationwide, which was detected in nine governorates out of the twelve governorates under study, followed by pathotype PTTNS which was detected in four governorates (Table 3).

In 2017/18, out of leaf rust pathotypes identified during this season, a total of 27 pathotypes were detected in Bani Swif population, thus it showed the highest number of the identified pathotypes and considered to be the largest population size rather than the other pathogen population studied represented by 26.73% frequency. Also, the three populations; Beheira, Kafr Elsheikh and Sohag, each showed 16.83% frequency. On the other hand, the lowest number of the identified pathotypes was found in Alexandria (2.97% frequency) and Qalyubia (4.95% frequency). Pathotype PTTTT is the most common and widely distributed pathotype, as it was geographical distributed in seven governorates, followed by pathotype PTTGS, which was detected in six governorates (Table 3).

Similarity and dissimilarity between the genetic structure of leaf rust populations:

The relationships between the 12 leaf rust populations in different locations under study, based on the presence and absence of the identified pathotypes in these populations were estimated and illustrated in Fig. (1A and 1B). Cluster analysis of similarities and dissimilarities between pathotypes in 2016/17 growing season, two main clusters were formed. The first cluster divided into two additional sub-clusters. The first sub-cluster includes five populations, i.e. Domiatta, Sohag, Alexandria, Kafr El-Sheikh and Bani Sweif, while the second includes the two locations, i.e. Sharqiya and Gharbiya. The second main cluster also divided into two sub-clusters, the first one includes four locations, i.e. Beheira, Qalyubiya, Minufiya and Fayoum. While, the second sub-cluster contains only one location, i.e. Dakahlia (Fig. 1A).

Table 3. Geographical distribution and frequency (%) of *Puccinia triticina* races identified in 12 Egyptian governorates during 2016/17 and 2017/18 growing seasons.

No.	Governorate	Season / Identified races and their frequency (%)			
		2016/17		2017/18	
		Identified races and frequency (%)	Total No. of races	Identified races and frequency (%)	Total No. of races
1	Alexandria	PTJPT (0.47), PTTPM (1.40), STTTK (1.40) and TPKPS (0.47).	4 (3.20%)	NTTTS (0.78), SSQJQ (0.39) and STTST (0.39)	3 (2.97%)
2	Beheira	CTTTT (0.47), FTKTS (0.47), FTTSS (0.47), GBTMT (0.47), KTSPT (0.47), MTTPS (0.47), NJTPK (0.47), NKTSS (0.47), NPTNK (0.47), NTKTS (0.47), NTTSR (0.47), PKKTT (0.93), PKTPR (0.47), PKTST (0.47), PPTPT (0.47), PRSTT (0.47), PTTNS (1.40), PTTPS (0.47), PTTPT (1.40), PTTSS (0.93), PTTST (0.47), PTTTS (0.47), STTTK (1.40) and TTTMS (0.47).	24 (19.20%)	MTTTT (0.78), NKTKS (1.18), NTTJT (0.78), NTTKT (0.78), NTTST (1.57), NTTTT (0.78), PKTJT (0.39), PMKGC (0.39), PPTBT (0.78), PSTTT (0.39), PTTGS (2.35), PTTTT (6.27), TTCJT (0.78), TTKGS (0.39), TTTBT (1.18), TTTKT (0.39) and TTTTT (0.78).	17 (16.83%)
3	Kafr-Elsheikh	JSLTT (0.47), PHTTT (1.87), PTKTH (0.47), STBPK (0.47) and STTTK (0.93).	5 (4.00%)	BTTTK (0.39), FTTKT (0.39), LTTTT (0.39), MTTBT (0.39), NBFDJ (0.39), NRTJT (0.39), NRTSS (0.39), NSPDT (0.39), NSTGN (0.39), NTTKT (0.39), PFKTT (0.39), PRTKF (0.39), PTMBT (0.39), PTTJT (0.39), PTTTT (1.18), SRTKC (0.39) and TTTKT (1.57).	17 (16.83%)
4	Domiatta	NRRLS (0.47), TTTNK (1.40), PTTTF (0.47) and STTTK (0.93).	4 (3.20%)	JTTJS (0.39), NKKJS (0.39), NTTTS (0.39), NTTTT (1.57), PTTGS (0.78), PTTST (0.39), PTTTT (0.39), SGTJS (0.39), STTST (0.39), TTKCT (0.39) and TTKJS (0.39).	11 (10.89%)
5	Dakahlia	DHTTT (0.47), FFTPS (0.47), FKRNK (0.47), FTTNS (0.47), JKKNJ (0.47), LJJNT (0.47), LJSLN (0.47), NDTNS (0.47), NTTPN (0.47), NTTPT (1.40), PFKNT (0.47), PJSFT (0.47), PKJTT (0.47), PRTNT (0.93), PTRPT (0.47), PTTSS (0.47), STTTK (0.47), TPPSS (0.47) and TPTMP (0.47).	19 (15.20%)	KTTJT (0.39), (0.39), NRTJT (0.39), NTTBT (0.39), PGTJC (0.39), PTFBT (0.39), PTTGS (0.78), PTTST (0.39), STJDD (0.39) and TTTKT (0.39).	9 (8.91%)
6	Sharqiya	CPPTB (0.47), DFTPS (0.47), FFTSS (0.47), LFCTF (0.47), PKTTR (1.40), PPTPR (0.47), PTTPQ (0.93), STTTK (1.40) and TFSTR (0.47).	9 (7.20%)	DSTJS (0.39), MKMFT (0.39), NTTTT (0.39), PTTJT (0.39), PTTTT (0.78), STBBR (0.39), TDFBQ (0.39) and TTTTT (0.39).	8 (7.92%)
7	Gharbiya	BDGGK (0.47), FKRPD (0.47), HSTPT (0.47), LDDLD (0.47), NKMPM (0.47), PSTPR (0.93), STRTQ (0.47), STTTK (1.40), TFKTR (0.93), TLSPQ (0.47) and TQTTQ (0.47).	11 (80%)	LTJTS (0.39), NTTJT (0.78), NTTKT (0.39), NTTTT (0.39), PKSSB (0.39), PKTSS (0.39), PRSJT (0.39), PSTST (0.39), PTTGS (0.39), QHTQT (0.39) and TTTTT (0.39).	11 (10.89%)
8	Minufiya	CTTTT (0.47), FTSNS (0.47), KTSPT (0.47), NHTPT (0.47), NKTSS (0.47), NTKTS (0.47), PFTNS (0.47), PFTNT (0.47), PKTPQ (0.47), PKTPT (1.40), PKTST (0.47), PKTTF (0.47), PKTTT (0.47), PPTNT (0.47), PRSTT (0.47), PTSLS (0.47), PTTNJ (0.47), PTTNS (0.47), PTTPR (0.47), PTTPS (0.93), PTTSS (0.47), PTTST (0.47), STTTK (0.93) and TTTMS (0.47).	24 (19.20%)	DTKBB (0.39), LSCBT (0.39), MTTTT (0.39), NNHJP (0.39), NTTTT (0.39), PHTKT (0.39), PQTBT (0.39), PTKDQ (0.39), PTTJT (0.39), QRTQT (0.39), RDTDT (0.39), SQTJD (0.39), TTSTQ (0.39) and TTTTT (1.18).	14 (13.86%)
9	Qalyubia	CTTTT (0.47), KTSPT (0.47), NNKSS (0.47), NTKTS (0.47), NTRTR (0.47), PFGLS (0.47), PKTST (0.47), PNGNJ (0.47), PPKNT (0.47), PRHNK (0.47), PRSTT (0.47), PTKTS (0.47), PTQST (0.47), PTTNS (0.47), PTTSP (0.47), PTTST (0.47), STTTK (0.93), TJQTL (0.47), TKTNK (0.47), TKTPR (0.47), TPSNT (0.47), TSSPM (0.47) and TTTST (1.87).	23(18.14%)	BTTFT (0.39), GTCBK (0.39), LSLTB (0.39), STTLT (0.39) and TTTTT (0.78).	5 (4.95%)
10	Fayoum	BGJFL (0.47), BNQSF (0.47), BPKST (0.47), BPLSN (0.47), DBBDF (0.47), FRTDT (0.47), FRTPJ (0.47), FTSSS (2.34), KMTTT (0.47), KTSPT (0.47), LFGLL (0.47), MTKSS (0.47), NPTNK (0.47), PFTST (0.47), PKFNT (0.47), PKTKS (0.47), PKTPT (0.93), PKTST (0.47), PSTLN (0.47), PTJNP (0.47), PTPTN (0.47), PTPTT (0.47), PTSTS (0.47), PTTNT (0.47), PTTPT (0.47) and TTTMS (0.47).	26 (20.80%)	GJBDJ (0.39), HPFDT (0.39), LPTGT (0.39), LTKTP (0.39), MTTKT (0.39), NTTJT (0.39), NTTST (0.39), PTSDS (0.39), PTTGS (0.39), PTTJT (0.39) and PTTTT (1.18).	11 (10.89%)
11	Bani Sweif	FJQRS (0.47), NKSPT (0.47), PDTPS (0.47), PFPNT (0.47), PTSNS (1.40) and TTKTS (1.40).	6 (4.80%)	DSTTS (0.39), DTFJM (0.39), JTTKT (1.18), LTPCS (0.39), NRKDS (0.39), NRTKT (0.39), NTFTT (1.18), NTTBK (0.39), NTTDS (0.39), NTTJT (1.96), NTTKT (1.57), NTTST (0.39), NTTTS (0.39), PKTJT (0.78), PRTST (0.39), PSTTT (0.78), PTKGT (0.39), PTTGS (0.39), PTTJT (0.39), PTTST (0.78), PTTTT (1.57), SBFST (0.39), STTTK (1.18), SKTSS (0.39), TTTBT (4.70), TTTKT (0.39) and TTTTT (1.18).	27 (26.73%)
12	Sohag	GBHLD (1.87), PMTTS (0.47), PPQST (1.87) and PTTNS (0.47).	4 (3.20%)	BTBBB (0.39), LTSKT (0.39), MTKJT (0.39), MTTGT (0.39), NFSST (0.39), NRSJT (0.39), NTSDT (0.39), NTTST (0.39), PTSJT (0.39), PTTCT (4.31), PTTJT (0.39), PTTST (0.39), PTTTT (0.78), TSPSF (0.39), TTBFQ (0.39), TTTBB (4.31) and TTTTT (0.39).	17 (16.83%)
	Total		[a]159		[a]150

[a]Total No. of pathotypes in 2016/17 is 125 and in 2017/18 is 101 and the differences between these numbers is due to the present of some pathotypes in more than one location.

In 2017/18 growing season, the similarity between identified races in the studied locations divided also into two main groups. The first group includes tow sub-cluster, the first one includes 9 locations, i.e. Kafr-Elsheikh, Sohag, Beheira, Dakahlia, Sharqiya, Domiatta, Gharbiya, Fayoum, Minufiya. While, the second sub-cluster includes two locations, i.e. Alexandria and Qalyubia. Finlay the second main cluster includes only one location, i.e. Bani Sweif (Fig. 1B).

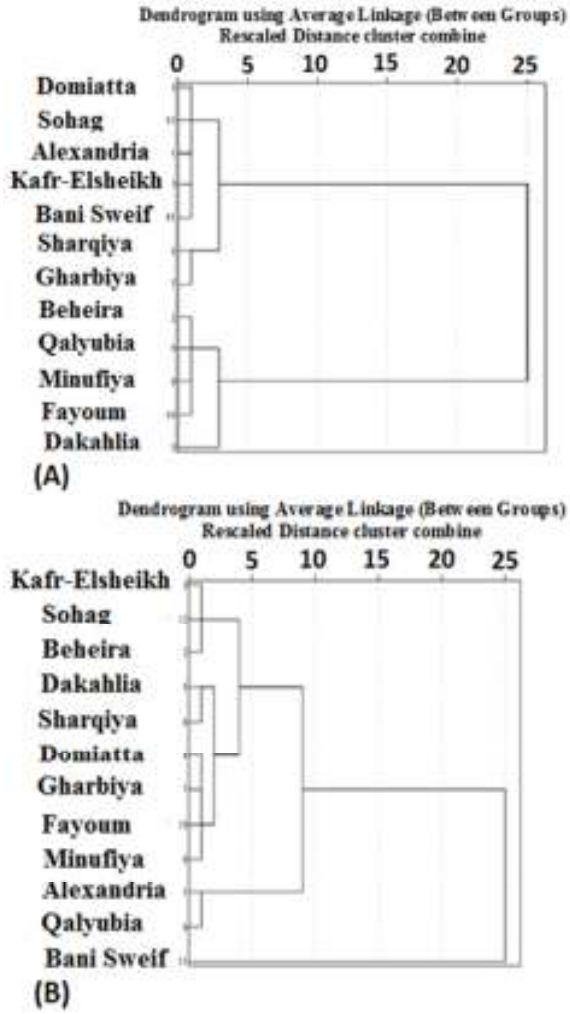

Fig. 1. Dendrogram of similarity and distribution of *P. triticina* races in 12 Egyptian governorates during 2016/2017 (A) and 2017/18 (B) growing seasons.

Virulence frequency (%) of the tested pathotypes:

Out of the tested pathotypes only 17 pathotypes were common in leaf rust populations during 2016/17 and 2017/18 growing seasons, as they found in at least five isolates within populations and showed the most frequency in their populations (Table 4). Race STTTK was the most frequent (9.81% frequency) in 2016/17, but race PTTTT was the most frequent (12.16% frequency) in 2017/18. Virulence frequency (%) of the 17 tested pathotypes ranged from 60% to 100%. Pathotype TTTTT was the most aggressive pathotype, where it found to be virulent to all 20 monogenic lines (*Lr*'s) and showed the highest virulence frequency (100.00%), followed by the five pathotypes PTTTT, TTTKT, NTTTT, PTTST and STTTK which showed virulence frequency reached to 90% and more. While, pathotype TTTBB showed the lowest virulence frequency (60.00%).

Diversity between pathogen populations under study:

Three indexes of diversity, *i.e.* Shannon (H_{SH}), Gleason (H_G) and Simpson (H_S) were mainly used to measure the phenotypic variation between different populations under study. These indexes were calculated for the 12 populations during the two growing seasons, *i.e.* 2016/17 and 2017/18 (Table 5 and Fig. 2).

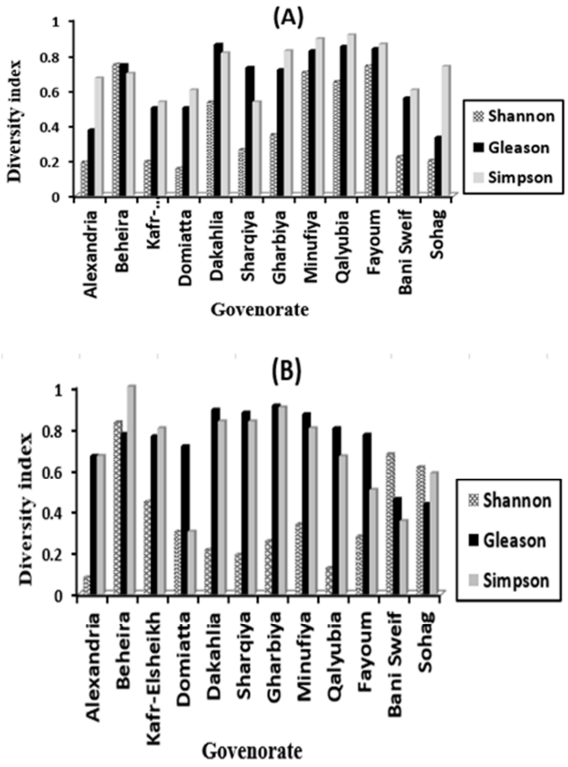

Fig. 2. The Shannon, Gleason and Simpson indexes of phenotypic diversity for 12 populations of *Puccinia triticina* in Egypt during (A) 2016/17 and (B) 2017/18 growing seasons.

In 2016/17 growing season, the obtained values of Shannon index ranged from 0.154 to 0.745, while, Gleason index values found to be ranged from 0.333 to 0.857. Also the Simpson index values ranged from 0.533 to 0.910. The highest phenotypic diversity of Shannon index was observed in Beheira, Fayoum, Minufiya and Qalyubia populations which showed 0.745, 0.736, 0.701 and 0.647 values, respectively. While, the lowest values of this index were found in Domiatta, Alexandria, Kafr El-Sheikh and Sohag populations, *i.e.* 0.154, 0.188, 0.193 and 0.199, respectively (Table 5 and Fig. 2).

The Gleason index values of Dakahlia, Qalyubia, Fayoum Minufiya populations were high, as it was 0.857, 0.846, 0.833 and 0.821 for these four populations, respectively. While, the two pathogen populations of Sohag and Alexandria governorates showed the lowest values of Gleason index, *i.e.* 0.333 and 0.375, respectively. On the other hand, leaf rust populations of the five governorates, *i.e.* Qalyubia, Minufiya, Fayoum, Gharbiya Dakahlia

showed in general the highest values of Simpson index (more than 0.80), as they were 0.910, 0.889, 0.859, 0.821 and 0.809 in the four pathogen populations, respectively. While, the two pathogen populations in Kafr-Elsheikh and Sharqiya governorates showed the lowest values of Simpson index (each with only 0.533) (Table 5 and Fig. 2).

In 2017/18 growing season, Shannon index values ranged from 0.081 to 0.828, while Gleason index values ranged from 0.432 to 0.909. Also, the Simpson index values ranged from 0.300 to 0.999. The highest phenotypic diversity values of Shannon index were found in Beheira which showed 0.828. While, the lowest Shannon index values were found in Alexandria and Qalyubia, *i.e.* 0.081 and 0.125, respectively. Gleason index values in most populations, *i.e.* Gharbiya, Dakahlia, Sharqiya, Minufiya, Qalyubia, Beheira, Fayoum, Kafr-Elsheikh, Domiatta and Alexandria were high as they showed 0.909, 0.889, 0.875, 0.867, 0.800, 0.773, 0.769, 0.762, 0.714 and 0.667 values, respectively. While, the two pathogen populations in Sohag and Bani Sweif showed the lowest values, *i.e.* 0.432 and 0.456, respectively. Simpson index values were high in Beheira, Gharbiya, Dakahlia, Sharqiya, Kafr-Elsheikh and Minufiya, as they showed 0.999, 0.900, 0.833, 0.833, 0.800 and 0.800 values, respectively. While, the two pathogen populations in the two governorates Domiatta and Bani Sweif showed the lowest values, *i.e.* 0.300 and 0.351, respectively (Table 5 and Fig. 2).

Table 4. Virulence formula, number of isolates, frequency within population (%) and virulence frequency (%) of the most common *Puccinia triticina* pathotypes in Egypt during 2016/17 and 2017/18 growing seasons.

No.	[a]Pathotype	Growing season / Virulence formula, number of isolates, frequency (%) & virulence frequency (%) of pathotypes					No. of ineffective genes	[b]Virulence frequency (%)
		Virulence formula (ineffective genes)	2016/17		2017/18			
			No. of isolates	Frequency (%)	No. of isolates	Frequency (%)		
1	FTSSS	*Lr*2c, 3a, 3ka, 9, 10, 11, 14b, 15, 16, 17, 18, 21, 24, 26, 36	5	2.34	-	-	15	75.00
2	NTTJT	*Lr*1, 2c, 3ka, 9, 11, 14b, 15, 16, 17, 18, 21, 24, 26, 30, 36, 42	-	-	10	3.92	16	80.00
3	NTTKT	*Lr*1, 2b, 2c, 3ka, 9, 11, 14b, 15, 16, 17, 18, 21, 24, 26, 30, 36, 42	-	-	8	3.14	17	85.00
4	NTTST	*Lr*1, 2c, 3ka, 9, 10, 11, 14b, 15, 16, 17, 18, 21, 24, 26, 30, 36, 42	-	-	7	2.75	17	85.00
5	NTTTT	*Lr*1, 2b, 2c, 3ka, 9, 10, 11, 14b, 15, 16, 17, 18, 21, 24, 26, 30, 36, 42	-	-	9	3.53	18	90.00
6	PKTPT	*Lr*1, 2b, 2c, 3a, 3ka, 10, 11, 14b, 15, 16, 17, 21, 24, 26, 30, 36, 42	5	2.34	-	-	17	85.00
7	PTTCT	*Lr*1, 2b, 2c, 3a, 3ka, 9, 11, 14b, 15, 16, 17, 24, 26, 30, 36, 42	-	-	11	4.31	16	80.00
8	PTTGS	*Lr*1, 2c, 3a, 3ka, 9, 11, 14b, 15, 16, 17, 18, 24, 26, 30, 36	-	-	13	5.10	15	75.00
9	PTTJT	*Lr*1, 2c, 3a, 3ka, 9, 11, 14b, 15, 16, 17, 18, 21, 24, 26, 30, 36, 42	-	-	6	2.35	17	85.00
10	PTTNS	*Lr*1, 2c, 3a, 3ka, 9, 10, 11, 14b, 15, 16, 17, 21, 24, 26, 30, 36	6	2.8	-	-	16	80.00
11	PTTST	*Lr*1, 2c, 3a, 3ka, 9, 10, 11, 14b, 15, 16, 17, 18, 21, 24, 26, 30, 36, 42	-	-	5	1.96	18	90.00
12	PTTTT	*Lr*1, 2b, 2c, 3a, 3ka, 9, 10, 11, 14b, 15, 16, 17, 18, 21, 24, 26, 30, 36, 42	-	-	31	12.16	19	95.00
13	STTTK	*Lr*1, 2a, 2b, 2c, 3ka, 9, 10, 11, 15, 16, 17, 18, 21, 24, 26, 30, 36, 42	21	9.81	-	-	18	90.00
14	TTTBB	*Lr*1, 2a, 2c, 3a, 3ka, 9, 11, 16, 17, 24, 26, 30	-	-	11	4.31	12	60.00
15	TTTBT	*Lr*1, 2a, 2c, 3a, 3ka, 9, 11, 14b, 15, 16, 17, 24, 26, 30, 36, 42	-	-	15	5.88	16	80.00
16	TTTKT	*Lr*1, 2a, 2b, 2c, 3a, 3ka, 9, 11, 14b, 15, 16, 17, 18, 21, 24, 26, 30, 36, 42	-	-	7	2.75	19	95.00
17	TTTTT	*Lr*1, 2a, 2b, 2c, 3a, 3ka, 9, 10, 11, 14b, 15, 16, 17, 18, 21, 24, 26, 30, 36, 42	-	-	13	5.10	20	100.00
Others (less than 5 isolates)			176	82.63	109	42.74	-	-
Total			213	100	255	100	-	-

[a]Pathotypes that did not found of at least 5 isolates within population were excluded from the Table.

[b]Virulence frequency (%) calculated by divided the number of ineffective genes for each pathotype to the total number of differential genes (20 genes) X 100.

Table 5. Phenotypic diversity of wheat leaf rust races in collections of 12 Egyptian governorates during 2016/17 and 2017/18 growing seasons.

Governorate	Season / Diversity index					
	2016/17			2017/18		
	Shannon	Gleason	Simpson	Shannon	Gleason	Simpson
Alexandria	0.188	0.375	0.667	0.081	0.667	0.667
Beheira	0.745	0.742	0.694	0.828	0.773	0.999
Kafr-Elsheikh	0.193	0.500	0.533	0.443	0.762	0.800
Domiatta	0.154	0.500	0.600	0.299	0.714	0.300
Dakahlia	0.532	0.857	0.809	0.212	0.889	0.833
Sharqiya	0.264	0.727	0.533	0.190	0.875	0.833
Gharbiya	0.348	0.714	0.821	0.255	0.909	0.900
Minufiya	0.701	0.821	0.889	0.335	0.867	0.800
Qalyubia	0.647	0.846	0.910	0.125	0.800	0.666
Fayoum	0.736	0.833	0.859	0.277	0.769	0.500
Bani Sweif	0.220	0.555	0.600	0.676	0.456	0.351
Sohag	0.199	0.333	0.733	0.613	0.432	0.584

Data in Table (6) revealed the correlations of the four components of diversity i.e. sample size collected for each region, number of isolates, number of races and standard deviation of race frequency to the three diversity indexes, *i.e.* Shannon, Gleason and Simpson over the two growing seasons of the study. Significant correlation of Shannon index was found between each of the sample size collected for each region, number of isolates, number of races and standard deviation of race frequency, indicating that this index was sensitive to sample size (r = 0.892), number of isolates (r = 0.959), number of races (r = 0.927) and standard deviation of race frequency (r = 0.973). While, no correlations were present between the four components of diversity to the other two diversity indexes, *i.e.* Gleason and Simpson. So, It can concluded that the Shannon index was the most important to calculate phenotypic diversity in *Puccinia triticina* populations under Egyptian conditions.

Table 6. Correlation between independent components of diversity and three indexes of diversity from 12 populations of *Puccinia triticina* in Egypt during 2016/17 and 2017/18 growing seasons.

Diversity index	Components of diversity			
	Sample size	No. of isolates	Race number	Standard deviation of race frequency
Shannon	0.892*	0.959*	0.927*	0.973*
Gleason	-0.213^{ns}	-0.069^{ns}	0.338^{ns}	-0.104^{ns}
Simpson	0.102^{ns}	0.061^{ns}	0.289^{ns}	0.114^{ns}

*** Coefficients are significant at 0.05. ns = Non-significant.**

DISUSSION

Leaf rust is the most widespread foliar disease, as it occurs annually in most of the commercial wheat varieties cultivated in all of the Egyptian governorates. Yield losses due to such disease may be reached to approximately 50 % on the highly susceptible wheat cultivars (Abdel-Hak *et al*. 1980; German *et al*., 2007 and El-Orabey *et al*., 2017). Host-genetic resistance or growing resistant cultivars is still the economical, environmentally safe, and the most effective method to control leaf rust of wheat. The failure of genetic resistance in some wheat cultivars after it's release and wide cultivation in the commercial fields was mainly due to the dynamic nature and the high evolutionary potential in the populations of leaf rust pathogen (McVey *et al*., 2004).

The dynamic nature of such pathogen led to continuous producing and emergency of new virulent races, that can rapidly increase in their frequency and able to overcome resistance genes newly deployed in the released wheat cultivars. Thus, resistance of these newly released cultivars subjected to a rapid loss in it's efficacy and these cultivars become susceptible in a short duration due to the sudden emergency of the new and more aggressive races of the causal pathogen. Therefore, it is essential to an early detection of the existence of new races in a pathogen population that able to breakdown resistance genes, before it becomes more prevalent, more frequent and can to be the cause of significant losses. Moreover, the early detection of new races within it's population would be of primary importance for planning and directing a successful breeding strategy for leaf rust resistance (Kolmer, 1996). As it was reported in the previous studies, there is a period of years from the first detection of a pathotype (first appearance) to a significant crop loss that cause during which alternative resistance cultivars can be released or promoted, and recommendation of replacement these resistant cultivars can occur (Kolmer, 1996). Successful breeding program for rust resistance especially wheat leaf rust requires full understanding of the genetic structure of pathogen populations and changes occurring in these populations, *i.e.* virulent races present and their frequencies of virulence and occurrence within different geographical areas in the country (Kolmer, 2005).

In the present study, leaf rust pathotype STTTK was the most common pathotype in it's population with frequency of 9.81%, during 2016/17 growing season. Meanwhile, pathotype PTTTT was the most dominant pathotype represented by 12.16% frequency in 2017/18 growing season. Similar results were previously obtained by Negm *et al*. (2013) who reported that the two leaf rust pathotypes PTTT and TTTT were the most frequent in Egypt during the two growing seasons 2009/10 and 2010/11. These two races were comprised by 12.21 and 15.84 % frequency, respectively in 2009/10 and 10.90 and 12.80 % frequency, respectively in 2010/11. Moreover, McVey *et al*. (2004) found that leaf rust race MCDLQ was the most common race in Egypt from 1997/1998 through 1999/2000. Also, races MCDLL and TCDML were found in Israel. While, races BBBLL, MBBLL and MBDLQ were found in Turkey as well as in Egypt, but the frequencies of these races in Egypt never exceeded up to1.2%. Also, race MBBLL was found in Sudan in 1998. In this study they concluded that, the common races in Egypt also were found in Romania and Israel, suggesting that windborne urediniospores of leaf rust pathogen move between Egypt and Israel and some of primary inocula may come to Egypt from either Israel or Eastern Europe. Also, El-Orabey *et al*. (2015) found that leaf rust races STTST and TKTTT were the most frequent (each with 2.54% frequency) during 2011/12. While, the three races;

PKTST, TTTT and TTTST were the most common races (6.63, 7.83 and 10.24% frequency, respectively) during 2012/13. Moreover, the two races; FKTTT and PTTTT were the most frequent during 2013/14 with frequency of 4.92 and 11.47%, respectively. In recent study by El-Orabey (2018) who reported that, the leaf rust races KTSPT and CTTTT were the most frequent races during 2015/16 growing season in Egypt, as they showed 30 and 85% frequency, respectively.

The obtained data in the current study also showed in general that, Fayoum governorate has the highest number of the identified leaf rust pathotypes, as it represented 20.80% frequency of the total races in a pathogen population during 2016/17 growing season, followed by Minufiya, Beheira and Qalyubia where they showed 19.20%, 18.20% and 18.14% frequency of the total races in population, respectively. While, the three governorates; Alexandria, Domiatta and Sohag showed the lowest number of the identified pathotype (each with 3.20%). Meanwhile, in the second growing season of the study (2017/18), Bani Swif showed the highest number of identified pathotypes, as it represented by 26.73%, followed by the three governorates, *i.e.* Beheira, Kafr Elsheikh and Sohag which showed 18.83% frequency for each. On the other hand, the lowest numbers of identified pathotypes were found in Alexandria (3.97%) and Qalyubia (4.95%). On the other hand, pathotype STTTK is the most common and widespread race in most locations, nationwide, as it was found in nine governorates in 2016/17. While, in 2017/18, PTTTT is the most geographically distributed pathotype, which was recorded in seven governorates. Similar results were previously obtained by El-Orabey (2018) who found that, five pathotypes were detected in Minufiya, while, four pathotypes were detected in Qalubiya and Fayom, but only three pathotypes were detected in Beheira. The most common and widespread pathotype in all locations under study was KTSPT, followed by NPTNK (only found in two locations, *i.e.* Beheira and Fayoum) and TTTMS (in Minufiya and Fayoum) during 2016/17 growing season.

The diversity observed in leaf rust populations within different locations during the two growing seasons of the study should depends to large extent, on the source of primary inocula that usually come each year by wind, from the external sources, due to the long-distance dispersal (LDD) of pathogen populations (Kolmer, 2005) and to the obvious shifts in the genetic structure of the recipient pathogen population, especially when such pathogen cannot persist or survive the summer in the country (Burdon and Silk, 1997). In addition, previous and early reports in Egypt supported the conclusion that, wheat leaf rust urediniospores do not persist or cannot survive the summer in Egypt (Abdel-Hak *et al*., 1974; Nazim *et al*., 1976; McVey *et al*., 2004 and Negm *et al*., 2013). Where the summer temperatures are very high and preclude a survival of such spores. Furthermore field observations in Egypt supported by early reports of Melcher, (1932) and Abdel-Hak *et al*. (1974) revealed that alternative hosts of wheat leaf rust, volunteer plants and stubs do not found in the country. As well as, the obtained data in this study support the conclusion that the primary inoculum of leaf rust pathogen usually arrives in Egypt from an external source each year. Nevertheless, data from the current study do not provide a conclusive evidence to positively or definitely identify the external sources of initial inoculum that arrives annually to Egypt. However, further studies are needed to emphasize and support this debatable issue.

Diversity indexes a quantitative measure that reflects how many different types (such pathogen races) found in a community or in population and simultaneously takes into account how pathotype are distributed among those types (Andrivon and de Vallavieille-Pope, 1995). The diversity of any pathogen population is one of it's important characteristics. According to Groth and Roelfs (1987) an optimal diversity index should satisfy several conditions. A pathogen population is more diverse (higher diversity index values) if it consists of a larger number of pathotypes (races). Also, when it is characterized by an even distribution of pathotypes, the low diversity alternative being the case where a small number of pathotypes dominants in the population, all other races being rare. Finally, the number of differences in virulence between pathotypes is larger (Groth and Roelfs, 1987).

In this study, three diversity indexes, *i.e.* Shannon, Simpson and Gleason were firstly used to estimate phenotypic diversity of *Puccinia triticina* populations in 12 different locations in Egypt. In general, Shannon index values are low and differed from the other two indexes, *i.e.* Simpson and Gleason. This mainly due to the low number of isolates in most populations and low values of frequency (%) of most pathotypes in their populations.

Gleason and Simpson indexes showed in general high values, this means that high diversity was found for the studied populations in most of the tested locations during the two growing seasons. The high diversity in different leaf rust populations under study might be explained by the long-term and wide cultivation of the commercial Egyptian wheat cultivars that have a high leaf rust resistance. Therefore, this make a high selection pressure levels on *P. triticina* pathotypes. This issue was previously supported by the findings of Singh *et al*. (2004) as they reported that the use of the high resistant cultivars places a high selection pressure on *P. triticina* populations for new virulent pathotypes. Moreover, common wheat cultivars with race-specific resistance genes lead to produce new races of *P. triticina* in an average of 3 years. Also, diversity may be due other evolutionary force, *i.e.* long distance dispersal or migration of leaf rust spores by wind-dispersal from different external sources

Meanwhile, the highest diversity values in Egyptian *P. triticina* pathotypes identified annually may be due to another reason, such as the differential sets used for nomenclature of *P. triticina* pathotypes in Egypt had to be identical for the entire period and detect most of the virulence diversity of *P. triticina* in Egypt. The first three subsets of the nomenclature of *P. triticina* pathotypes are the same all over the world (Kolmer, 1991) and the last two subsets are changed according the efficacy of the leaf rust monogenic lines in each country. So, the two leaf rust monogenic lines in the last sets, *i.e.* set four containing *Lr*10, *Lr*18, *Lr*21 and *Lr*2b and set five containing *Lr*14b, *Lr*15, *Lr*36 and *Lr*42 should be changed and adapted according to the efficacy of leaf rust resistance genes under Egyptian conditions. Moreover, the leaf rust resistance genes *Lr*11 in set 3 and *Lr*18 in set 4 that used in the nomenclature of leaf rust pathotypes are sensitive to high temperature and must be tested below 18 ^{O}C (Dyck and Johnson, 1983).

Ultimately, the three diversity indexes used in this study are little else in common and represent real choices in how best to characterize diversity of pathogen populations. Thus, choice the suitable index must depend on the objectives of the study and the properties of the population(s) of the target pathogen to be characterized or compared with others. However, if populations of plant pathogen are being characterized the kind of diversity indexes used will mainly depend upon the number of pathotypes, degree of dominance and size of samples (Groth and Roelfs, 1987). The Gleason index is the most sensitive to the number of pathotypes, in addition, it is usually used if frequency data are not to be included. So, the use and applied of more than one index to describe and

estimate the phenotypic diversity of pathogen populations is desirable, if several of the meanings of diversity are of interest. In particular, either the Simpson or Shannon index might be calculated for each of the two populations to assess absolute magnitude of diversity (Groth and Roelfs, 1987).

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التنوع وديناميكية القدرة المرضية داخل عشائر الفطر *Puccinia triticina* في مصر

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يعتبر مرض صدأ الأوراق المتسبب عن *الفطر Puccinia triticina* من أكثر الأمراض إنتشاراً علي القمح في مصر والعالم. أجري في هذة الدراسة تحليل 468 عزلة ممرضة من الفطر المسبب تم الحصول عليها من 193 عينه نباتية مصابة بالمرض وذلك من 12 محافظة مصرية ، لتحديد التركيب الجيني لعشائر الفطر المسبب. حيث تم تعريف 125 ، 101 سلالة من سلالات فطر صدأ الأوراق في القمح خلال موسمي الزراعة 2017/2016 و 2018/2017. وقد كانت أكثر السلالات تكرارية داخل عشيرة الفطر هي السلالة STTK (9.81%) يليها السلالة PTTNS (2.80%) وذلك في موسم 2017/2016. بينما كانت السلالة PTTTT هي أكثر السلالات تكرارية (12.16%) يليها السلالات TTTBT ، PTTGS ، TTTTT ، PTTCT بنسبة (5.88 ، 5.10 ، 5.10 ، 4.31%) بالترتيب وذلك خلال الموسم الزراعي 2018/2017. ومن ناحية أخري فقد أظهرت النتائج المتحصل عليها بتلك الدراسة أن السلالة STTK كانت أكثر السلالات إنتشاراً ، حيث تم تعريفها في تسع محافظات في موسم 2017/2016 ، بينما كانت السلالة PTTTT أكثر السلالات إنتشاراً في سبع محافظات في موسم 2018/2017. وبمقارنة عشائر الفطر المختلفة داخل مصر ، وجد أن أعلي تشابة في تلك العشائر كان بين المحافظات الخمس التالية: دمياط ، سوهاج ، الإسكندرية ، كفر الشيخ ، بني سويف في موسم 2017/2016. بينما في موسم 2018/2017 فقد وجد أن أعلي تشابة في عشائر الفطر المسبب ، كان بين محافظات كفر الشيخ ، سوهاج ، البحيرة ، الدقهلية ، الشرقية ، دمياط ، الغربية ، الفيوم ، المنيا. ومن ناحية أخري فقد تم قياس التنوع أو التباين بين عشائر الفطر الممرض وذلك بإستخدام ثلاثة مؤشرات وهي Shannon ، Gleason ، Simpson وقد أوضحت النتائج المتحصل عليها في ذلك المجال أن مؤشر Shannon أكثر تلك المؤشرات ثباتاً ودلالةً علي قياس التنوع في عشائر الفطر المختلفة ، حيث إتضح أن هذا المؤشر كان أكثر تلك المؤشرات حساسية لحجم العينة وعدد العزلات وعدد السلالات والإنحراف القياسي للسلالات.