OPEN ACCESS Int. J. Phytopathol. 08 (03) 2019. 111-122 DOI: 10.33687/phytopath.008.03.3087

Available Online at EScience Press
International Journal of Phytopathology
ISSN: 2312-9344 (Online), 2313-1241 (Print)
https://esciencepress.net/journals/phytopath

VIRULENCE AND MOLECULAR POLYMORPHISM OF *PUCCINIA TRITICINA* PATHOTYPES IN EGYPT

[a]Walid M. El-Orabey*, [b]Aladdin Hamwieh, [a]Mohamed A. Gad, [b,c]Shaimaa M. Ahmed
[a] *Wheat Diseases Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt.*
[b] *International Centre for Agricultural Research in the Dry Areas (ICARDA), Giza, Egypt*
[c] *Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt.*

**Corresponding Author Email: walid_elorabey2014@hotmail.com*

A B S T R A C T

Forty wheat leaf rust (*Puccinia triticina*) pathotypes were collected from eleven Egyptian governorates during the two growing seasons 2016/2017 and 2017/2018 were analyzed based on both virulence and molecular marker analysis. Virulence analysis was carried out based on infection type of the tested pathotypes on 20 differential monogenic lines, each carrying single leaf rust resistance genes (*Lr*). Six simple sequence repeats (SSR) markers were used for molecular characterization of *P. triticina* to detect the genotypic variation among pathotypes. Almost all of the tested pathotypes were phenotypically and genetically varied that confirms a high diversity within Egyptian leaf rust populations. Cluster analysis based on both virulence analysis and molecular patterns classified the tested pathotypes into three main groups. A relatively weak correlation was found between virulence and molecular analysis (r = 0.03). High similarity was found between leaf rust populations in the three governorates; Sohag, Bani Swear, and Fayoum. Also, the high similarity was found between leaf rust populations in the five; Egyptian governorates; Minufiya, Kafr-Elsheikh, Gharbiya, Alexandria, and Qalyubia, while, wide variation was found between leaf rust populations of the three governorates; Beheira, Sharqiya and Dakahlia. The results of this study support using molecular markers analysis to estimate genetic diversity between *P. triticina* pathotypes.

Keywords: *Puccinia triticina*, virulence, molecular markers, genetic diversity, leaf rust resistance genes.

INTRODUCTION

Leaf rust caused by *P. triticina* is the most common and widespread rust disease as it was annually found everywhere wheat (*Triticum aestivum* L.) is grown in Egypt and worldwide. Severe yield losses in the susceptible wheat cultivars due to leaf rust infection may be reached to approximately 50% under favorable environmental conditions for disease incidence and development (Germán *et al.*, 2007). In Egypt, experimentally grain yield losses as a result of artificial leaf rust infection reached up to 32% on the highly susceptible wheat cultivars (Shahin and El-Orabey, 2016; El-Orabey *et al.*, 2017).

reeding for resistance or growing resistant cultivars is still the most effective and preferred control method for minimizing the annual grain yield losses due to rust diseases, especially leaf rust (Kolmer *et al.*, 2012). So, a successful breeding program for leaf rust resistance requires specific information about the evolution and virulence of the new *P. triticina* pathotypes.

Due to the high evolutionary potential of leaf rust populations and the large population sizes that consisted of a large number of pathotypes, it is essential to understand and measure the diversity of *P. triticina* pathotypes within its populations. However, wide variations and high diversity of virulence in *P. triticina* populations in Egypt, mainly due to high selection pressure and migration, are the main two evolutionary forces for the emergence of many new leaf rust pathotypes (McVey *et al.*, 2004). Recently, a total of 226 leaf rust pathotypes were detected during the 2016/17 and 2017/18 growing seasons from 12 Egyptian governorates (El-Orabey *et al.*, 2018).

Diversity characteristics and analysis of the genetic structure of pathogen populations for rust fungi, in general, are very important to make a proper and

correct decision for the planning and direction of the successful breeding programs for rust resistance especially leaf rust. However, knowledge on the diversity of leaf rust populations in Egypt is very limited; thus, little information is available about the diversity of *P. triticina* pathotypes occurring within its pathogen population in the country. Also, few studies were conducted on the molecular characterization of *P. triticina* pathotypes in Egypt. Out of these studies, El-Orabey *et al.* (2018) studied phenotypic diversity on *P. triticina* populations in Egypt using the three main and widely used indexes i.e. Shannon, Gleason, and Simpson. This study revealed that, there was a high diversity among leaf rust populations in Egypt due to the selection pressure and migration.

Molecular marker analysis has been used to assess and measure genetic variations among *P. triticina* populations using simple sequence repeat (SSR) markers (Szabo and Kolmer, 2007). Several different molecular markers were used as the most effective method to estimate the genetic variation in *P. triticina* populations which provide direct information concerning the effects of host selection in the potential effectiveness of leaf rust resistance genes (Kolmer, 1999).

The main objectives of this study were to determine the polymorphism and diversity among 40 selected leaf rust pathotypes by using virulence analysis as well as DNA markers; simple sequence repeat marker (SSR). Also, to study the relationship among the tested pathotypes using virulence analysis and molecular marker.

MATERIALS AND METHODS

***P. triticina* pathotypes:** Forty pathotypes of *P. triticina* were selected from the Egyptian leaf rust populations during the 2016/17 and 2017/18 growing seasons (El-Orabey *et al.*, 2018). Some of these chosen pathotypes were dominant in its population, as they represented by more than one isolate, and the other was less frequent and represented by only one isolate during these two growing seasons.

Determination of virulence phenotypes: Urediniospores of each of the forty selected leaf rust pathotypes were used to inoculate seven-day-old seedlings of the highly susceptible wheat variety; Morocco as described by Kolmer *et al.* (2009) to multiply and increase the urediniospores that used for virulence analysis and DNA isolation. To detect virulence phenotypes of leaf rust pathotypes under study, five sets of four Thatcher monogenic lines of wheat leaf rust; set 1 (*Lr* 1, *Lr* 2a, *Lr* 2c, and *Lr* 3a); set 2 (*Lr* 9, *Lr* 16, *Lr* 24 and *Lr* 26); set 3 (*Lr* 3ka, *Lr* 11, *Lr* 17, and *Lr* 30); set 4 (*Lr* 10, *Lr* 18, *Lr* 21, and *Lr* 2b); set 5 (*Lr* 14b, *Lr* 15, *Lr* 36, and *Lr* 42) were used (Long and Kolmer, 1989; McVey *et al.*, 2004). The inoculation and disease assessment were done according to Long and Kolmer (1989) and Kolmer *et al.* (2009). Virulence phenotypes were determined after 10 - 12 days of inoculation for each pathotype on the 20 differentials monogenic lines using the 0-4 scale (Long and Kolmer, 1989). Pathotypes with infection types (IT·s) 0, 0;, 1 and 2 were classified as avirulent pathotypes, while those with infection types 3 and 4 were classified as virulent pathotypes. Each pathotype was given a five-letter code based on virulence or avirulence to the 20 differential monogenic lines (Long and Kolmer, 1989; McVey *et al.*, 2004).

Molecular marker characterization of *P. triticina* pathotypes: DNA isolation method was carried out according to the procedure of Niazmand *et al.* (2013) from the urediniospores of each of the 40 pathotypes under study using 20 mg of the stored urediniospores. Cell walls of the spores were crushed using 20 mg autoclaved carborundum powder by adding the carborundum powder to the urediniospores for each of the tested pathotypes to 1.5 ml micro-centrifuge tubes. The tubes were placed into a medium-sized mortar and liquid nitrogen was added. Frozen urediniospores were ground using plastic mini-pestles mounted in an electric drill under a low speed for 20 sec. After grinding, 500 µl of extraction buffer (200 m mol Tris-HCl (pH 7.5), 250 m mol NaCl, 25 m mol EDTA, 0.5% SDS) was added to the cracked spores, which were then homogenized for 5 min at maximum speed using Vortex-Genie 2. Then, 350 µl phenol was added and the tubes inverted gently four times. Subsequently, 250 µl chloroform was added and the tubes inverted gently 40 times. The tubes were centrifuged at 4 °C for 30 min. After centrifugation, the aqueous supernatants were decanted into new tubes. The samples were treated with 2 µl RNase (20 µg ml TE) and incubated at 37 °C for 10 min. An equal volume of chloroform was added and mixed gently. The tubes were centrifuged at 4 °C for 10 min. The aqueous layer was transferred to a new Eppendorf tube, to which a 0.54 volume of cold isopropanol was added for precipitation of the DNA. The tubes were centrifuged at 4°C for 5 min. The supernatants were poured into a sink gently and 100 µl cold 70% ethanol was added to pellets, which were

then centrifuged at 4 °C for 5 min; the ethanol was then removed from tubes. Each pellet was dried in an incubator at 37 °C for 30 min and dissolved in (50 µl) sterile double-deionized water. The subsequent DNA yields and quality were assessed by standard electrophoresis through a 1% (w/v) ethidium bromide-stained agarose gel. DNA for each of the tested pathotype was amplified according to the protocol of Niazmand *et al.* (2013) using five SSR primers (Table 1). Amplification products were separated on 7 % polyacrylamide gel.

Table 1. Sequences of six SSR primers used for molecular analysis of 40 *P. triticina* pathotypes.

Marker	Primer sequence (5`-3`)	No. of allele	No. of polymorphic allele	Product (bp)
PtSSR68	F: GACTCAGCCCACTGCTAA R: GATGGCGACGTATTTGGTCT	5	5	362, 360, 356, 347, 337
PtSSR154	F: ACGGTCAACAGCCAACTACC R: CCTCGTCATCCTGGTTGAGT	5	5	287, 281, 279, 276, 260
PtSSR164	F: GTGGAAGTGAGCGGAAGAAG R: GGAGATGGGCAGATGAGGTA	3	2	218, 220
PtSSR173	F: CTCAGCGACCTCAAAGAACC R: GAGACGACGGATGTCAACAA	4	4	220, 217, 212, 210
RB10	F: AAGATTGGTGGTATGTGGTGGA R: TTGTCTTTCATCTCATCCAGCC	1	1	218
RB29	F: CTCACCAAACATCAAGCACC R: GAGCCTAGCATCAGCATC	9	2	118-129

Data analysis: The infection type data (0-4, scale) were converted into a binary code of 0 for avirulence and 1 for virulence of the tested pathotypes on the differential monogenic lines. Moreover, a binary data matrix was generated for all SSR markers based on the presence (1) or absence (0) of amplification products. A matrix cluster of both virulence and molecular data were derived with GenAlex 6 (Peakall and Smouse, 2006). Correlation between the SSR marker data and virulence data was determined by a comparison of the two similarity matrices using the MXCOMP, program of NTSYS-pc software.

RESULTS

Geographical distribution of the tested leaf rust pathotypes: A total of eight pathotypes were detected in Beheira population, which showed 20.00% of the whole population, followed by Fayoum, which has six pathotypes and showed 15.00% of the entire population. While, the five governorates; Alexandria, Kafr-Elsheikh, Gharbiya, Sharqiya and Sohag showed the lowest number of pathotypes i.e. 1 (2.50), 2 (5.00 %), 2 (5.00 %), 2 (5.00 %) and 2 (5.00 %), respectively. The other tested governorates showed a moderate number of pathotypes; ranged from 3 (7.50 %) to 5 (12.50 %) (Table 2).

Frequency (%) of the tested leaf rust pathotypes: The two leaf rust pathotypes; STTTK and TTTTT were the most common with relatively high frequency, as they showed 9.81% and 5.10% frequency within the pathogen population during the two growing seasons of the study. While, twenty of the tested pathotypes showed the lowest frequency (%), i.e. 0.47%, each represented by only one isolate in leaf rust population. The frequency (%) of the other pathotypes ranged from 0.93% to 2.80% frequency (Table 2).

Virulence analysis: A total of 40 leaf rust pathotypes were collected during the 2016/17 and 2017/18 growing seasons survey in Egypt. Virulence analysis of the tested pathotypes was conducted based on the reaction of each pathotype to 20 differentials leaf rust monogenic lines. Out of the forty tested pathotypes, only pathotype TTTTT was the most aggressive, as it proved to be virulent to all 20 wheat leaf rust monogenic lines; *Lr* 1, *Lr* 2a, *Lr* 2b, *Lr* 2c, *Lr* 3a, *Lr* 3ka, *Lr* 9, *Lr* 10, *Lr* 11, *Lr* 14b, *Lr* 15, *Lr* 16, *Lr* 17, *Lr* 18, *Lr* 21, *Lr* 24, *Lr* 26, *Lr* 30, *Lr* 36 and *Lr* 42, while, pathotype GBHLD was virulent to only five monogenic lines; *Lr* 2a, *Lr* 10, *Lr* 11, *Lr* 30 and *Lr* 36. The other most aggressive pathotype; TTTST was virulent to 19 leaf rust monogenic lines; *Lr* 1, *Lr* 2a, *Lr* 2c, *Lr* 3a, *Lr* 3ka, *Lr* 9, *Lr* 10, *Lr* 11, *Lr* 14b, *Lr* 15, *Lr* 16, *Lr* 17, *Lr* 18, *Lr* 21, *Lr* 24, *Lr* 26, *Lr* 30, *Lr* 36 and *Lr* 42. The other tested pathotypes were virulent to 11-18 leaf rust monogenic lines (Table 3).

Table 2. Geographical distribution and frequency (%) of leaf rust pathotypes identified in 11 Egyptian governorates during the 2016/17 and 2017/18 growing seasons.

No.	Governorate	Identified Pathotype and Frequency (%)	No. of Pathotypes and Percentage (%)
1	Alexandria	STTTK (9.81)	1 (2.50 %)
2	Kafr-Elsheikh	PHTTT (1.87), PTKTH (0.47)	2 (5.00 %)
3	Beheira	CTTTT (1.40), GBTMT (0.47), NJTPK (0.47), NTTSR (0.47), PKKTT (0.93), PKTPR (0.47), PPTPT (0.47), PRSTT (1.40)	8 (20.00 %)
4	Gharbiya	PSTST (0.93), TTTTT (5.10)	2 (5.00 %)
5	Minufiya	MTTTT (1.18), PKTTF (0.47), PKTTT (0.47), PTTPR (0.47), PTTPS (1.40),	5 (12.50 %)
6	Qalyubia	NTKTS (1.40), PTKTS (0.47), PTTSP (0.47), TTTST (1.87)	4 (10.00 %)
7	Sharqiya	DFTPS (0.47), PTTPQ (0.93),	2 (5.00 %)
8	Dakahlia	DHTTT (0.47), FTTNS (0.47), PJSFT (0.47), PTTSS (1.87), TPTMP (0.47)	5 (12.50 %)
9	Fayoum	KTSPT (0.47), NPTNK (0.93), PKTST (1.87), PTJNP (0.47), PTTNT (0.47), TTTMS (1.40)	6 (15.00 %)
10	Bani Sweif	NRKDS (0.93), PTKGT (0.93), PTSNS (1.40),	3 (7.50 %)
11	Sohag	GBHLD (0.47), PTTNS (2.80)	2 (5.00 %)
Total			40 (100.00 %)

Table 3. Virulence phenotypes of forty *Puccinia triticina* pathotypes isolated from Egypt during 2016/17 and 2017/18 growing seasons and used in this study.

No.	Pathotype[a]	Virulence (*Lr* genes)	No. of ineffective genes
1	CTTTT	*3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 2b, 14b, 15, 36, 42*	17
2	DFTPS	*2c, 24, 26, 3ka, 11, 17, 30, 10, 21, 2b, 14b, 15, 36*	13
3	DHTTT	*2c, 16, 26, 3ka, 11, 17, 30, 10, 18, 21, 2b, 14b, 15, 36, 42*	15
4	FTTNS	*2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 21, 14b, 15, 36*	15
5	GBHLD	*2a, 11, 30, 10, 36*	5
6	GBTMT	*2a, 3ka, 11, 17, 30, 10, 2b, 14b, 15, 36, 42*	11
7	KTSPT	*2a, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 10, 21, 2b, 14b, 15, 36, 42*	17
8	MTTTT	*1, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 2b, 14b, 15, 36, 42*	18
9	NJTPK	*1, 2c, 16, 24, 3ka, 11, 17, 30, 10, 21, 2b, 15, 36, 42*	14
10	NPTNK	*1, 2c, 9, 24, 26, 3ka, 11, 17, 30, 10, 21, 15, 36, 42*	14
11	NRKDS	*1, 2c, 9, 16, 26, 11, 17, 30, 21, 14b, 15, 36*	12
12	NTKTS	*1, 2c, 9, 16, 24, 26, 17, 30, 10, 18, 21, 2b, 14b, 15, 36*	15
13	NTTSR	*1, 2c, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 14b, 15, 42*	16
14	PHTTT	*1, 2c, 3a, 16, 26, 3ka, 11, 17, 30, 10, 18, 21, 2b, 14b, 15, 36, 42*	17
15	PJSFT	*1, 2c, 3a, 16, 24, 3ka, 11, 17, 21, 2b, 14b, 15, 36, 42*	14
16	PKKTT	*1, 2c, 3a, 16, 24, 26, 11, 17, 30, 10, 18, 21, 2b, 14b, 15, 36, 42*	17
17	PKTPR	*1, 2c, 3a, 16, 24, 26, 3ka, 11, 17, 30, 10, 21, 2b, 14b, 15, 42*	16
18	PKTST	*1, 2c, 3a, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 14b, 15, 36, 42*	17
19	PKTTF	*1, 2c, 3a, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 2b, 36, 42*	16
20	PKTTT	*1, 2c, 3a, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 2b, 14b, 15, 36, 42*	18

21	PPTPT	*1, 2c, 3a, 9, 24, 26, 3ka, 11, 17, 30, 10, 21, 2b, 14b, 15, 36, 42*	17
22	PRSTT	*1, 2c, 3a, 9, 16, 26, 3ka, 11, 17, 10, 18, 21, 2b, 14b, 15, 36, 42*	17
23	PSTST	*1, 2c, 3a, 9, 16, 24, 3ka, 11, 17, 30, 10, 18, 21, 14b, 15, 36, 42*	17
24	PTJNP	*1, 2c, 3a, 9, 16, 24, 26, 11, 17, 10, 21, 14b, 36, 42*	14
25	PTKGT	*1, 2c, 3a, 9, 16, 24, 26, 11, 17, 30, 18, 14b, 15, 36, 42*	15
26	PTKTH	*1, 2c, 3a, 9, 16, 24, 26, 11, 17, 30, 10, 18, 21, 2b, 15, 42*	16
27	PTKTS	*1, 2c, 3a, 9, 16, 24, 26, 11, 17, 30, 10, 18, 21, 2b, 14b, 15, 36*	17
28	PTSNS	*1, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17,10, 21, 14b, 15, 36*	15
29	PTTNS	*1, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 21, 14b, 15, 36*	16
30	PTTNT	*1, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 21, 14b, 15, 36, 42*	17
31	PTTPQ	*1, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 21, 2b, 14b, 15*	16
32	PTTPR	*1, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30,10, 21, 2b, 14b, 15, 42*	17
33	PTTPS	*1, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 21, 2b, 14b, 15, 36*	17
34	PTTSP	*1, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 14b, 36, 42*	17
35	PTTSS	*1, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 14b, 15, 36*	17
36	STTTK	*1, 2a, 2c, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 2b, 15, 36, 42*	18
37	TPTMP	*1, 2a, 2c, 3a, 9, 24, 26, 3ka, 11, 17, 30, 10, 2b, 14b, 36, 42*	16
38	TTTMS	*1, 2a, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 2b, 14b, 15, 36*	17
39	TTTST	*1, 2a, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 14b, 15, 36, 42*	19
40	TTTTT	*1, 2a, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30, 10, 18, 21, 2b, 14b, 15, 36, 42*	20

[a] Nomenclature of leaf rust pathotypes according to Long and Kolmer (1989) and McVey *et al*. (2004).

Virulence polymorphism: Based on the virulence/avirulence formula to 20 leaf rust monogenic lines, the tested pathotypes could be divided into three main groups, belonging to three main clusters (Figure 1). The first cluster consisted of only one pathotype; NTTSR which was avirulent to the four leaf rust resistance genes; *Lr* 2a, *Lr* 2b, *Lr* 3a and *Lr* 36. The second cluster also contained only one pathotype; PSTST that was avirulent to the three leaf rust resistance genes; *Lr* 2a, *Lr* 2b and *Lr* 26.

The third cluster consists of 38 pathotypes and contained two sub-clusters. The first sub-cluster contained 28 pathotypes and separated into two sub-sub-clusters. The first sub-sub-cluster contained 25 pathotypes i.e. PTKTS, KTSPT, PJSFT, NJTPK, PRSTT, PPTPT, NPTNK, GBTMT, GBHLD, TPTMP, TTTMS, DFTPS, PTTPQ, PKTPR, PTTPR, PTTPS, MTTTT, CTTTT, PTKTH, PKKTT, PKTTF, PHTTT, DHTTT, PKTTT and PKTST. The second sub-sub-cluster contained only three pathotypes, i.e. TTTTT, TTTST and STTTK, which were the most aggressive pathotypes and were virulent to 20, 19 and 18, respectively of the 20 differential monogenic lines.

The second sub-cluster contained 10 pathotypes and separated into two sub-sub-clusters. The first one sub-sub-cluster contained seven pathotype i.e. PTTNS, FTTNS, PTSNS, PTTSS, PTKGT, NRKDS, and NTKTS, and these pathotypes were avirulent to *Lr* 2a. The second sub-sub-cluster contained only three pathotypes, i.e. PTTSP, PTJNP and PTTNT which were avirulent to the two leaf rust resistance genes *Lr* 2a and *Lr* 2b.

Molecular analysis: Based on the six SSR primers, the genetic similarities between the 40 tested pathotypes and the Jaccard similarity coefficient, cluster analysis was done using software NT sys (Ver. 2.02), three main groups were obtained (Figure 2). Group 1 and group 2 each included four pathotypes, i.e. PKKTT, NTTSR, FTTNS, DHTTT and PTKTS, PTJNP, PRSTT and PPTPT, respectively.

Group 3 included two sub-clusters, sub-cluster 1 included 30 pathotypes i.e. NJTPK, MTTTT, TTTTT, PTKGT, PKTST, PSTST, NRKDS, PTTPR, PTTNT, KTSPT, CTTTT, PTTSS, PTTPQ, PJSFT, PKTPR, TTTMS, PTKTH, GBHLD, TTTST, PKTTT, PTTNS, PKTTF, PTSNS, PHTTT, DFTPS, NTKTS, GBTMT, NPTNK, PTTSP and TPTMP. Sub-cluster 2 included only two pathotypes, i.e. STTTK and PTTPS.

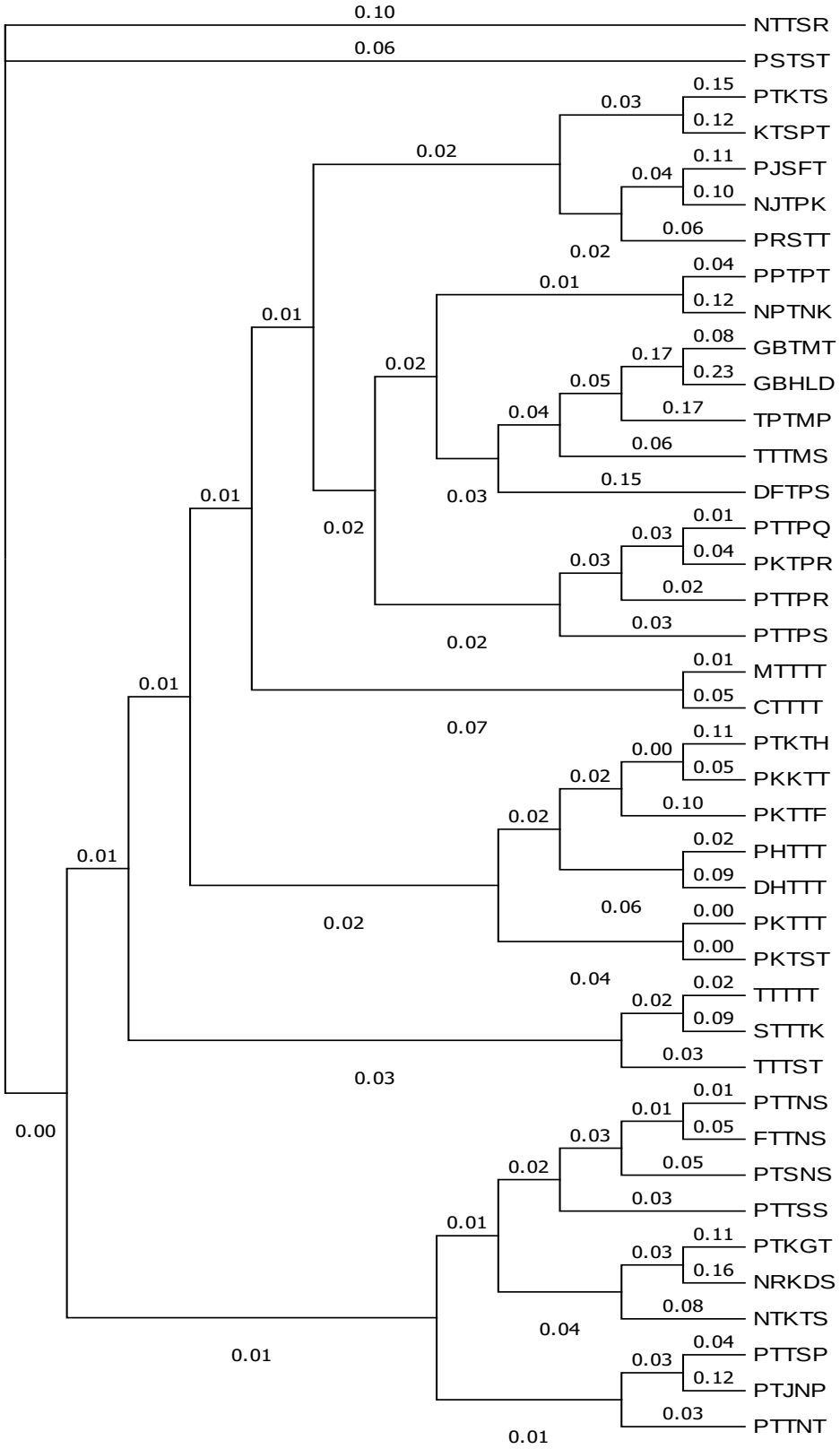


Figure 1. Dendogram of 40 *P. triticina* pathotypes based on virulence to 20 Thatcher isogenic lines with different leaf rust resistance genes of wheat.

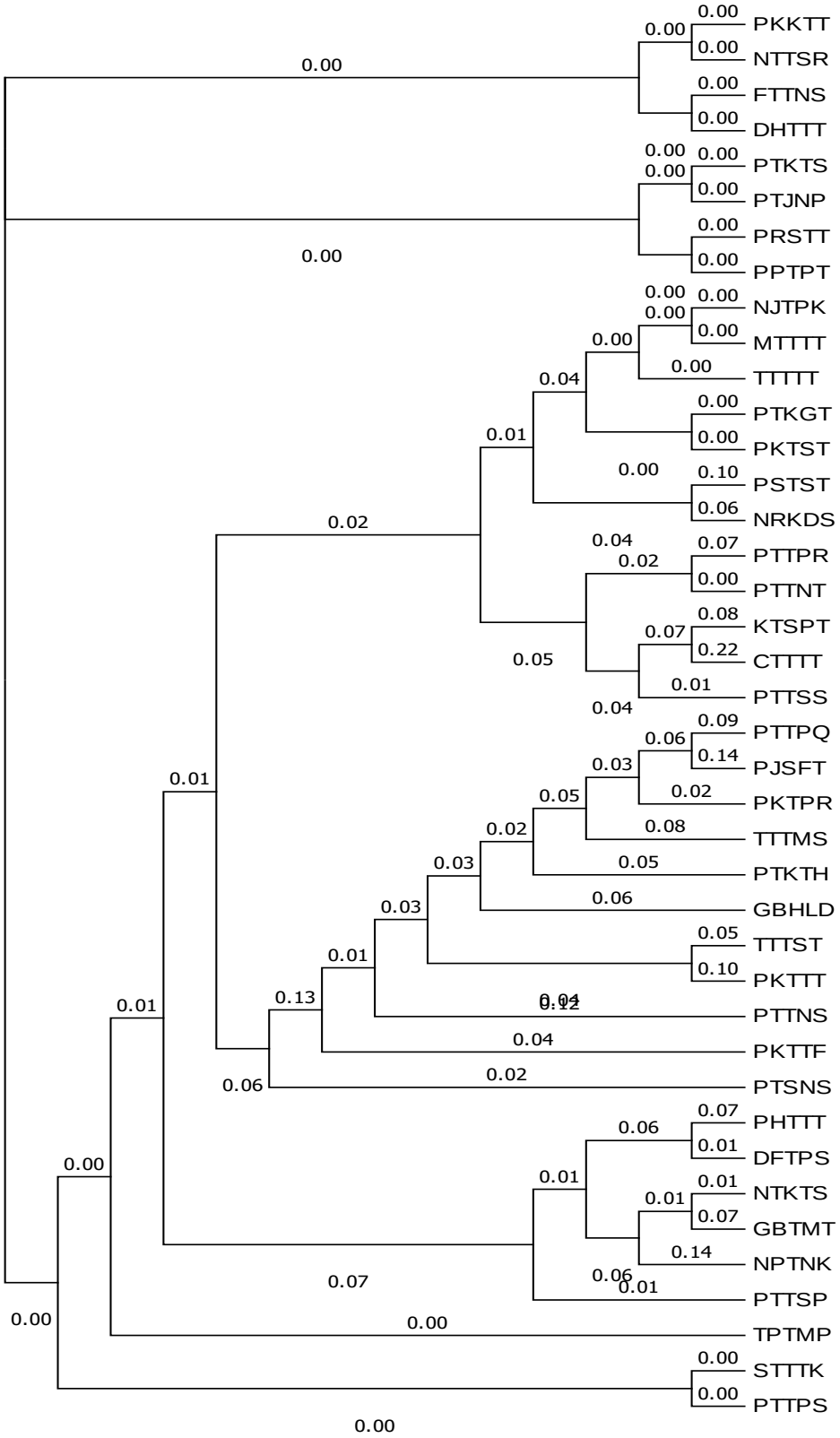
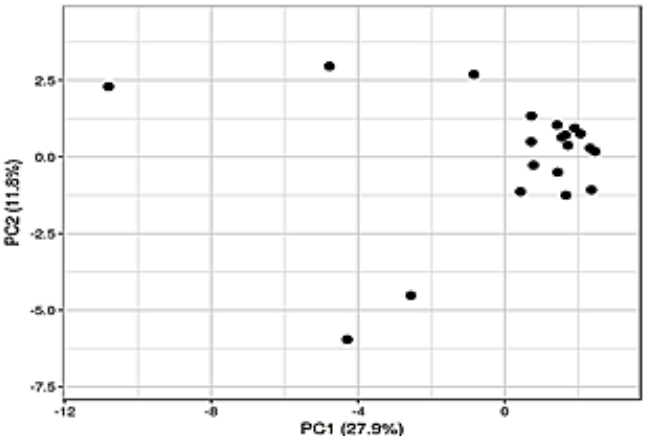


Figure 2. Dendogram of 40 *P. triticina* pathotypes based on simple sequence (SSR) repeat using Nei،s genetic distance between groups collected from 11 Egyptian governorates.

Diversity among the tested leaf rust pathotypes:

Diversity based on virulence analysis: Principal coordinate analysis in Figure 3 grouped the tested leaf rust pathotypes depending on their virulence to 20 leaf rust monogenic lines. The X-axis is principal coordinates (PC1), and Y-axis is the second principal coordinates (PC2) which were accounted for 27.9 and 11.8% of the total variation, respectively in the tested leaf rust pathotypes.

Diversity based on molecular marker pattern: Diversity and genetic distance among the tested populations based on molecular marker pattern data were calculated by Power Marker software (Table 4). Results of molecular analysis of variance showed that the genetic variation among populations is 97% and difference among populations (from the different governorates) is 3% and very high gene flows among populations exist.



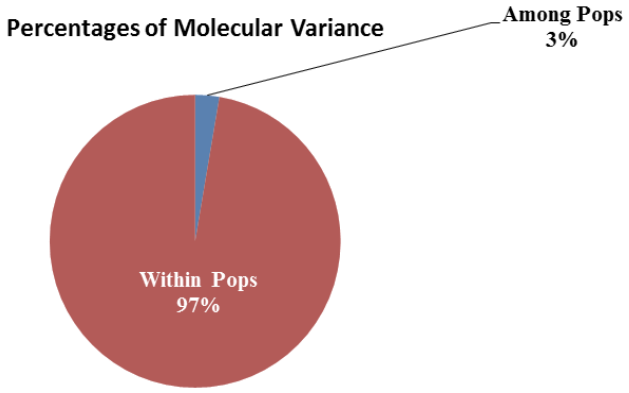


Figure 3. Principal coordinate analysis (PCA) plot of 40 leaf rust pathotypes isolated from Egypt during 2016/17 and 2017/18 growing seasons based on virulence differences to 20 leaf rust differential lines.

Table 4. The calculated values for genetic diversity between and within leaf rust populations.

Source	Df	SS	MS	Est. Var.	%
Among Pops	9	27.641	3.071	0.076	3%
Within Pops	29	80.667	2.782	2.782	97%
Total	38	108.308		2.858	100%

Geographical distribution of the tested pathotypes: The cluster analysis of the distribution of the tested 40 leaf rust pathotypes in the 11 locations were carried out based on similarities and dissimilarities of the tested pathotypes and illustrated in Figure 4. Three main clusters were formed; the first and second clusters contain only one location for each Beheira and Sharqiya, respectively. The second cluster included nine locations and divided into two sub-clusters. The first sub-clusters divided into two sub-sub-clutters. The first sub-sub-clutter consists of three locations i.e. Sohag, Bani Sweif and Fayoum. The second sub-sub-clutter included five locations Minufiya, Kafr-Elsheikh, Gharbiya, Alexandria and Qalyubia. On the other hand, the second sub-cluster contains only one location i.e. Dakahlia.

Correlation between the tested pathotypes based on virulence analysis and molecular characterization: The relationship between virulence analysis data and molecular characterization data to detect variations between 40 *Puccinia triticina* pathotypes was illustrated in Figure 5. The correlation between virulence analysis and molecular characterization data was very low ($R^2 = 0.03$).

DISCUSSION

Leaf rust disease occurred annually under the Egyptian field conditions in the second half of February, while it was recorded in most of the commercial wheat cultivars, nationwide. This disease causes significant yield losses under Egyptian filed conditions (Shahin and El-Orabey, 2016; El-Orabey *et al.*, 2017). Host-genetic resistance or using resistance cultivars for

controlling leaf rust in wheat is the most effective and common control method for reducing yield losses (Kolmer *et al.*, 2012). Breakdown and/or overcome host resistance especially race-specific resistance, found to be mainly due to the emergence and evolution of new leaf rust pathotypes (McVey *et al.*, 2004).

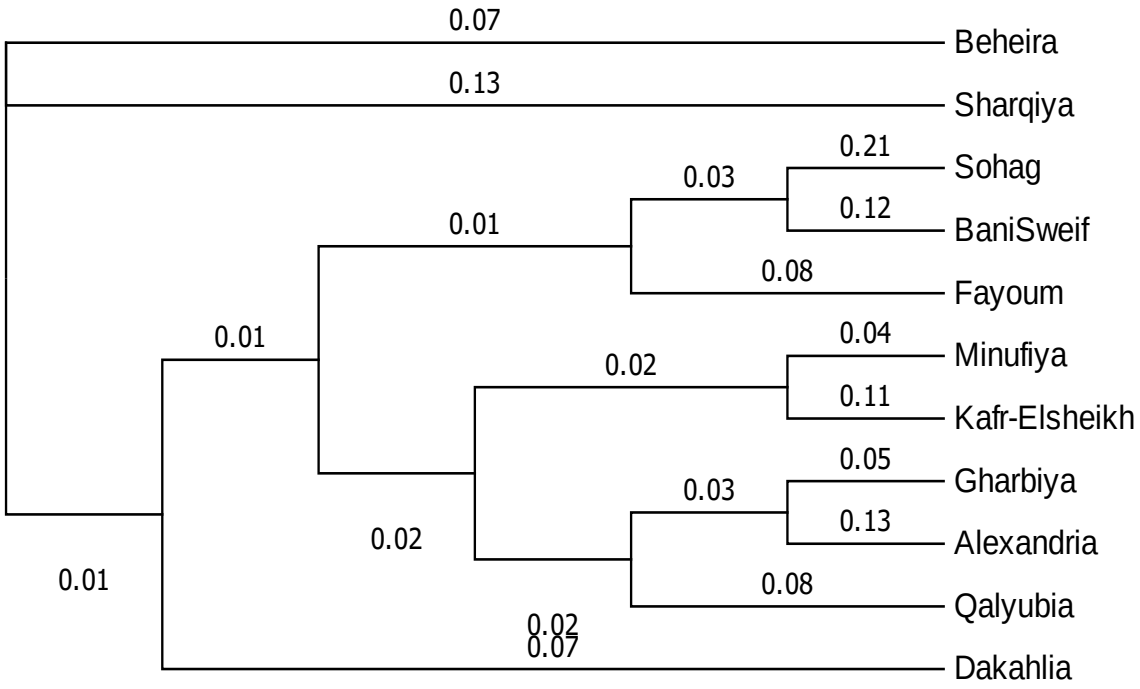


Figure 4. Dendogram of 40 *P. triticina* pathotypes based on the presence and absence of the tested pathotypes in each of the 11 locations.

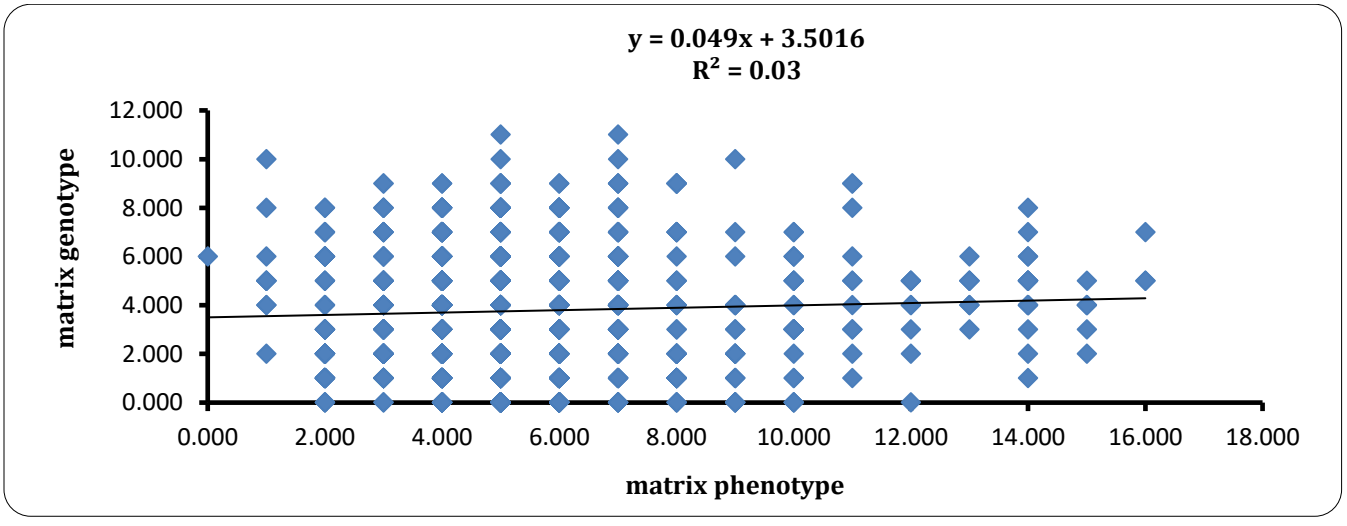


Figure 5. Association between virulence analysis data and molecular characterization data to detect variations between 40 *Puccinia triticina* pathotypes.

An annual survey of leaf rust pathotypes in wheat is essential to be carried out to identify and detect virulence pathotypes that may be introduced to different regions in the country. Also, it provides useful information about the effectiveness of leaf rust resistance gene (s) that were widely used in the

commercial wheat cultivars and the new resistance genes which not yet deployed in these cultivars (Park *et al.*, 2011). The dynamic nature of the causal pathogen and variation or diversity of genetic structure of leaf rust populations was mainly due to some evolutionary potential forces such as; migration, sexual recombination and mutation (McVey *et al.*, 2004; El-Orabey *et al.*, 2015). Moreover, a selection pressure, cultivation of wheat cultivars having a high level of resistance promoted the selection of virulent pathotypes (Burdon and Silk, 1997). All of these evolutionary forces usually are occurred, except sexual reproduction that did not occurred in Egypt, because of the absence of the alternative hosts, volunteer plants and stubs. The primary inoculum for leaf rust comes from external sources each year from the neighboring countries and the spores of leaf rust pathotypes did not survive summers in Egypt (McVey *et al.*, 2004). El-Orabey *et al.* (2018) estimated the diversity of leaf rust pathotypes using the three indexes; Shannon, Gleason and Simpson. They found that, highest diversity values for the Egyptian leaf rust population during 2016/2017 and 2017/2018. Analysis of diversity of leaf rust pathotypes was carried out by virulence analysis or using molecular techniques i.e. SSR markers (Agarwal *et al.*, 2018). Analysis of the diversity of plant pathogens based on molecular markers enables understanding the taxonomy and structure of populations. Also, it helps in knowing the genetic structure and relationship among plant pathogens pathotypes (Agarwal *et al.*, 2018).

Two methods are commonly used for the study of genetic diversity within and among wheat rust populations. First, study virulence and avirulence analysis of pathotypes on the differential lines and the second is using molecular markers (Kolmer, 2001).

In this study, the analysis of the tested leaf rust pathotypes using virulence analysis on the 20 leaf rust resistance genes and molecular markers confirmed the presence of high variation in pathogenicity and genetic diversity among these pathotypes. Moreover, virulence characteristics on resistance genes are more applicable and more useful in race analysis but because of virulence properties under strong selection provide incorrect estimations. Using DNA markers to study characteristics of leaf rust populations is very important because these markers are highly informative and more accurate tool (McDonald, 1997). Virulence analysis data revealed that, all tested pathotypes which were similar in virulence behavior were present in the same group except for some pathotypes which were in the same cluster but not identical in the same virulence behavior. This may be due to the Egyptian differential sets used for the nomenclature of *P. triticina* pathotypes should be changed especially for the last two subsets. The first three subsets are the same all over the world (Kolmer, 1991) and the last two subsets (4 and 5) are changed worldwide according to the effectiveness of the other leaf rust monogenic lines in each country. So, leaf rust monogenic lines in set four contain *Lr* 10, *Lr* 18, *Lr* 21 and *Lr* 2b and set five include *Lr* 14 b, *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 two leaf rust resistance genes *Lr* 11 in subset 3 and *Lr* 18 in subset 4 of nomenclature of *P. triticina* pathotypes in Egypt are sensitive to temperature and must be tested below 18 ℃ and temperature in the greenhouse is higher than 18 ℃ especially at the end of February and during March (Dyck and Johnson, 1983).

Molecular marker data showed that, all the tested pathotypes were genetically different and produced a unique SSR allele. Similar results were found by Gultyaeva *et al.* (2018) studied 46 isolates of *P. triticina* using 12 SSR markers and they found that, a high variation among the tested *P. triticina* pathotypes in Russia. Mantovani *et al.* (2010) tested 24 isolates of *P. triticina* from Italy using 15 SSR markers. They found that, the tested isolates were found into three groups. The first group, included isolates which were virulent and collected from durum wheat. The second group, included isolates that had virulence similar to the isolates from common wheat but were distinct for SSR genotypes compared to the isolates from durum wheat and common wheat. Isolates in the third group had virulence phenotypes and SSR genotypes closely related to the isolates from common wheat. Also, they found that virulence phenotypes and molecular genotypes were highly correlated with r = 0.74.

CONCLUSION

Based on the results of this study, leaf rust pathotypes in Egypt is very diverse and had very high evolutionary potential. Thus, the ability of single gene resistance probably is short. The strategy for breeding for resistance to leaf rust disease should be established based on the use of quantitative genes (partial resistance genes; race-nonspecific resistance genes) with other

race specific resistance genes resistance. In Egypt, effective leaf rust resistance genes such as *Lr* 17, *Lr* 18, *Lr* 21 and *Lr* 28 should be used in combination with non-specific genes such as *Lr* 34, *Lr* 46, *Lr* 67 and *Lr* 68 could create a more effective and stability of resistance. Moreover, a more powerful technique for detecting molecular polymorphism such as amplified fragment length polymorphism, may allow more discrimination between and within leaf rust pathotypes. Correlation between molecular variation and diversity in pathotypes based on virulence analysis was low and this correlation may be improved by using large numbers of markers.

ACKNOWLEDGMENTS

We thank Prof. Dr. Osama Boulot and Prof. Dr. Mohamed Anis Naguib (Plant Pathology Research Institute, ARC, Giza, Egypt) for language corrections and valuable suggestions on the manuscript revision.

Conflict of Interest: The authors declare that they have no conflicts of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by the authors.

REFERENCES

Agarwal, R., S. Sharma, S. Gupta, S. Banerjee, B. M. Bashyal and S. C. Bhardwaj. 2018. Molecular characterization of predominant Indian wheat rust pathotypes using URP and RAPD markers. Indian Journal of Biotechnology, 17: 327-36.

Burdon, J. J. and J. Silk. 1997. Sources and patterns of diversity in plant-pathogenic fungi. Phytopathology, 87: 664-69.

Dyck, P. L. and R. Johnson. 1983. Temperature sensitivity of genes for resistance in wheat to *Puccinia recondita*. Canadian Journal of Plant Pathology, 5: 229-34.

El-Orabey, W., R. Omara and M. Abou-Zeid. 2018. Diversity and virulence dynamics within *Puccinia triticina* populations in Egypt. Journal of Plant Protection and Pathology, 9: 735-45.

El-Orabey, W. M., N. I. A. El-Malik, M. A. Ashmawy and M. A. Abou-Zeid. 2017. Reduction in grain yield caused by leaf rust infection in seven Egyptian wheat cultivars. Minufiya Journal of Plant Protection, 2: 71-81.

El-Orabey, W. M., M. E. Sallam, R. I. Omara and N. I. Abd El-Malik. 2015. Geographical distribution of *Puccinia triticina* physiologic races in Egypt during 2012-2014 growing seasons. African Journal of Agricultural Research, 10: 4193-203.

Germán, S., A. Barcellos, M. Chaves, M. Kohli, P. Campos and L. de Viedma. 2007. The situation of common wheat rusts in the southern cone of America and perspectives for control. Australian Journal of Agricultural Research, 58: 620-30.

Gultyaeva, E. I., E. L. Shaydayuk, V. P. Shamanin, A. K. Akhmetova, V. A. Tyunin, E. R. Shreyder, I. V. Kashina, L. A. Eroshenko, G. A. Sereda and A. I. Morgunov. 2018. Genetic structure of Russian and Kazakhstani leaf rust causative agent *Puccinia triticina* erikss. populations as assessed by virulence profiles and SSR markers. Sel'skokhozyaistvennaya Biologiya, 53: 85-95.

Kolmer, J. A. 1991. Evolution of distinct populations of *Puccinia recondita* f. sp. *tritici* in Canada. Phytopathology, 81: 316-22.

Kolmer, J. A. 1999. Physiologic specialization of *Puccinia triticina* in Canada in 1997. Plant Disease, 83: 194-97.

Kolmer, J. A. 2001. Molecular polymorphism and virulence phenotypes of the wheat leaf rust fungus *Puccinia triticina* in Canada. Canadian Journal of Botany, 79: 917-26.

Kolmer, J. A., A. Hanzalova, H. Goyeau, R. Bayles and A. Morgounov. 2012. Genetic differentiation of the wheat leaf rust fungus *Puccinia triticina* in Europe. Plant Pathology, 62: 21-31.

Kolmer, J. A., D. L. Long and M. E. Hughes. 2009. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2007. Plant Disease, 93: 538-44.

Long, D. L. and J. A. Kolmer. 1989. A north American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology, 79: 525-29.

Mantovani, P., M. Maccaferri, R. Tuberosa and J. Kolmer. 2010. Virulence phenotypes and molecular genotypes in collections of *Puccinia triticina* from Italy. Plant Disease, 94: 420-24.

McDonald, B. A. 1997. The population genetics of fungi: Tools and techniques. Phytopathology, 87: 448-453.

McVey, D. V., M. Nazim, K. J. Leonard and D. L. Long. 2004. Patterns of virulence diversity in *Puccinia triticina* on wheat in Egypt and the United States in 1998-2000. Plant Disease, 88: 271-79.

Niazmand, A., D. Choobineh and S. Hajmansoor. 2013. A simple and rapid method to extract genomic DNA

from urediniospores of rust diseases for molecular analysis. STAPFIA, 99: 235-38.

Park, R., T. Fetch, D. Hodson, Y. Jin, K. Nazari, M. Prashar and Z. Pretorius. 2011. International surveillance of wheat rust pathogens: Progress and challenges. Euphytica, 179: 109-17.

Peakall, R. and P. E. Smouse. 2006. Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6: 288-95.

Shahin, S. I. and W. M. El-Orabey. 2016. Assessment of grain yield losses caused by *Puccinia triticina* in some Egyptian wheat genotypes. Minufiya Journal of Agricultural Research, 41: 29-37.

Szabo, L. J. and J. A. Kolmer. 2007. Development of simple sequence repeat markers for the plant pathogenic rust fungus *Puccinia triticina*. Molecular Ecology Notes, 7: 708-10.