Virulence of Leaf Rust Physiological Races in Iran From 2010 to 2017

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Abstract

The wheat leaf rust fungus, *Puccinia triticina*, has widespread geographical distribution in Iran within the Fertile Crescent region of the Middle East where wheat was domesticated and *P. triticina* originated. Therefore, it is of great importance to identify the prevalence and distribution of *P. triticina* pathotypes in this area. From 2010 to 2017, 241 single-uredinium isolates of *P. triticina* were purified from 175 collections of *P. triticina* made from various hosts in 14 provinces of Iran, and they were tested on 20 Thatcher near-isogenic lines carrying single-leaf rust resistance genes. In total, 86 pathotypes were identified, of which the pathotypes FDTTQ, FDKPQ, FDKTQ, and FDTNQ were most prevalent. No virulence for *Lr2a* was detected, whereas virulence for *Lr1*

was found only on bread wheat in a few provinces in 2016. Only isolates from durum wheat and wild barley were virulent to *Lr28*. Although virulence for *Lr9*, *Lr20*, and *Lr26* was observed in some years, the virulence frequency for these genes was lower than that of the other *Lr* genes. *P. triticina* collections from host plants with different ploidy levels or genetically dissimilar backgrounds were grouped individually according to genetic distance. Based on these results, collections from barley, durum wheat, oat, triticale, and wild barley were different from those of bread wheat.

Keywords: Iran, leaf rust, Middle East, virulence phenotypes, wheat

Bread wheat (*Triticum aestivum* L.) is the dominant cereal crop in Iran, and it is cultivated in >7 million ha (FAO 2018). Leaf rust caused by *Puccinia triticina* Erikss. is one of the most common and widely distributed diseases of this crop worldwide (Roelfs et al. 1992). However, leaf rust epidemics in Iran are very sporadic mainly because of different weather conditions, where the arid climate in some areas makes this disease less important than in other countries. Nevertheless, under favorable conditions, many parts of the country can experience a rapid increase and spread of this disease (Torabi et al. 2001), where virulent isolates are detected over a wide geographical area.

Iran is the 15th leading wheat producer in the world, with its production totaling 14.5 metric tons in 2016 (FAO 2018). The country is located in the northeastern part of the Fertile Crescent region, where the natural ranges of the primary (*Triticum* species) and alternative (*Thalictrum* species) hosts overlap (D'Oliveira and Samborski 1966). Previous studies have indicated that the center of origin of *P. triticina* is likely somewhere in the Fertile Crescent region in southwest Asia (Arthur 1929), where both sexual and asexual reproduction prevail (Kolmer et al. 2011). However, possible sexual recombination events are rare in the world (Kolmer et al. 2011). According to the studies by Huerta-Espino et al. (2011), Kolmer (2013), and Ordoñez et al. (2010), populations of *P. triticina* become widespread worldwide through clonal reproduction. Therefore, there is a high probability that urediniospores could migrate thousands of kilometers to and also, from Iran. These foreign introductions could

cause exotic races to appear and affect *P. triticina* populations in the country. The other main source of variation in the pathogen population is mutation. Both exotic and newly mutated races can overcome resistance genes, render current cultivars susceptible (Kolmer 2005), and consequently, lead to leaf rust epidemics. The origin of new *P. triticina* races is not always known. To address this question, worldwide race surveys are conducted. Because the wheat rust fungi spread easily within and between continents, it is essential to study virulence phenotypes in wheat-producing countries in a concerted effort and a standard way to facilitate the exchange of data more easily. Some researchers in various countries conduct annual rust surveys to provide information about the prevalence, epidemiology, and frequency of known rust pathotypes as well as to detect new and potentially dangerous ones (Kolmer and Hughes 2018; McCallum et al. 2017).

The specific interactions between resistance genes and avirulence genes serve as extremely useful markers for characterizing rust populations. Globally, differential sets consisting of near-isogenic lines or wheat cultivars with known resistance genes have been widely adopted to identify races in rust populations (Dyck and Kerber 1985; McIntosh et al. 1995). Despite reports of leaf rust occurrence in Iran in 1973 by Bamdadian (1973), populations of *P. triticina* have not been fully studied, and little information about yearly prevalence of virulence phenotypes is available (Dadrezaei et al. 2012; Niazmand et al. 2010). Studies in this context were mainly limited to the effectiveness/ineffectiveness of a certain *Lr* gene to a population that is a mixture of genotypes and for which pathotypes have not been fully characterized (Afshari et al. 2006; Elyasi-Gomari 2010). From 2010 onward, no investigations of *P. triticina* pathotypes were conducted. Similarly, no study on *P. triticina* pathotypes isolated from durum wheat has been performed. Therefore, this study was conducted to i) characterize *P. triticina* populations over a period of 8 years (2010 to 2017) and study population dynamics of the pathogen by comparing annual and local populations; ii) specify and differentiate populations originating from bread wheat, durum wheat, barley, oat, triticale, and wild barley; iii) compare Iranian *P. triticina* virulence phenotypes with those from other countries and discuss potential wind routes through which urediniospores may move to and from Iran; and iv) examine changes in the frequency of virulence for *Lr* genes among different phenotypes of *P. triticina* in Iran across different regions and years.

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*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table is published online.

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Materials and Methods

Purification and increase of *P. triticina*. In total, 169 *P. triticina* samples were originally collected from fields from 2010 to 2016. Additionally, six samples were collected on wild barley (*Hordeum vulgare* ssp. *spontaneum* Thell.) in Bamou National Park and the Margoon Protected Region in Fars province in 2017 (Nemati et al. 2017). Uredinial samples of *P. triticina* were also collected from various *Gramineae*, such as bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.), barley (*H. vulgare* L.), oat (*Avena sativa* L.), triticale (*Triticosecale* Wittm. ex A. Camus), and wild barley (*H. vulgare* ssp. *spontaneum* Thell.). The surveyed area spanned 14 provinces as follows (Fig. 1): in 2010, 20 samples from Ilam, Khuzestan, Khorasan Razavi, Kurdistan, Lorestan, Hamedan, and Mazandaran provinces; in 2011, 19 samples from Ardabil, Golestan, Khorasan Razavi, Khuzestan, Kurdistan, and Mazandaran provinces; in 2012, 17 samples from West Azerbaijan, Fars, Golestan, Khorasan Razavi, Khuzestan, Lorestan, and Mazandaran provinces; in 2013, 19 samples from Golestan, and Mazandaran provinces; in 2014, 43 samples from Ardabil, West Azerbaijan, East Azerbaijan, Golestan, Kerman, Khuzestan, Lorestan, Mazandaran, and Zanjan provinces; in 2015, 9 samples from Khuzestan and Mazandaran provinces; and in 2016, 42 samples from Ardabil, Ilam, Khorasan Razavi, Khuzestan, Lorestan, and Mazandaran provinces. One or two single uredinia were derived from each sample, and in the end, they totaled 241 isolates (Supplementary Table S1). These isolates were then increased on the susceptible bread wheat cultivar Boolani in the greenhouse (20 to 24°C) using the methods described by Kolmer et al. (2009). Urediniospores were dried in a desiccator for 2 days and stored at -80°C for later use.

Virulence phenotypes. Differential sets consisting of near-isogenic lines in a Thatcher background were used to determine virulence phenotypes. These included set 1: *Lr1*, *Lr2a*, *Lr2c*, and *Lr3*; set 2: *Lr9*, *Lr16*, *Lr24*, and *Lr26*; set 3: *Lr3ka*, *Lr11*, *Lr17*, and *Lr30* (Long and Kolmer 1989); set 4: *LrB*, *Lr10*, *Lr14a*, and *Lr18* (Kolmer et al. 2017); and set 5: *Lr3bg*, *Lr14b*, *Lr20*, and *Lr28* (Kolmer et al. 2014). Approximately 6 to 10 seeds of each genotype were planted in clumps in 12-cm-diameter pots. Plants were inoculated 12 days after planting with urediniospores of each isolate suspended in light mineral oil (0.3 ml liter⁻¹). The inoculated materials were incubated at 18°C and 100% RH for 24 h, after which

they were moved to a growth cabinet with a temperature of 22 ± 2°C. The susceptible Boolani was used as a negative control, which was inoculated along with Thatcher isogenic lines. Infection types (ITs) were recorded 12 days postinoculation following the method by Long and Kolmer (1989), where ITs 0 to 2+ (immune response to medium-sized uredinia with chlorosis and necrosis) were classified as avirulent and ITs 3 to 4 (medium to large uredinia without chlorosis or necrosis) were classified as virulent. Pathotype nomenclature was performed according to the North American system described by Long and Kolmer (1989). In this method, each isolate was given a five-letter code based on avirulence/virulence to the 20 near-isogenic Thatcher lines.

Data analysis. A binary data matrix was also generated for all virulence phenotypes based on the presence (1) or absence (0) of virulence for *Lr* genes. In total, 241 isolates were grouped in 13 populations based on the year and host or origin. The dissimilarity matrix for virulence was used to construct dendrograms using Nei's distance coefficient method in unweighted pair group arithmetic mean method (UPGMA) with POWER MARKER v. 3.25 (Liu and Muse 2005).

Results

***P. triticina* phenotyping.** In total, 241 single-uredinium isolates of *P. triticina* were obtained from samples collected in 14 provinces of Iran from 2010 to 2017 (Fig. 1). Based on their reaction on the 20 Thatcher differential genotypes, 86 pathotypes of *P. triticina* were identified (Table 1). The highest numbers of samples came from Mazandaran (65) and Khuzestan (48) provinces, which were differentiated into 27 and 25 different pathotypes, respectively. The fewest samples came from Zanjan (2) and Hamedan (2) provinces, which were differentiated pathotypes FDTSQ and CDKRQ, respectively. In general, the most common pathotypes found in Iran were FDKPQ, FDTNQ, FDKLQ, FDKNQ, FDKRQ, and FDKTQ, which were distributed across different provinces and years in Iran (Supplementary Table S1).

None of these 86 pathotypes were found across all provinces in all years studied. FDKPQ was the most widely distributed across all years of the study, except 2010 and 2017. This pathotype, collected from bread wheat fields in Kurdistan (2011), Kerman (2013), Lorestan (2014 and 2016), and Mazandaran (2011, 2012, and 2013)

Fig. 1. A, Map of the routes of wind blowing to Iran. B, Map of Iran. The provinces from which collections of *Puccinia triticina* were sampled from 2010 to 2017 are marked with stars.

Table 1. Virulence phenotypes of *Puccinia triticina* in 14 provinces of Iran from 2010 to 2017 identified by 20 differential lines of wheat with single genes for leaf rust resistance

Phenotype/virulence	2010	2011	2012	2013	2014	2015	2016	2017
BDKLQ <i>3bg, 11, 14b, 17, 24, 30, B</i>	0	0	0	4 ^a (4%) ^b	0	0	0	0
BDKQQ <i>3bg, 10, 11, 14b, 17, 24, 30, B</i>	0	8, 12 (8.7%)	0	0	0	0	0	0
BDTPG <i>3ka, 11, 14a, 14b, 17, 18, 24, 30, B</i>	0	0	0	0	0	9 (13.3%)	0	0
BKKNG <i>11, 14a, 14b, 16, 17, 24, 26, 30, B</i>	0	0	0	0	0	4 (26.7%)	0	0
CBMLQ <i>3a, 3bg, 3ka, 14b, 30, B</i>	0	0	0	0	9 (2%)	0	0	0
CDDLQ <i>3a, 17, 24, B</i>	0	4 (4%)	0	0	0	0	0	0
CDFMQ <i>3a, 3bg, 14b, 17, 18, 24, 30, B</i>	0	0	0	0	1 (3.9%)	0	0	0
CDHLQ <i>3a, 3bg, 11, 14b, 24, 30, B</i>	0	0	0	4, 12 (12%)	0	0	0	0
CDKQQ <i>3a, 3bg, 10, 11, 14b, 17, 24, 30, B</i>	0	0	8 (21.8%)	0	0	0	0	0
CDKRQ <i>3a, 3bg, 10, 11, 14b, 17, 18, 24, 30, B</i>	10 (13%)	0	0	0	0	0	0	0
CDKSR ^c <i>3a, 3bg, 10, 11, 14a, 14b, 17, 24, 28, 30, B</i>	0	0	0	0	0	0	9 (4.7)	0
CDRLQ <i>3a, 3bg, 3ka, 11, 14b, 24, 30, B</i>	0	0	0	0	9 (2%)	0	0	0
CDTPG <i>3a, 3ka, 11, 14a, 14b, 17, 18, 24, 30, B</i>	0	0	0	0	0	9 (6.7%)	0	0
CDKQQ <i>3a, 3bg, 10, 11, 14b, 17, 24, 30, B</i>	0	0	8 (21.8%)	0	0	0	0	0
CFDLB <i>3a, 17, 24, 26, B</i>	0	4 (4%)	0	0	0	0	0	0
CJTTG <i>3a, 3ka, 10, 11, 14a, 14b, 16, 17, 18, 24, 30, B</i>	0	0	0	0	0	12 (13.4%)	0	0
CSKPQ <i>3a, 3bg, 9, 11, 14a, 14b, 16, 17, 18, 24, 30, B</i>	0	0	0	0	0	12 (13.4%)	0	0
DDPRQ <i>2c, 3bg, 3ka, 10, 14b, 17, 18, 24, 30, B</i>	0	9 (8%)	0	0	0	0	0	0
DJTRQ <i>2c, 3bg, 3ka, 10, 11, 14b, 16, 17, 18, 24, 30, B</i>	0	0	0	0	4 (3.9%)	0	0	0
DKKSQ <i>2c, 3bg, 10, 11, 14a, 14b, 16, 17, 24, 26, 30, B</i>	0	0	0	0	0	0	12 (3.1%)	0
DKTSS ^d <i>2c, 3bg, 3ka, 10, 11, 14a, 14b, 16, 17, 20, 24, 26, 30, B</i>	0	0	0	0	0	0	9 (4.7%)	0
FBHMQ <i>2c, 3a, 3bg, 11, 14b, 18, 30, B</i>	9, 11 (6.4%)	0	0	0	0	0	0	0
FBMNQ <i>2c, 3a, 3bg, 3ka, 14a, 14b, 30, B</i>	9 (3.2%)	0	0	0	0	0	0	0
FBMTQ <i>2c, 3a, 3bg, 3ka, 10, 14a, 14b, 18, 30, B</i>	9 (9.7%)	0	0	0	0	0	0	0
FBPMQ <i>2c, 3a, 3bg, 3ka, 14b, 17, 18, 30, B</i>	8 (6.4%)	0	0	0	0	0	0	0
FDCQS ^e <i>2c, 3a, 3bg, 10, 14b, 20, 24, 30, B</i>	0	0	0	0	0	0	6 (4.7%)	0
FDFLQ <i>2c, 3a, 3bg, 14b, 17, 24, 30, B</i>	0	4 (4%)	0	0	0	0	0	0
FDFQQ <i>2c, 3a, 3bg, 10, 14b, 17, 24, 30, B</i>	0	10 (8%)	0	0	0	0	0	0
FDFTQ ^f <i>2c, 3a, 3bg, 10, 14a, 14b, 17, 18, 24, 30, B</i>	0	0	0	0	0	0	0	3 (33.3%)

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^a Number of province: 1 = Ardabil, 2 = East Azerbaijan, 3 = Fars, 4 = Golestan, 5 = Hamedan, 6 = Ilam, 7 = Kerman, 8 = Khorasan Razavi, 9 = Khuzestan, 10 = Kurdistan, 11 = Lorestan, 12 = Mazandaran, 13 = West Azerbaijan, and 14 = Zanjan.

^b Percentage of virulence frequency of leaf rust per year.

^c Leaf rust populations on durum wheat.

^d Leaf rust populations on oat.

^e Leaf rust populations on wild barley.

^f Leaf rust populations on barley.

^g Leaf rust populations on triticale.

Table 1. (Continued from previous page)

Phenotype/virulence	2010	2011	2012	2013	2014	2015	2016	2017
FDFTR ^c	0	0	0	0	0	0	0	3 (33.3%)
2c,3a,3bg,10,14a,14b,17,18,24,28,30,B								
FDHLQ	0	0	0	12 (4%)	12 (2%)	0	0	0
2c,3a,3bg,11,14b,24,30,B								
FDHTQ	0	0	0	0	12 (2%)	0	0	0
2c,3a,3bg,10,11,14a,14b,18,24,30,B								
FDKGQ	0	0	0	0	0	0	11 (1.6%)	0
2c,3a,3bg,10,11,14b,17,24,30,B								
FDKLQ	0	0	4, 9 (8.7%)	4, 12 (12%)	7 (1.9%)	0	0	0
2c,3a,3bg,11,14b,17,24,30,B								
FDKMQ	0	0	0	4 (8%)	0	0	11 (1.6%)	0
2c,3a,3bg,11,14b,17,18,24,30,B								
FDKNQ	6, 9 (9.7%)	10, 12 (16%)	12 (8.7%)	0	0	0	0	0
2c,3a,3bg,11,14a,14b,17,24,30,B								
FDKPQ	0	10, 12 (20%)	12 (4.3%)	12 (4%)	7, 10, 12 (5.8%)	0	11 (1.6%)	0
2c,3a,3bg,11,14a,14b,17,18,24,30,B								
FDKRQ	12 (3.2%)	0	0	4 (8%)	4, 12 (3.8%)	0	0	0
2c,3a,3bg,10,11,14b,17,18,24,30,B								
FDKTQ	0	0	0	4, 12 (16%)	12 (3.8%)	0	1, 11 (7.8%)	0
2c,3a,3bg,10,11,14a,14b,17,18,24,30,B								
FDPLL	0	0	0	0	12 (1.9%)	0	0	0
2c,3a,3bg,3ka,17,24,30,B								
FDPNQ	8 (6.4%)	1, 8 (20%)	0	0	0	0	0	0
2c,3a,3bg,3ka,14a,14b,17,24,30,B								
FDRNQ	6, 9, 11 (9.7%)	0	0	0	0	0	0	0
2c,3a,3bg,3ka,14a,14b,11,24,30,B								
FDTLQ	0	0	0	0	1, 12 (5.8%)	0	0	0
2c,3a,3bg,3ka,11,14b,17,24,30,B								
FDTNQ	12 (3.2%)	1, 12 (12%)	12 (4.3%)	12 (8%)	7, 11 (7.7%)	0	0	0
2c,3a,3bg,3ka,11,14a,14b,17,24,30,B								
FDTPQ	11 (3.2%)	0	0	0	0	0	0	0
2c,3a,3bg,3ka,11,14a,14b,17,18,24,30,B								
FDTSQ	9 (6.5%)	0	0	0	13, 14, 5 (7.7%)	0	0	0
2c,3a,3bg,3ka,10,11,14a,14b,17,24,30,B								
FDTTL	9 (3.2%)	0	0	0	0	0	0	0
2c,3a,3bg,3ka,10,11,14a,17,18,24,30,B								
FDTTQ	8, 9, 12 (16.1%)	0	0	0	0	0	0	0
2c,3a,3bg,3ka,10,11,14a,14b,17, 18,24,30,B								
FFFLQ	0	4 (4%)	0	0	0	0	0	0
2c,3a,3bg,14b,17,24,26,30,B								
FFFQQ	0	0	3 (4.3%)	0	0	0	0	0
2c,3a,3bg,10,14b,17,24,26,30,B								
FFFRQ	0	0	3 (8.6%)	0	0	0	0	0
2c,3a,3bg,10,14b,17,18,24,26,30,B								
FFKRQ	0	0	4, 12 (8.6%)	0	0	0	0	0
2c,3a,3bg,10,11,14b,17,18,24,26,30,B								
FFKTQ	0	0	0	0	0	0	6 (1.5%)	0
2c,3a,3bg,10,11,14a,14b,17,18,24,26,30,B								
FGCLL	0	0	0	0	11 (3.8%)	0	0	0
2c,3a,3bg,16,30,B								
FGCLQ	0	0	0	0	11, 12 (5.8%)	0	0	0
2c,3a,3bg,14b,16,30,B								
FGQRS	0	0	0	0	2 (3.8%)	0	0	0
2c,3a,3bg,3ka,10,11,14b,16,18,20,B								
FHTQQ	0	0	0	4 (8%)	0	0	0	0
2c,3a,3bg,3ka,10,11,14b,16,17,26,30,B								
FJTSQ	0	0	0	0	0	0	9 (3.1%)	0
2c,3a,3bg,3ka,10,11,14a,14b,16,17,24,30,B								
FJHPQ	0	0	0	4 (8%)	0	0	0	0
2c,3a,3bg,11,14a,14b,16,18,24,30,B								
FJKNQ	0	0	0	0	0	12 (20%)	0	0
2c,3a,3bg,11,14a,14b,16,17,24,30,B								
FJKPQ ^c	0	0	0	0	0	0	6 (4.7%)	0
2c,3a,3bg,11,14a,14b,16,17,18,24,30,B								
FJKRQ ^c	0	0	0	0	0	0	9 (3.1%)	0
2c,3a,3bg,10,11,14b,16,17,18,24,30,B								

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Table 1. (Continued from previous page)

Phenotype/virulence	2010	2011	2012	2013	2014	2015	2016	2017
FIPSQ	0	0	0	0	1, 12 (5.8%)	0	0	0
2c,3a,3bg,3ka,10,14a,14b,16,17,24,30,B								
FJTNQ ^c	0	0	0	0	0	0	9 (3.1%)	0
2c,3a,3bg,3ka,11,14a,14b,16,17,24,30,B								
FJTTQ ^f	0	0	0	0	0	0	9 (3.1%)	0
2c,3a,3bg,3ka,10,11,14a,14b,16,17,18,24,30,B								
FKTTQ	0	0	0	0	0	0	1, 9, 11 (15.6%)	0
2c,3a,3bg,3ka,10,11,14a,14b,16,17,18,24,26,30,B								
FKFFQ	0	0	3, 9 (13%)	0	0	0	0	0
2c,3a,3bg,14a,14b,16,17,18,24,26,30,B								
FKKTQ	0	0	0	0	0	0	6, 9 (3.1%)	0
2c,3a,3bg,10,11,14a,14b,16,17,18,24,26,30,B								
FKTMQ	0	0	11, 12 (8.7%)	0	0	0	0	0
2c,3a,3bg,3ka,11,14b,16,17,18,24,26,30,B								
FKTNQ ^{e,f}	0	0	0	0	0	9 (6.7%)	3 (50%)	0
2c,3a,3bg,3ka,11,14a,14b,16,17,24,26,30,B								
FKTPQ ^f	0	0	0	0	0	0	9 (1.6%)	0
2c,3a,3bg,3ka,11,14a,14b,16,17,18,24,26,30,B								
FKTTQ	0	0	0	0	0	0	1, 9, 11 (15.6%)	0
2c,3a,3bg,3ka,10,11,14a,14b,16,17,18,24,26,30,B								
FNMLS	0	0	0	0	12 (5.8%)	0	0	0
2c,3a,3bg,3ka,9,14b,20,24,30,B								
FPKNQ	0	0	0	12 (4%)	0	0	0	0
2c,3a,3bg,9,11,14a,14b,17,24,26,30,B								
FSTTS	0	0	0	0	1 (1.9%)	0	0	0
2c,3a,3bg,3ka,9,10,11,14a,14b,16,17,18,20,24,30,B								
FTKNQ	0	0	0	12 (4%)	0	0	0	0
2c,3a,3bg,9,11,14a,14b,16,17,24,26,30,B								
LJKQS ^g	0	0	0	0	0	0	9 (3.1%)	0
1,3bg,10,11,14b,16,17,20,24,30,B								
NJKSQ	0	0	0	0	0	0	12 (3.1%)	0
1,2c,3bg,10,11,14a,14b,16,17,24,30,B								
PDTSQ	0	0	0	0	0	0	1 (1.5%)	0
1,2c,3a,3bg,3ka,10,11,14a,14b,17,24,30,B								
PJKTQ ^e	0	0	0	0	0	0	11 (3.1%)	0
1,2c,3a,3bg,10,11,14a,14b,16,17,18,24,30,B								
PJKTS	0	0	0	0	0	0	1 (3.1%)	0
1,2c,3a,3bg,10,11,14a,14b,16,17,18,20,24,30,B								
PJTSQ	0	0	0	0	0	0	1, 11 (3.1%)	0
1,2c,3a,3bg,3ka,10,11,14a,14b,16,17,24,30,B								
PJTSS	0	0	0	0	0	0	12 (1.5%)	0
1,2c,3a,3bg,3ka,10,11,14a,14b,16,17,20,24,30,B								
PKTSQ	0	0	0	0	0	0	1 (1.5%)	0
1,2c,3a,3bg,3ka,10,11,14a,14b,16,17,24,26,30,B								
PKTSS	0	0	0	0	0	0	12 (1.5%)	0
1,2c,3a,3bg,3ka,10,11,14a,14b,16,17,20,24,26,30,B								
PKTTS	0	0	0	0	0	0	8 (3.1%)	0
1,2c,3a,3bg,3ka,10,11,14a,14b,16,17,18,20,24,26,30,B								

provinces, was virulent for *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr11*, *Lr14a*, *Lr14b*, *Lr17*, *Lr18*, *Lr24*, *Lr30*, and *LrB*. Similarly, none of the 86 pathotypes were detected across all provinces surveyed in the study (Table 1). Eighteen were phenotyped as the FDTTQ with virulence to *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr17*, *Lr18*, *Lr24*, *Lr30*, and *LrB* (Table 1), which were collected from bread wheat and had a wide distribution in Iran. Other common pathotypes distributed widely among years included FDKTQ with virulence for *Lr2c*, *Lr3a*, *Lr3bg*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr17*, *Lr18*, *Lr24*, *Lr30*, and *LrB*. It was detected in samples from Ardabil (2016), Golestan (2013), Lorestan (2016), and Mazandaran (2012 and 2013) provinces. FDTNQ, with virulence for *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr11*, *Lr14a*, *Lr14b*, *Lr17*, *Lr24*, *Lr30*, and *LrB*, was detected frequently from 2010 to 2014 in Ardabil (2011), Kerman (2014), Lorestan (2014), and Mazandaran (from 2010 to 2013) provinces. Although PKTTS and PKTSS pathotypes with virulence for 17 and 16 *Lr* genes, respectively, were the most virulent ones, they

had low frequencies in Khorasan Razavi and Mazandaran provinces in 2016. Pathotypes CDDLb and CFDLb (with virulence for only four and five *Lr* genes, respectively) had the narrowest virulence spectrum found in the survey, and they were originally collected in Golestan province in 2011 (Table 1).

Isolates of *P. tritici* collected from a range of different hosts in the *Gramineae* family (durum wheat, barley, oats, triticale, and wild barley) were also assayed for their virulence on the Thatcher differential set. These samples were collected across several regions in different years. None of these pathotypes were common within a specific species or between them and bread wheat, except for pathotype FKTNQ, which was common between barley and wild barley (Table 1). Isolates from durum wheat were collected from different cultivars in Ilam and Khuzestan provinces in 2016. Six pathotypes were identified on durum wheat and included FDKQS and FJKPQ from Ilam province and CDKSR, FJKRQ, FJTNQ, and FJTSQ from Khuzestan province. Three and two isolates were collected from oat

and triticale, respectively, from Khuzestan province in 2016. The respective pathotypes identified were DKTSS and LJKQS. Isolates from barley were collected in Khuzestan province in 2015 and 2016, from which three pathotypes were identified: FJTQ, FKTNQ, and FKTPQ. Lastly, isolates from wild barley collected in Lorestan province in 2016 and Fars province (Bamou National Park and the Margoon Protected Region) in 2017 keyed to pathotypes PJKTQ, FKTNQ, FDFTR, and FDFTQ (Table 1).

According to Nei's genetic distance from the UPGMA tree (Fig. 2), pathotypes from each host were separated from each other, especially with respect to those from triticale and oat. In total, four groups were observed in the UPGMA dendrogram and included a triticale group, an oat group, a population from bread wheat 2010 to 2014 with durum wheat groups, and populations from bread wheat 2015 to 2017 with barley and wild barley groups. The yearly population collected from 2010 to 2014 comprised a group distinct from the yearly populations from 2015 to 2017. The increased frequency for high virulence for genes, such as *Lr1*, *Lr16*, *Lr18*, *Lr20*, and *Lr26*, has caused the annual population of wheat to be divided into two groups.

Virulence frequencies to *Lr* genes. The virulence frequencies for *Lr* genes were compared among the regional populations of *P. triticina* on bread wheat and other *Poaceae* species separately. With respect to bread wheat (Fig. 3), virulence for *Lr2a* was not found in any

studied area or year, whereas virulence for *Lr1* was detected only in 2016 and with a low frequency in Ardabil, Khorasan Razavi, and Mazandaran provinces. Similarly, virulence for *Lr9* was found with a low frequency in Mazandaran province from 2013 to 2015, and it was detected in Ardabil province only in 2014. No virulence for *Lr16* was observed before 2012. Yet, in 2012, virulence for this gene was found in Khuzestan, Lorestan, and Mazandaran provinces, and since then, it has spread to different provinces. The frequency of virulence for *Lr18* was moderate (range of 28 to 61%) in all areas and years (Fig. 3). Virulence for *Lr20* was detected for the first time in East Azerbaijan in 2014, and thereafter, it was found in Ardabil and Mazandaran provinces from this year forward. Virulence for *Lr20* was lower than for other *Lr* genes. The Thatcher near-isogenic line with *Lr26* conferred moderate levels of resistance (range of 0 to 48%) to Iranian isolates of *P. triticina* (Fig. 3). Virulence for *Lr26* was first found in Golestan province in 2011, and it was subsequently reported from different areas. No virulence was reported for *Lr28* during this 8-year survey period. Virulence frequency for both *LrB* and *Lr30* was the highest in all studied areas and years. Races virulent for *Lr2c*, *Lr3a*, *Lr3bg*, *Lr11*, *Lr14b*, and *Lr17* were also observed frequently.

None of the isolates collected from barley, durum wheat, oat, triticale, and wild barley were virulent for either *Lr2a* or *Lr9*. Virulence for *Lr1* was found in isolates from triticale and two isolates from wild

Fig. 2. The unweighted pair group arithmetic mean method dendrogram of relationships among annual populations of *Puccinia triticina* in Iran from 2010 to 2017. The unweighted pair group method with arithmetic means dendrogram of Nei's genetic distance was adapted for virulence of *P. triticina* populations based on virulence to 20 wheat leaf rust differentials. Numbers on branch lengths indicate Nei's genetic distance. *P. triticina* populations from other hosts have been labeled: B, barley; DI, durum wheat from Ilam province; DKH, durum wheat from Khuzestan province; O, oat; T, triticale; WB, wild barley.

barley in Lorestan province. Virulence for *Lr16* was frequently detected among all of the other hosts of *Poaceae*. Virulence for *Lr18* was not detected among isolates from oat or triticale, whereas a high frequency of virulence for this gene was observed in samples collected from barley, durum wheat, and wild barley. The frequency of virulence for *Lr20* was low among isolates collected on durum wheat, oat, and triticale. Virulence for *Lr26* was found on samples from oat, barley, and wild barley. Lastly, virulence for *Lr28* was detected in low frequency from durum wheat and wild barley.

Frequency of virulence for *Lr* genes was estimated from 2010 to 2016. No virulence was detected for *Lr2a*. Virulence frequency for *Lr* genes, such as *Lr1*, *Lr9*, *Lr20*, and *Lr28*, remained <20% across all years (Fig. 3). Virulence for *Lr3ka*, *Lr10*, and *Lr18* varied between 20 and 80%. For *Lr16*, the virulence frequency was <20% until 2014 but then, increased rapidly to >20 and <80% in different years (Fig. 3). Virulence for *Lr26* was <20% for most years with the exception of 2012 and 2016, when it was >20% (Fig. 3). The frequency of virulence for *Lr11* and *Lr14a* changed to >80% in 2013 and 2015, respectively (Fig. 3). Finally, the frequency of virulence for the genes *Lr2c*, *Lr3*, *Lr3bg*, *Lr14b*, *Lr17*, *Lr24*, *Lr30*, and *LrB* was as high as 80% in all studied years (Fig. 3).

Discussion

Wheat is the main cereal crop in Iran, and it has been domesticated and cultivated in this region since ancient times, where its progenitors, such *Triticum monococcum* L and *Aegilops* spp., are still found growing throughout the region. Because both wheat and *P. triticea* have coevolved in the area, it is plausible that leaf rust has been established in the Middle East for thousands of years (Kolmer et al. 2011). The inoculum source for this disease could be clonal and/or sexually derived. Sexual reproduction occurs on alternate hosts, such as *Thalictrum* species (Roelfs et al. 1992). Despite the reports for existence of different species of *Thalictrum* in Iran (Pakravan et al. 2014), the extent and role of the sexual stage remain to be fully studied. Moreover, Iranian populations of *P. triticea* are thought to be influenced by migration of urediniospores from neighboring countries—even those quite distant.

The results of this study indicated that urediniospores could migrate between Iran and its northern neighbor Russia. However, it is also possible that inoculum could emanate from locations further afield (i.e., China, India, and even Africa) and Europe. Wheat is mainly cultivated almost all over the country where leaf rust greatly impacts its production in some years. The wind route map in Figure 1 indicates that Iran is generally influenced by winds from almost all directions (i.e., northerly winds from Russia and Turkey as well as westerly, southwesterly, and northeasterly winds known as trade winds). Some Iranian pathotypes of *P. triticea* in this study are similar to those previously reported in Russia, and they could be attributable to the northerly winds that blow from Russia to the north of Iran (Fig. 1) (Gulyaeva et al. 2017; Kolmer et al. 2014). Because Russia is located north of Iran, the presence of similar phenotypes in both countries could indicate the probability of reciprocal migration (Fig. 1). This can be illustrated by the fact that the pathotype PDTSQ detected in northern Iran (Ardabil province 2016) with virulence for the genes *Lr1*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr3bg*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr17*, *Lr24*, *Lr30*, and *LrB* is similar to the most common virulence phenotype in the central region of Russia: that is, PBPSQ (2010) with virulence for genes *Lr1*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr3bg*, *Lr10*, *Lr14a*, *Lr14b*, *Lr17*, *Lr30*, and *LrB*. Virulence comparison between these two pathotypes indicates that they differ only in two *Lr* genes: *Lr11* and *Lr24*. Likewise, pathotypes FBPMQ (Khuzestan province), FBPMQ (Khorasan Razavi province), and FDTTQ in Iran were very similar to Russian pathotypes FBPNQ/FBPSQ and -TTQ (Gulyaeva et al. 2017; Kolmer et al. 2014). These pathotypes differ in just one or two *Lr* genes from those of Russia. The likely reason is that the cultivars deployed in each country differ, and as a consequence, their cultivation leads to the emergence of new phenotypes owing to mutation followed by migration.

Westerly winds, which blow from Turkey and the Mediterranean Sea, are likely a major cause of urediniospores dispersion in Iran.

Therefore, common virulence types between the two countries are expected. Iranian pathotypes, such as FJTNTQ, FBPMQ, FJTSTQ, FSPSQ and FJTNTQ, were similar to FBTNTQ, FBPSQ, FHTSQ, FHPSQ, and PGTSS, respectively, as reported in Turkey by Kolmer et al. (2011). Northeasterly winds blowing from the Indian peninsula to Pakistan and Iran probably facilitate both short- and long-distance dissemination of *P. triticea*. Similarity between *P. triticea* pathotypes found in Iran and Pakistan could indicate migration between the two countries. For example, the pathotypes FHTQQ (Golestan province 2013) and FJPSQ (Ardabil and Mazandaran provinces 2014) were very similar to pathotypes KHPQQ (2008 and 2010), FHPSQ (2013), and KHMQQ (2011) from Pakistan (Kolmer et al. 2017). Iranian pathotypes differed from the Pakistani ones with respect to virulence for *Lr2a* but avirulence for *Lr11* and *Lr17*, whereas virulence for the genes *Lr11* and *Lr17* was common among Iranian pathotypes in this study. The genes *Lr9*, *Lr11*, *Lr18*, and *Lr28* were effective against Pakistani pathotypes in Pakistan, but in this research, the genes *Lr1*, *Lr2a*, *Lr9*, *Lr20*, and *Lr28* were effective against Iranian pathotypes.

Long-distance dissemination of *P. triticea* into Iran has likely occurred from as far away as China and India. The pathotype FHTQQ from Iran (Golestan province 2013) was similar to collections of *P. triticea* from China in 2007 (FHTS, FHTR, and FHDQQ) as reported by Huerta-Espino et al. (2011). Furthermore, the pathotype FHTQQ in Iran was very similar to FHTTQ from India (Bhardwaj et al. 2010c). Also, -TTQ, -TTS, and, -TSS pathotypes, such as FDTTQ, FKTTQ, PKTTS, or PKTSS from Iran, were similar to THTTQ, THTTS, and PHTTL pathotypes from India and Nepal (Bhardwaj et al. 2010b, c). Minor differences between Iranian and

Fig. 3. Virulence frequencies in *Puccinia triticea* isolates collected from common wheat from 2010 to 2016 in Iran. Parts A, B, and C contain various *Lr* genes.

Indian pathotypes were on virulence/avirulence for the genes *Lr1*, *Lr2a*, *Lr16*, *Lr24*, and *Lr26*. Virulence for *Lr2a* has not been detected in Iran, whereas virulence for *Lr1*, *Lr16*, and *Lr26* was observed in 2016, 2012, and 2011, respectively. It seems that Indian wheat cultivars carry *Lr* genes, such as *Lr1* and *Lr26*, and consequently, pathotypes with virulence for these genes have developed (Bhardwaj et al. 2010a). Although virulence for *Lr24* was in high frequency among Iranian isolates, this gene is reported to be still effective in India (Bhardwaj et al. 2010c; Manjunatha et al. 2015). The presence of common pathotypes among Iran, India and the Far Eastern country of China clearly suggests long-distance dissemination of pathotypes beyond country borders. Northeasterly winds (trade winds) and local winds, which blow from the Middle East to India, usually cause urediniospores to disperse to the east. In the case of yellow rust, new pathotypes were introduced into India from the Middle East (Bhardwaj 2012; Singh et al. 2011).

There are grounds supporting continental migration of *P. tritici* between Africa, Europe, and Asia. In many respects, the existence of common pathotypes between Egypt and Iran can be clearly seen in the case of intercontinental migration (Fig. 1). The only difference between pathotypes was the existence of virulence for *Lr1* in Egyptian isolates (McVey et al. 2004). This suggests that the deployment of *Lr1* in Iran has had a strong selective effect on virulence in the pathogen population, because virulence to *Lr1* was not detected in Iran before 2016. Furthermore, pathotype DDPRQ collected from Khuzestan province (southwestern Iran) in 2011 was common with pathotype DDPR in Tunisia in 2007 (Huerta-Espino et al. 2011). This clearly indicates the existence of similar pathotype patterns between both countries. Additionally, the most frequent pathotype in South Africa (pathotype PDR) virulent to *Lr20* was similar to PDTSQ (avirulent to *Lr20*) (Terefe et al. 2009), which was detected in Ardabil province (northwest of Iran) in 2016. Therefore, it is quite likely that southwesterly winds dispersed urediniospores of *P. tritici* from central and eastern parts of Africa to the west and southwest of Iran. Conversely, northeasterly winds could have dispersed urediniospores from Iran to wheat production regions in Africa (Fig. 1). Interestingly, similarity was seen between pathotypes in Iran and those of Europe, especially the Czech Republic (Kolmer et al. 2012). European pathotypes, such as FBMSQ, FCPNQ, FHMQQ, and FHPNQ, differed for virulence to just one *Lr* gene with Iranian ones. Generally, these results support previous research on the leaf rust migration over considerable distances worldwide.

A high level of variability was detected for virulence to *Lr* genes from 2010 to 2017, and it was accompanied by an increased virulence rate from 2010 to 2016 (Fig. 3). Virulence frequencies were generally high for *Lr2c*, *Lr3*, *Lr3bg*, *Lr11*, *Lr14a*, *Lr14b*, *Lr17*, *Lr24*, *Lr30*, and *LrB*; intermediate for *Lr3ka*, *Lr10*, *Lr16*, *Lr18*, and *Lr26*; and low for *Lr1*, *Lr9*, *Lr20*, and *Lr28*, whereas virulence for *Lr2a* was not detected despite previous reports of low IT for this gene in Iran (Afshari et al. 2006; Dadrezaei et al. 2012; Niazmand et al. 2010). Similar to Iran, no virulence for *Lr2a* was reported in Russia (Kolmer et al. 2014), whereas low virulence for this gene was also reported in Europe and South America (Hanzalová et al. 2017; Huerta-Espino et al. 2011; Kolmer et al. 2012). Virulence for *Lr1* and *Lr2a* in countries such as Egypt and India caused minor differences compared with Iranian *P. tritici* pathotypes. It is worth mentioning that no virulence for *Lr1* was detected from 2010 to 2015, but this gene became ineffective for the first time in 2016 when new pathotypes with virulence for this gene emerged (Table 1). The *Lr1*-virulent pathotypes were collected on triticale in Khuzestan, wild barley in Lorestan, and bread wheat in Mazandaran, Ardabil, Lorestan, and Khorasan Razavi provinces. The resistance gene *Lr9* was reported to be effective from 2010 to 2012, consistent with previous studies (Afshari et al. 2006; Dadrezaei et al. 2012; Elyasi-Gomari 2010; Niazmand et al. 2010); however, limited virulence was detected in Ardabil and Mazandaran provinces in 2013 and 2014, respectively, where FNMLS, FPKNQ, FSTTS, and FTKNQ pathotypes with virulence for *Lr9* were detected. Virulence for this gene was only detected in races FNMLS, FPKNQ, FSTTS, and

FTKNQ, all of which were collected from common wheat in Ardabil in 2014, whereas no virulence for this gene was found in 2016 and 2017. Low ITs for *Lr9* have been reported worldwide. For example, no or low virulence for *Lr9* was detected in China (Kolmer 2015), India (Bhardwaj et al. 2010b), Pakistan (Kolmer et al. 2017), South Africa (Huerta-Espino et al. 2011), Europe (Huerta-Espino et al. 2011; Hanzalová et al. 2017), and Canada (McCallum et al. 2017), which makes it potentially an appropriate resistance gene for deployment in wheat breeding programs in combination with other resistance genes. Virulence to *Lr16* increased slightly from 2012 to 2017 in Iran, consistent with previous reports (Afshari et al. 2006; Dadrezaei et al. 2012). Such a slight increase in virulence frequency for *Lr16* has been recorded worldwide (Kolmer et al. 2014; Kolmer 2015; McCallum et al. 2017). Low virulence frequency for *Lr20* was recorded in the north and northwestern regions of Iran in 2014; then, it was found in northeastern Iran in low frequency in 2016. Previous studies had reported virulence for this gene in parts of the country in 2004 and 2010 (Afshari et al. 2006; Dadrezaei et al. 2012). The gene *Lr28* had been previously reported to be effective in Iran (Afshari et al. 2006; Dadrezaei et al. 2012) and still remains effective today. It seems that this gene is effective in many regions worldwide, because either no virulence or low-virulence frequency for *Lr28* has been found in countries, such as China, India, Russia, Syria, and Europe (Hanzalová et al. 2017; Huerta-Espino et al. 2011; Kolmer 2015; Kolmer et al. 2012, 2014). In contrast, the virulence frequency for this gene has been reported to be intermediate or high in the United States (Kolmer and Hughes 2018). In this study, the virulence frequency for *Lr26* was found to be moderate. The number of pathotypes with virulence for *Lr26* fluctuated. Although no virulence for *Lr26* was reported in Iran from 2002 to 2004 (Afshari et al. 2006), a marked increase in the frequency of virulence to this gene has been observed since 1999 (Torabi et al. 2001). Commercial wheat cultivars with the 1RS.1BL translocation and hence, *Lr26* were introduced in the 1950s, and since then, >400 wheat cultivars with this translocation have been produced (Kosman et al. 2004). Iranian wheat cultivars have gained this translocation directly or indirectly from CIMMYT germplasm in which this gene has been used in high frequency. In a study by Dadkhodaie et al. (2011), 58% of accessions in a CIMMYT-derived wheat nursery were reported to carry this translocation.

The variation was widely distributed across local and annual populations. Iranian populations of leaf rust are being affected by international migration of other leaf rust populations, which in turn, account for the variation of the pathogen. Different introductions of *P. tritici* have occurred in various regions. Urediniospores migration exerts an influence on Iranian populations of *P. tritici* in the north, east, and west of Iran. However, the wide distribution of *P. tritici* pathotypes across the major wheat production in Iran caused diverse populations. After all, geographical barriers in Iran, such as mountains or deserts, could have triggered the local populations to emerge. The virulence frequency for *Lr* genes varied in different regions of Iran, but *Lr* genes, such as *Lr1*, *Lr2a*, *Lr9*, *Lr16*, *Lr20*, and *Lr28*, were still effective in some areas. Previous studies confirmed that some Iranian commercial cultivars carry leaf rust resistance genes, such as *Lr1*, *Lr9*, and *Lr26* (Aliakbari Sadeghabad et al. 2016; Kadkhodaie et al. 2012). As a consequence of deploying such resistance genes, distinct populations of *P. tritici* pathotypes can be found in Iran that overcome the genes *Lr1* and *Lr26* in some areas. For example, regional populations of *P. tritici* differ in virulence to these genes.

The pathotypes derived from durum (CDKSR, FDCQS, FJKPQ, FJKRQ, FJTQ, and FJTSQ) produced low ITs on *Lr3ka*, *Lr18*, *Lr20*, and *Lr28*, but they were completely avirulent for *Lr1*, *Lr2a*, *Lr9*, and *Lr26*. These findings are in agreement with those of Goyeau et al. (2012), who showed that isolates from durum wheat were avirulent to *Lr1*, *Lr2a*, *Lr9*, *Lr16*, and *Lr26* in France. Generally, ITs of *P. tritici* pathotypes from durum wheat in Iran were similar to those reported in other studies (Goyeau et al. 2012; Singh et al. 2004). This could indicate that Iranian pathotypes from durum wheat might have originated from other countries and

evolved by mutation and selection, two possibilities discussed above.

Interestingly, isolates from barley and wild barley were the closest to the 2016 bread wheat isolates in 2016. The FKTNQ pathotype from barley and wild barley, virulent for *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr11*, *Lr14a*, *Lr14b*, *Lr16*, *Lr17*, *Lr24*, *Lr26*, *Lr30*, and *LrB*, was similar to pathotypes FKTMQ and FKTTQ from bread wheat. This similarity among virulence phenotypes of wheat and barley could indicate the absence of specific host selection for the pathogen. Collections isolated from wild barley in Bamu National Park and the Margoon Protected Region, Fars province in 2017 are suspected as being derived from a sexual population (ongoing project). This diverse population of *P. tritricina* could be because of the heterogeneous host environment of bread wheat, durum wheat, and other *Poaceae* hosts in Iran and gene flow among them. Increased sampling for other *Poaceae* hosts would have likely detected more pathotypes and increased information about them.

Based on these findings, the pathogen could overwinter at lower latitudes on both wild barley and other *Poaceae*, and the following season, it could migrate to wheat-producing areas in the region. Also, leaf rust infections become established in the fall on winter wheat cultivars that are grown in the southern countries in Asia, such as Iran, and the urediniospores are subsequently wind dispersed to the northern latitudes the following spring and summer by the southerly winds. It is possible that new pathotypes of *P. tritricina* could emerge from established populations through mutation, move to new locations by wind, and cause epidemics. Moreover, the *P. tritricina* populations in Iran are likely influenced by selection owing to the deployment by leaf rust resistance genes in currently grown wheat cultivars. This study gives an indication of different pathotypes prevalent in Iran. Therefore, different resistance genes must be excluded from wheat breeding programs, whereas the combinations of some genes could be still useful in new cultivars of wheat to prevent yield losses. Additionally, new emerging pathotypes in different areas are being reported. Therefore, new genes must be characterized and incorporated in wheat cultivars. The cultivation of wheat cultivars in one region can have a direct effect on the *P. tritricina* population in a region thousands of kilometers away. If wheat cultivars with different resistance genes are grown in different areas, they can prolong the effective lifespan of these genes by decreasing *P. tritricina* epidemiological regions.

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