Race and Virulence Analysis of *Puccinia triticina* in China During 2011 to 2013

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Abstract

Wheat leaf rust, caused by *Puccinia triticina*, is a common fungal disease of wheat in China. In order to identify races and determine the individual virulence of isolates in different wheat-growing regions in China, leaf rust samples collected from 18 provinces in 2011 to 2013 were tested on 37 Thatcher near-isogenic lines each carrying a different single leaf rust resistance gene. A total of 158 races were identified. Races THTT (19.5%), THTS (16.9%), PHTT (7.7%), THJS (5.0%), THJT (4.2%), and PHTS (4.0%) were the most predominant races in 2011 to 2013. All of these races were avirulent to resistance genes *Lr9* and *Lr24*. The two most frequent races, THTT and THTS, were widely distributed. The frequencies of the isolates with virulence to *Lr1*, *Lr2c*, *Lr3*, *Lr16*, *Lr26*, *Lr17*, *LrB*, *Lr10*, *Lr14a*, *Lr3bg*, *Lr14b*,

Lr33, *Lr37*, and *Lr50* exceeded 90%. Frequencies of virulence to *Lr2a*, *Lr3ka*, *Lr11*, *Lr30*, *Lr2b*, and *Lr32* exceeded 70% but were less than 90%. Frequencies of virulence to *Lr18*, *Lr21*, *Lr15*, *Lr23*, *Lr33+34*, *Lr36*, *Lr39*, and *Lr44* were below 70%, whereas the frequency of virulence to *Lr25* was less than 1%. All isolates were avirulent to *Lr9*, *Lr19*, *Lr24*, *Lr28*, *Lr42*, *Lr29*, *Lr38*, and *Lr47*. The identified races and individual virulence frequencies provide a basis for selection of effective leaf rust resistance genes for use in breeding programs and can also provide information for the study of race evolution of *P. triticina*.

Keywords: leaf rust, *Puccinia triticina*, races, virulence, wheat

Wheat leaf rust, caused by *Puccinia triticina* Erikss., is a common fungal disease of wheat (*Triticum aestivum* L.) that causes significant yield losses in major wheat-growing regions worldwide (Bolton et al. 2008; Johnson 1987; Kolmer 2005; Saari and Prescott 1985). Yield losses can vary from 7 to 30% depending upon the level of epidemics and the level of cultivar resistance and may even exceed 50% in exceptionally severe epidemics (Huerta-Espino et al. 2011).

China is the largest wheat producing country in the world with a crop area of 24.1 million hectares and a production of 132 million tons in 2019 (USDA-FAS, <https://www.fas.usda.gov/>). Leaf rust is a common wheat disease in China, especially in the North China Plain, middle and lower reaches of the Yangtze River, and southwest and northwest regions (Liu and Chen 2012). Severe leaf rust epidemics in winter wheat regions of North China and spring wheat regions of Northeast China caused large economic losses from the 1960s to 1980s (Dong 2001; Hu and Roelfs 1985). Since the late 1990s, leaf rust has become increasingly severe in the major wheat production regions, including the North China Plain and southern part of the Huang-Huai Valleys (Zhao et al. 2008). The disease was severe and widespread in 2008 and 2009, relatively low in 2010 to 2011, but became widespread throughout the country again in

2012. The 2012 epidemic affected more than 15 million hectares and caused approximately 3 million metric tons of yield loss (Wu et al. 2019; Zhou et al. 2013). In 2013, leaf rust epidemic occurred in Shandong, Henan, and some areas of Xinjiang (Zhang et al. 2015). The epidemic in 2015 was the most serious in the past 20 years (Zhang et al. 2020). Intermittent epidemics are caused by favorable climatic conditions that support high carryover of uredinal inoculum between crop seasons, and/or loss of resistance in widely deployed wheat cultivars (Wang et al. 2015; Zhang et al. 2015). As the pathogen is most clonally propagated, the selection for virulence to a single resistance gene can result in significant changes in the frequencies of virulence or avirulence for many other genes. Therefore, in addition to the study of resistance genes in wheat cultivars, there is a need to conduct race surveys and determine virulence frequencies for better understanding of population trends in the pathogen in order to predict the risk of epidemics and manage the disease. Race surveys are conducted to monitor the population dynamics of the pathogen, determine whether new races are increasing due to virulence to deployed resistance genes, determine race distribution in major wheat-growing regions, and provide meaningful information to breeders and industry (Kolmer and Hughes 2018). As indicated by Kolmer (2019), continuous long-term data from pathogen virulence surveys provide a valuable resource for population biology studies of *P. triticina* and for the development of leaf rust resistant wheat cultivars. Annual race surveys of the wheat leaf rust pathogen in the United States began in 1926 (Johnston et al. 1968; Mains and Jackson 1926), and the most recent summary of the race situation in the United States from 2000 to 2017 was published by Kolmer (2019). Similar surveys have been conducted in Canada since 1931 (Johnson 1956; Kolmer 1991), and McCallum et al. (2016) summarized the race dynamics of *P. triticina* in Canada from 1999 to 2010. Race surveys have been conducted to analyze the race dynamics of *P. triticina* in Egypt (El-Orabey et al. 2018; McVey et al. 2004) and India (Bhardwaj et al. 2019).

In China, race survey of *P. triticina* began in the 1940s. Wang (1947) first identified three races from the samples collected in Yunnan Province, based on the eight wheat differential genotypes chosen by Johnston and Mains (1932). The later studies in China

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Funding: Key Projects of Hebei Education Department, grant no. ZD2019130; National Key Basic Research Program of China, grant no. 2013CB127700, 2017YFD0300906, and 2018YFD0300502; Natural Science Foundation of Hebei Province, grant no. C2019204040; and Hebei Province Wheat Industry System, grant no. HBCT2018010204.

The author(s) declare no conflict of interest.

Accepted for publication 8 March 2020.

reported the race and virulence dynamics of *P. triticina* from 1986 to 2015 by using a set of Thatcher near-isogenic lines as differentials (Chen et al. 1993, 1998, 2004; Hu and Roelfs 1989; Kolmer 2015; Liu and Chen 2012; Yang et al. 2004; Zhang et al. 2020). We have

analyzed the races of *P. triticina* in China during 2014 and 2015 (Zhang et al. 2020), but the collections of 2011 to 2013 have not been systematically studied for their races and virulence. Therefore, the objectives of this study were to identify races of *P. triticina* from

Fig. 1. Wheat leaf rust collection sites from 2011 to 2013 are shown with black triangles. HE, Hebei; HA, Henan; JS, Jiangsu; SD, Shandong; SX, Shanxi; AH, Anhui; HB, Hubei; ZJ, Zhejiang; FJ, Fujian; SC, Sichuan; GZ, Guizhou; YN, Yunnan; SN, Shaanxi; GS, Gansu; QH, Qinghai; XJ, Xinjiang Autonomous Region; NM, Inner Mongolia Autonomous Region; JL, Jilin. Provincial capitals are marked with red circles.

Table 1. Distribution and frequencies of predominant races of *Puccinia triticina* among single uredinal isolates taken from leaf rust samples collected from wheat fields from 2011 to 2013

Races	Zhejiang		Yunnan		Hebei		Henan		Jiangsu		Shandong		Shaanxi		Shanxi		Sichuan	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
THTT	0	0.0	7	25.9	22	12.2	33	16.0	5	19.2	13	7.9	1	25.0	4	8.2	3	50.0
THTS	0	0.0	5	18.5	29	16.1	48	23.3	0	0.0	39	23.6	0	0.0	1	2.0	1	16.7
PHTT	0	0.0	2	7.4	19	10.6	9	4.4	6	23.1	4	2.4	0	0.0	12	24.5	0	0.0
THJS	0	0.0	1	3.7	9	5.0	15	7.3	0	0.0	13	7.9	0	0.0	1	2.0	0	0.0
THJT	0	0.0	0	0.0	2	1.1	8	3.9	4	15.4	3	1.8	1	25.0	5	10.2	1	16.7
PHTS	0	0.0	2	7.4	11	6.1	12	5.8	0	0.0	3	1.8	0	0.0	4	8.2	0	0.0
PHJT	0	0.0	1	3.7	7	3.9	0	0.0	5	19.2	0	0.0	0	0.0	1	2.0	0	0.0
THPS	0	0.0	0	0.0	0	0.0	5	2.4	0	0.0	9	5.5	0	0.0	0	0.0	0	0.0
KHTS	0	0.0	1	3.7	8	4.4	3	1.5	0	0.0	2	1.2	0	0.0	0	0.0	0	0.0
PHJS	0	0.0	2	7.4	3	1.7	2	1.0	0	0.0	1	0.6	0	0.0	3	6.1	0	0.0
THDS	0	0.0	0	0.0	1	0.6	4	1.9	0	0.0	5	3.0	0	0.0	0	0.0	0	0.0
THTN	0	0.0	0	0.0	2	1.1	3	1.5	0	0.0	3	1.8	0	0.0	1	2.0	0	0.0
PHRT	1	33.3	0	0.0	6	3.3	1	0.5	0	0.0	0	0.0	0	0.0	1	2.0	0	0.0
PHGT	0	0.0	0	0.0	1	0.6	0	0.0	1	3.8	3	1.8	0	0.0	0	0.0	0	0.0
Others	2	66.7	6	22.2	60	33.3	63	30.6	5	19.2	67	40.6	2	50.0	16	32.7	1	16.7
Total	3	...	27	...	180	...	206	...	26	...	165	...	4	...	49	...	6	...

(Continued on next page)

the collections made in 2011 to 2013, determine individual virulence frequencies in China, and to clarify the variation of *P. triticina* by comparing these data with the previous data.

Materials and Methods

Leaf rust occurrence and sample collection. Leaf rust samples were collected from 18 provinces from 2011 to 2013 by researchers from the College of Plant Protection, Hebei Agricultural University, and cooperators from various regions (Fig. 1). Samples were from naturally infected agricultural experiment station plots in various regions, wheat cultivar demonstration plots, and farm fields. Each sample consisted of one to several leaf rusted leaves from a single wheat cultivar or breeding line. The leaves were air dried at room temperature and stored at 4°C until processed.

Multiplication of single-uredinial isolates. Multiplication of single-uredinial isolates was performed as described by Zhang et al. (2020). Urediniospores were collected from dried leaves in each sample and used to inoculate 7-day-old seedlings of susceptible wheat cultivar Zhengzhou 5389. The pots containing inoculated seedlings were then placed in individually isolated containers and incubated at 18°C and 100% relative humidity in darkness for 16 to 24 h. The pots were then moved to a greenhouse at 20 ± 5°C with 12 to 14 h of supplemental fluorescent light. Each pot of seedlings was covered by an open glass cylinder with four layers of gauze at the top to provide ventilation but prevent cross-contamination between pots. When flecks were evident prior to sporulation, one leaf for each sample was trimmed to a single uredinium to represent each isolate. Urediniospores from each isolated uredinium were used to inoculate new seedlings of 'Zhengzhou 5389.' Urediniospores were then collected 10 to 12 days after inoculation and placed in glass tubes for drying, lyophilization, and storing at -20°C.

Race identification. Race identifications were performed as described by Zhang et al. (2020). Two sets of Thatcher near-isogenic lines, the first including 16 lines used as standardized differentials (with individual resistance genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3*; *Lr9*, *Lr16*, *Lr24*, *Lr26*; *Lr3ka*, *Lr11*, *Lr17*, *Lr30*; *LrB*, *Lr10*, *Lr14a*, and *Lr18*) and the second comprising 21 extra differentials (individually with resistance genes *Lr2b*, *Lr3bg*, *Lr14b*, *Lr15*, *Lr19*, *Lr20*, *Lr21*, *Lr23*, *Lr25*, *Lr28*, *Lr29*, *Lr32*, *Lr33*, *Lr33+34*, *Lr36*, *Lr37*, *Lr38*, *Lr42*, *Lr44*, *Lr47*, and *Lr50*), were used to identify races and determine virulence frequencies to individual resistance genes. Thatcher was used as a susceptible control. The wheat lines were planted in 30 × 16 cm plastic trays containing soil mix. Each line was represented by 5 to 10 seedlings. Urediniospores of each isolate, mixed with talcum powder (1:10), were applied to premoistened leaves of 10-day-old seedlings of all 37 wheat lines and Thatcher. The

seedlings were then transferred to a closed humid container for incubation at 18 to 24°C for 16 to 24 h, and subsequently moved to a greenhouse at 20 ± 5°C and a 12- to 14-h photoperiod supplemented with fluorescent light.

Infection types (IT) on the differentials were recorded at 12 days postinoculation based on a 0 to 4 scale (Roelfs 1984), when the disease was fully developed on Thatcher. Races were described on the basis of the four-letter code outlined by Kolmer (1999). To analyze relationships between avirulence/virulence loci, individual infection types were converted to binary descriptors with "0" assigned for avirulent responses (IT 0, ;, 1, 2, and X) and "1" for virulent responses (IT 3 to 4). The population genetic diversity and Nei's population genetic distance were calculated with the software POPGENE version 1.32 (Yeh et al. 1997). A dendrogram (UPGMA, unweighted average method) was constructed based on the Nei's genetic distances using software MEGA 6.0 (Tamura et al. 2013).

Results

Leaf rust occurrence and isolate collection. A total of 2,296 leaf rust samples were obtained from 18 provinces in 2011 to 2013, including 253 samples in 2011, 1,408 in 2012, and 635 in 2013. The distribution and severity of leaf rust varied over the three years; the disease was sporadic and mild in 2011 probably due to dry conditions, but severe in the following years, especially in 2012.

Distribution of races. Nine hundred and seventy-six single-uredinial isolates, representing 42.5% of all collections, were obtained from the 2,296 samples, including 84 isolates in 2011, 547 in 2012, and 345 in 2013. A total of 158 races were identified from the isolates. The six most predominant races were THTT (19.5%), THTS (16.9%), PHTT (7.7%), THJS (5.0%), THJT (4.2%), and PHTS (4.0%); eight races (PHJT, THPS, KHST, PHJS, THDS, THTN, PHRT, and PHGT) had frequencies between 1.0 and 2.2%; and the remaining were less than 1% (Table 1). The number of races in 2011 was the lowest at 35, and the numbers were higher in 2012 and 2013 at 109 and 75, respectively, indicating a correlation between the numbers of samples and numbers of races. Races THTT and THTS predominated over the three years. However, the frequencies of THTT and THTS varied in different years or regions. For example, in 2011, the frequency of THTT (7.1%) was lower than that of THTS (28.6%), while in 2012 and 2013, the frequencies of THTT (17.2% and 26.1%) was higher than those of THTS (14.1% and 18.6%) in these years, respectively. The frequencies of THTT and THTS in Henan were higher than that in Hebei. This indicated that although the predominant races in different years or regions were similar, the proportions of the predominant races varied in different years or regions.

Table 1. (Continued from previous page)

Guizhou		Hubei		Jilin		Anhui		Gansu		Inner Mongolia		Xinjiang		Qinghai		Fujian		Total	
N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
18	37.5	8	27.6	0	0.0	8	38.1	13	27.7	4	7.1	46	50.0	1	33.3	4	30.8	190	19.5
3	6.3	3	10.3	0	0.0	1	4.8	3	6.4	19	33.9	13	14.1	0	0.0	0	0.0	165	16.9
5	10.4	7	24.1	0	0.0	3	14.3	3	6.4	0	0.0	4	4.3	0	0.0	1	7.7	75	7.7
0	0.0	1	3.4	1	100.0	2	9.5	4	8.5	1	1.8	0	0.0	0	0.0	1	7.7	49	5.0
6	12.5	1	3.4	0	0.0	1	4.8	7	14.9	1	1.8	1	1.1	0	0.0	0	0.0	41	4.2
3	6.3	1	3.4	0	0.0	0	0.0	0	0.0	3	5.4	0	0.0	0	0.0	0	0.0	39	4.0
3	6.3	1	3.4	0	0.0	0	0.0	2	4.3	1	1.8	0	0.0	0	0.0	0	0.0	21	2.2
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	5.4	0	0.0	0	0.0	0	0.0	17	1.7
0	0.0	0	0.0	0	0.0	1	4.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	15	1.5
0	0.0	0	0.0	0	0.0	1	4.8	0	0.0	2	3.6	0	0.0	0	0.0	0	0.0	14	1.4
0	0.0	0	0.0	0	0.0	1	4.8	0	0.0	2	3.6	0	0.0	0	0.0	1	7.7	14	1.4
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	3.6	2	2.2	0	0.0	0	0.0	13	1.3
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	2.2	0	0.0	0	0.0	11	1.1
1	2.1	0	0.0	0	0.0	0	0.0	0	0.0	1	1.8	2	2.2	1	33.3	0	0.0	10	1.0
9	18.8	7	24.1	0	0.0	3	14.3	15	31.9	17	30.4	22	23.9	1	33.3	6	46.2	302	30.9
48	...	29	...	1	...	21	...	47	...	56	...	92	...	3	...	13	...	976	...

Six races, THTT, THTS, PHTT, THJS, THJT, and PHTS, were not only high in frequency, but also widely distributed; they were present in many provinces and made up 57.3% of all isolates phenotyped during the period. In the three years, most provinces had at least one of the predominant races, while there were more predominant races in some major wheat-growing regions, such as North China Plain and the middle and lower reaches of the Yangtze River had all the predominant races. This may be related to different planting patterns and planting environments in different regions, such as

Fig. 2. Frequencies of predominant *Puccinia triticina* races during 2011 to 2015.

different wheat cultivars, planting areas, and climates. By comparing the races in different years, THTT, THTS, and PHTS were predominant in all three years, although the frequencies were different. The frequencies of races THJT, THJS, and PHTT increased significantly in 2012 and 2013 compared with 2011. THTT was detected in almost all provinces, except Hebei in 2011, Jilin in 2012, and Zhejiang and Inner Mongolia in 2013.

According to the survey results from 2011 to 2013 and from 2014 and 2015 (Zhang et al. 2020), the frequencies of predominant races fluctuated somewhat randomly apart from the decline of THTS from 2011 and general increase of THTT throughout the five-year period (Fig. 2).

Frequencies of individual virulence genes. All single-uredinial isolates were tested for virulence to 37 known *Lr* genes or gene combinations (Table 2). The virulence frequencies differed across regions and years. The frequencies of isolates virulent to *Lr1*, *Lr2c*, *Lr3*, *Lr16*, *Lr26*, *Lr17*, *LrB*, *Lr10*, *Lr14a*, *Lr3bg*, *Lr14b*, *Lr33*, *Lr37*, and *Lr50* exceeded 90% in all three years (2011 to 2013). The frequencies of isolates virulent to *Lr2a*, *Lr3ka*, *Lr11*, *Lr30*, *Lr2b*, and *Lr32* exceeded 70% but were less than 90% over the three years. The frequencies of virulence to *Lr18*, *Lr21*, *Lr15*, *Lr23*, *Lr33+34*, *Lr36*, *Lr39*, and *Lr44* were below 70%. The frequency of virulence to *Lr25* was less than 1% and no isolate was virulent for *Lr9*, *Lr24*, *Lr19*, *Lr28*, *Lr42*, *Lr29*, *Lr38*, and *Lr47*, indicating their effectiveness throughout the country.

Table 2. Frequency of virulence to wheat leaf rust resistance genes among 976 *Puccinia triticina* isolates taken from leaf rust samples collected from 2011 to 2013

Gene	Zhejiang		Yunnan		Hebei		Henan		Jiangsu		Shandong		Shaanxi		Shanxi		Sichuan	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
<i>Lr1</i>	1	33.3	22	81.5	159	88.3	198	96.1	22	84.6	160	97.0	3	75.0	45	91.8	6	100.0
<i>Lr2a</i>	1	33.3	14	51.9	107	59.4	172	83.5	13	50.0	131	79.4	3	75.0	20	40.8	6	100.0
<i>Lr2c</i>	3	100.0	27	100.0	178	98.9	197	95.6	26	100.0	155	93.9	4	100.0	48	98.0	6	100.0
<i>Lr3</i>	3	100.0	27	100.0	176	97.8	193	93.7	26	100.0	158	95.8	4	100.0	48	98.0	6	100.0
<i>Lr9</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<i>Lr16</i>	3	100.0	27	100.0	173	96.1	199	96.6	26	100.0	164	99.4	4	100.0	47	95.9	6	100.0
<i>Lr24</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<i>Lr26</i>	3	100.0	27	100.0	177	98.3	202	98.1	26	100.0	160	97.0	3	75.0	48	98.0	6	100.0
<i>Lr3ka</i>	2	66.7	22	81.5	133	73.9	147	71.4	14	53.8	111	67.3	2	50.0	29	59.2	4	66.7
<i>Lr11</i>	3	100.0	25	92.6	165	91.7	169	82.0	25	96.2	121	73.3	4	100.0	46	93.9	5	83.3
<i>Lr17</i>	2	66.7	25	92.6	165	91.7	201	97.6	24	92.3	160	97.0	4	100.0	44	89.8	5	83.3
<i>Lr30</i>	2	66.7	22	81.5	133	73.9	147	71.4	14	53.8	112	67.9	2	50.0	29	59.2	4	66.7
<i>LrB</i>	3	100.0	27	100.0	169	93.9	191	92.7	26	100.0	141	85.5	4	100.0	49	100.0	6	100.0
<i>Lr10</i>	3	100.0	27	100.0	164	91.1	181	87.9	26	100.0	133	80.6	4	100.0	44	89.8	6	100.0
<i>Lr14a</i>	3	100.0	26	96.3	175	97.2	199	96.6	25	96.2	158	95.8	4	100.0	45	91.8	6	100.0
<i>Lr18</i>	1	33.3	13	48.1	90	50.0	70	34.0	20	76.9	36	21.8	4	100.0	35	71.4	5	83.3
<i>Lr21</i>	2	66.7	23	85.2	86	47.8	75	36.4	19	73.1	65	39.4	2	50.0	35	71.4	5	83.3
<i>Lr28</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<i>Lr42</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<i>Lr2b</i>	2	66.7	21	77.8	152	84.4	135	65.5	23	88.5	123	74.5	3	75.0	42	85.7	6	100.0
<i>Lr3bg</i>	3	100.0	27	100.0	168	93.3	176	85.4	24	92.3	142	86.1	4	100.0	48	98.0	5	83.3
<i>Lr14b</i>	2	66.7	27	100.0	165	91.7	202	98.1	26	100.0	158	95.8	3	75.0	45	91.8	6	100.0
<i>Lr15</i>	1	33.3	13	48.1	65	36.1	90	43.7	4	15.4	84	50.9	3	75.0	25	51.0	4	66.7
<i>Lr19</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<i>Lr23</i>	2	66.7	20	74.1	131	72.8	173	84.0	18	69.2	135	81.8	2	50.0	21	42.9	3	50.0
<i>Lr25</i>	0	0.0	0	0.0	1	0.6	1	0.5	1	3.8	0	0.0	0	0.0	0	0.0	0	0.0
<i>Lr29</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<i>Lr32</i>	1	33.3	20	74.1	146	81.1	191	92.7	22	84.6	149	90.3	4	100.0	39	79.6	6	100.0
<i>Lr33</i>	3	100.0	27	100.0	178	98.9	200	97.1	25	96.2	142	86.1	4	100.0	48	98.0	6	100.0
<i>Lr33+34</i>	2	66.7	24	88.9	85	47.2	78	37.9	13	50.0	66	40.0	1	25.0	32	65.3	6	100.0
<i>Lr36</i>	0	0.0	14	51.9	121	67.2	154	74.8	15	57.7	116	70.3	2	50.0	20	40.8	3	50.0
<i>Lr37</i>	3	100.0	27	100.0	177	98.3	204	99.0	25	96.2	156	94.5	4	100.0	49	100.0	6	100.0
<i>Lr38</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<i>Lr39</i>	1	33.3	8	29.6	54	30.0	20	9.7	7	26.9	13	7.9	0	0.0	11	22.4	4	66.7
<i>Lr44</i>	1	33.3	12	44.4	99	55.0	146	70.9	21	80.8	71	43.0	4	100.0	25	51.0	2	33.3
<i>Lr47</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<i>Lr50</i>	3	100.0	27	100.0	170	94.4	191	92.7	24	92.3	142	86.1	4	100.0	49	100.0	6	100.0
Total	3	...	27	...	180	...	206	...	26	...	165	...	4	...	49	...	6	...

(Continued on next page)

The survey areas were divided into six regions: North China Plain (Hebei, Henan, Shandong, and Shanxi), middle and lower reaches of the Yangtze River (Jiangsu, Anhui, Hubei, Zhejiang, and Fujian), Southwest China (Yunnan, Sichuan, and Guizhou), Northwest China (Shaanxi, Gansu, Xinjiang, and Qinghai), Northeast China (Jilin), and Inner Mongolia region (Inner Mongolia). Nei's genetic distances were calculated for these regions. There were two major groups during 2011 to 2013 (Fig. 3). Isolates in the North China Plain and Inner Mongolia region were highly related, as were isolates in middle and lower reaches of the Yangtze River, Southwest China, and Northwest China. Isolates in Northeast China were classified as a separate group different from all other regions. Given the short period of three years, these data may show only small differences.

Discussion

Based on the race and virulence analyses, it is evident that the Chinese *P. trititica* population consists of diverse races with a complex virulence structure. A total of 158 races were identified from the collections made in 2011 to 2013. Although the number of races varied from year to year, the predominant races were similar as might be expected based on the frequencies of wheat cultivars. In general, leaf rust epidemics should be closely related to the emergence of new races that can overcome a widely used resistance gene or have a competitive advantage of superior fitness; otherwise, any changes should

be random. The results from 2011 to 2013 indicated that the predominant race structure remained largely unchanged. The frequencies of some new races were very low. Therefore, the epidemics of leaf rust in 2012 and 2013 were likely caused by leaf rust favorable climatic conditions rather than pathogen adaptation to host cultivar resistance. Long-term and large-scale planting of a single wheat cultivar with one or few resistance genes that causes an increase of the frequency of virulence to that gene(s) affects the frequencies of all other avirulence/virulence alleles with which the selected gene was associated because of the clonal propagation of the pathogen. Henan is the largest wheat-growing region in China, but the wheat cultivars planted during the period of 2011 to 2015 were relatively stable, mainly including 'Zhoumai 22,' 'Aikang 58,' 'Zhengmai 366,' 'Xinong 979,' 'Zhongmai 1,' 'Zhengmai 9023,' 'Yanzhan 4110,' 'Yumai 49-198,' 'Luomai 23,' 'Hengguan 35,' 'Zhoumai 16,' 'Xianmai 8,' 'Zhengmai 7698,' and 'Zhongmai 895,' which accounted for more than 75% of the total wheat area (>5.3 million hectares) (Zhang et al. 2020). These cultivars carry a small number of leaf rust resistance genes, such as *Lr1*, *Lr16*, and *Lr26*, which are generally ineffective. In addition to the effects of wheat cultivar resistance, climatic conditions also played an important role in the occurrence and epidemics of leaf rust, especially temperatures and precipitations. The obvious characteristics of the three years were that most of main wheat-growing regions had higher temperatures and precipitations than previous years. These weather conditions

Table 2. (Continued from previous page)

Guizhou		Hubei		Jilin		Anhui		Gansu		Inner Mongolia		Xinjiang		Qinghai		Fujian		Total	
N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
47	97.9	27	93.1	1	100.0	20	95.2	40	85.1	56	100.0	92	100.0	2	66.7	11	84.6	912	93.4
31	64.6	17	58.6	0	0.0	16	76.2	34	72.3	41	73.2	80	87.0	2	66.7	11	84.6	699	71.6
46	95.8	28	96.6	1	100.0	19	90.5	47	100.0	53	94.6	92	100.0	3	100.0	13	100.0	946	96.9
48	100.0	29	100.0	1	100.0	21	100.0	46	97.9	54	96.4	92	100.0	3	100.0	12	92.3	947	97.0
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
45	93.8	29	100.0	1	100.0	21	100.0	43	91.5	55	98.2	88	95.7	3	100.0	13	100.0	947	97.0
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
48	100.0	29	100.0	1	100.0	21	100.0	47	100.0	55	98.2	89	96.7	3	100.0	13	100.0	958	98.2
32	66.7	24	82.8	0	0.0	15	71.4	30	63.8	39	69.6	88	95.7	2	66.7	7	53.8	701	71.8
45	93.8	28	96.6	1	100.0	20	95.2	46	97.9	44	78.6	90	97.8	3	100.0	11	84.6	851	87.2
46	95.8	26	89.7	1	100.0	19	90.5	42	89.4	53	94.6	86	93.5	2	66.7	10	76.9	915	93.8
32	66.7	24	82.8	0	0.0	15	71.4	30	63.8	39	69.6	88	95.7	2	66.7	7	53.8	702	71.9
48	100.0	29	100.0	1	100.0	21	100.0	47	100.0	49	87.5	85	92.4	3	100.0	13	100.0	912	93.4
45	93.8	29	100.0	1	100.0	21	100.0	47	100.0	53	94.6	84	91.3	3	100.0	13	100.0	884	90.6
46	95.8	28	96.6	1	100.0	21	100.0	45	95.7	54	96.4	87	94.6	3	100.0	12	92.3	938	96.1
38	79.2	21	72.4	0	0.0	15	71.4	39	83.0	12	21.4	67	72.8	3	100.0	10	76.9	479	49.1
38	79.2	22	75.9	0	0.0	17	81.0	33	70.2	8	14.3	76	82.6	3	100.0	10	76.9	519	53.2
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
43	89.6	22	75.9	1	100.0	18	85.7	42	89.4	27	48.2	77	83.7	3	100.0	13	100.0	753	77.2
48	100.0	27	93.1	1	100.0	21	100.0	42	89.4	53	94.6	92	100.0	3	100.0	12	92.3	896	91.8
46	95.8	28	96.6	1	100.0	20	95.2	47	100.0	56	100.0	87	94.6	3	100.0	13	100.0	935	95.8
31	64.6	14	48.3	0	0.0	9	42.9	20	42.6	24	42.9	65	70.7	1	33.3	8	61.5	461	47.2
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
17	35.4	14	48.3	1	100.0	8	38.1	20	42.6	53	94.6	51	55.4	1	33.3	5	38.5	675	69.2
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.3
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
42	87.5	22	75.9	0	0.0	17	81.0	35	74.5	52	92.9	79	85.9	1	33.3	10	76.9	836	85.7
47	97.9	28	96.6	1	100.0	20	95.2	46	97.9	53	94.6	85	92.4	3	100.0	12	92.3	928	95.1
38	79.2	19	65.5	0	0.0	12	57.1	25	53.2	12	21.4	69	75.0	3	100.0	8	61.5	493	50.5
13	27.1	15	51.7	1	100.0	7	33.3	23	48.9	47	83.9	34	37.0	2	66.7	7	53.8	594	60.9
46	95.8	29	100.0	1	100.0	20	95.2	47	100.0	54	96.4	90	97.8	3	100.0	13	100.0	954	97.7
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
10	20.8	8	27.6	0	0.0	9	42.9	25	53.2	2	3.6	39	42.4	2	66.7	4	30.8	217	22.2
16	33.3	15	51.7	1	100.0	8	38.1	33	70.2	28	50.0	27	29.3	2	66.7	10	76.9	521	53.4
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
47	97.9	29	100.0	1	100.0	21	100.0	47	100.0	49	87.5	92	100.0	3	100.0	13	100.0	918	94.1
48	...	29	...	1	...	21	...	47	...	56	...	92	...	3	...	13	...	976	...

Fig. 3. Nei's genetic distances comparing virulence frequencies in *Puccinia triticina* isolates between six wheat growing regions of China from 2011 to 2013.

were conducive to the development and spread of wheat leaf rust. From 2011 to 2015, leaf rust was serious every year except 2011. Although the temperatures from 2011 to 2015 were similar, the precipitations in 2012 to 2015 were higher than normal, while that in 2011 was unusually lower (CMA, China Meteorological Administration) and thus leaf rust was low in 2011 but high in 2012 to 2015. Therefore, the general susceptibility of wheat cultivars and weather conditions mentioned above may be the main factors contributing to the epidemics of leaf rust from 2012 to 2015.

Compared with the results of previous surveys, the composition and frequencies of predominant races changed. The predominant races were THT, PHT, and FHB in 1997 to 1999 and PHT, THT, PHJ, and THJ in 2000 to 2006 (Chen et al. 2004; Liu and Chen 2012; Yang et al. 2004). In the present study, THT-, THJ-, and PHT- were still predominant in 2011 to 2013, but the frequencies were significantly different from previous years. For example, the frequency of THT- increased significantly compared with 2000 to 2006, while the frequencies of PHJ- and PHT- decreased. The frequency of THJ- was lower in 2011, but higher in 2012 and 2013 than in 2000 to 2006. Therefore, we speculated that this might be caused by mutations in PHT- or PHJ- form THT- or THJ-, which lead to an increase in the frequencies of THT- and THJ-, and mutations in other races cannot be excluded (Zhang et al. 2020). The difference between PHT- and THT- or between PHJ- and THJ- is virulence versus avirulence to *Lr2a*, so the increased frequencies of THT- and THJ- meant that the frequency of virulence to *Lr2a* was increasing. According to Liu and Chen (2012) and the present study, the frequency of virulence to *Lr2a* did increase from 2000 to 2013, indicating that this gene was continuously losing its effectiveness in different regions (Zhang et al. 2020). Meanwhile, race THT- began to become the most predominant race in China after 2011. Compared with the races in 2014 and 2015, the composition and frequencies of predominant races in 2011 to 2013 were also somewhat different. The difference was that in addition to THTT, THTS, THJT, and THJS, PHTS was the predominant race from 2011 to 2013, and PHJT was the predominant race in 2014 and 2015. We speculated that this was due to the increased frequency of virulence to *Lr18*. If the frequency of virulence to *Lr18* increased, race PHTS may evolve into PHTT, and PHJS into PHJT. From the results of 2011 to 2015, the frequencies of PHTT and PHJT were indeed increasing year after year, while the race frequencies of PHTS and PHJS decreased. During this period, the general trend of other predominant races increased year after year. However, no matter how the frequencies of these races changed, the frequencies of THTT and THTS were always at a high level, despite varying degrees of fluctuation, which indicated that both races have played an important role in the epidemics of wheat leaf rust in recent years. This is not only because of their high virulence, but also because they also have a wide range of distribution and sufficient inoculum, which promotes the spread of the pathogen. Therefore, based on this discussion, we speculate that they may still play a major role in the occurrence of wheat leaf rust in the near future (Zhang et al. 2020).

The tests of *P. triticina* isolates collected from 2011 to 2013 on 37 near-isogenic lines of Thatcher indicated that there were very high

frequencies (>90%) of virulence to genes *Lr1*, *Lr2c*, *Lr3*, *Lr16*, *Lr26*, *Lr17*, *LrB*, *Lr10*, *Lr14a*, *Lr3bg*, *Lr14b*, *Lr33*, *Lr37*, and *Lr50*. The frequencies of virulence to *Lr1*, *Lr2c*, *Lr3*, *Lr16*, *Lr26*, *Lr17*, *Lr10*, *Lr14a*, *Lr3bg*, and *Lr14b* were more than 80%, or even 90% during 2000 to 2006 (Liu and Chen 2012), indicating that these genes were largely ineffective before 2011. The frequencies of virulence to some *Lr* genes increased significantly during this period. For example, some wheat cultivars, such as 'Weimai 8,' 'Shannong 981,' 'Gailiang Yannong 15,' and 'Yumai 18-64,' may carry *Lr2a* or *Lr32*. However, although the frequency of virulence to *Lr2a* was only 29.4% in 2000 to 2006, it reached 71.6% in 2011 to 2013, and even exceeded 80% in 2014 and 2015. The frequency of virulence to *Lr32* was 64.6% in 2000 to 2006, 85.7% in 2011 to 2013, and more than 90% in 2014 and 2015. These data showed that these genes lost effectiveness around 2010. However, the reason for the loss of resistance of wheat cultivars is often related to its long-term and large-scale planting. For example, some common wheat cultivars planted in Henan, such as 'Zhoumai 22,' 'Aikang 58,' 'Zhengmai 7698,' 'Zhongmai 895,' 'Hengguan 35,' 'Zhongmai 1,' 'Zhoumai 16,' 'Yanzhan 4110,' 'Xianmai 8,' 'Zhengmai 366,' 'Xianmai 8,' 'Zhengmai 9023,' 'Zhengmai 369,' and 'Zhengmai 119,' are generally less resistant to leaf rust, and most of these cultivars carry genes *Lr26* or *Lr1*. The widespread application of *Lr26* in China was due to the introduction of the 1BL.1RS translocation in Chinese breeding programs in the 1970s (Li et al. 2010). *Lr1* was also present in many Chinese cultivars (Singh et al. 1999), probably having been introduced with key parents Funo and Abbonanza carrying *Lr1* during the 1950s and 1960s (Li et al. 2010; Zhang et al. 2017a, b). The long-term and widespread use of these genes along with *Lr16* presumably led to the loss of resistance and near-fixation of the virulence alleles in the pathogen population. The frequencies of virulence to leaf rust resistance genes *Lr9*, *Lr24*, and *Lr28* in the United States exceed 30% (Kolmer 2019), whereas virulence has not been recorded in China. Since the 1990s, the frequencies of virulence to *Lr16*, *Lr18*, and *Lr21* in China has been as high as 90% or more, whereas these genes still confer effective resistance in most areas of the United States. Moreover, the frequencies of virulence to *Lr2a*, *Lr2c*, *Lr26*, and *Lr11* in China are generally higher than those in the United States. The reasons for these differences are likely to be historic and then influenced by the genes deployed in wheat cultivars over time.

Based on the virulence frequencies, the Northeast region was separated into different groups (Fig. 3), which may be caused by different geographical environment, planting patterns, and wheat cultivars (Zhang et al. 2020). Inner Mongolia was very similar to the North China Plain in 2011 to 2013 but was classified as a separate group in 2014 (Zhang et al. 2020). The reason for this difference was not only related to the geographic isolation, but also related to the changes of the frequencies of virulence to many *Lr* genes, such as *Lr3ka*, *Lr30*, *Lr21*, *Lr2b*, *Lr15*, and *Lr33+34*, whose virulence frequencies increased significantly during this period. In the present study, the frequencies of virulence to *Lr1*, *Lr2c*, *Lr3*, *Lr16*, *Lr26*, *Lr11*, *Lr17*, *LrB*, *Lr10*, *Lr14a*, *Lr3bg*, *Lr14b*, *Lr32*, *Lr33*, *Lr37*, and *Lr50* ranged from 85.7 to 98.5% from 2011 to 2013. The

frequencies of virulence to these *Lr* genes were relatively stable and continued at high levels from 2011 to 2015. These results indicated that these genes were not effective in China during that period and had little value to breeding programs (Zhang et al. 2020). Resistance genes *Lr9*, *Lr24*, *Lr28*, *Lr42*, *Lr19*, *Lr25*, *Lr29*, *Lr38*, and *Lr47*, which showed high resistance from 2011 to 2015, are still potentially useful, especially if they can be used in combinations. Moreover, an approach of combining all-stage and adult plant resistance genes should be utilized in breeding programs for developing wheat cultivars with durable, high-level resistance to leaf rust.

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