Race and Virulence Dynamics of *Puccinia triticina* in China During 2000–2006

T. G. Liu and W. Q. Chen, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193

Abstract

Liu, T. G., and Chen, W. Q. 2012. Race and virulence dynamics of *Puccinia triticina* in China during 2000–2006. Plant Dis. 96:1601–1607.

Wheat leaf rust, caused by *Puccinia triticina*, is an important foliar disease of wheat in China. The dynamics of races and virulence in *P. triticina* populations in China during 2000 to 2006 were studied. Leaf rust samples were collected during surveys of wheat fields and trap nurseries in 16 provinces, and provided by coworkers throughout China. The virulence of single-pustule isolates was determined on near-isogenic Thatcher lines for leaf rust resistance genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3*, *Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, and *Lr30*, and races were denominated using the *Prt* code system. During 2000 to 2006, 79 races

were identified from a total of 613 isolates. Races PHT (23.7%), THT (14.7%), PHJ (11.4%), and THJ (4.2%) were the four common races, all avirulent to *Lr9* and *Lr24*. The frequency of isolates with virulence to *Lr1*, *Lr2c*, *Lr3*, *Lr11*, *Lr16*, *Lr17*, and *Lr26* was over 80%, and these isolates were widely distributed in China, whereas the frequencies of virulence to *Lr9*, *Lr19*, *Lr24*, *Lr25*, *Lr28*, and *Lr29* were 0.2 to 2.5%. The diversity of virulence phenotypes of Chinese *P. triticina* populations appeared to increase from 2000 to 2006. *P. triticina* races and virulences in China appear to be isolated from those in other countries.

Wheat leaf rust, caused by *Puccinia triticina* Erikss. (31), is the most common of the three rust diseases of wheat (*Triticum aestivum* L.) throughout the world. In China, it occurs annually on about 15 million ha of wheat, and the disease is especially common in the southwest and northwest areas, middle and lower Yangtze River Valley, and the southern Huang-Huai-Hai region (Huang [Yellow] River, Huai River, and Hai River regions). Severe leaf rust epidemics in 1969, 1973, and 1975 resulted in significant yield losses in north China (13). Monitoring the dynamics and variation of virulence to leaf rust resistance genes in the rust population provides the basis for anticipatory breeding of cultivars with optimum rust resistance, prediction of disease epidemics, and integrated disease control (7,22,25).

Surveys of *P. triticina* races in China were first carried out in the 1940s (35), and three races (race 1, 63, and 123) were identified from samples from Yunnan Province based on infection type (IT) to the eight international differentials used by Johnston and Mains (17). However, the differential set of eight cultivars was not adequate for fully differentiating Chinese races of *P. triticina*. In the early 1970s, race surveys of *P. triticina* were conducted by several institutions, such as the Institute of Plant Protection, Chinese Academy of Agricultural Sciences; Henan, Shandong, and Helongjiang Academies of Agricultural Sciences; and Hebei Agricultural University. In 1977, eight wheat genotypes (Lovrin 10, 6068, IRN66-331, Redman, Dongfanghong 3, Fengchan 3, Baiyoubao, and Taishan 4) were chosen as Chinese unified *P. triticina* differentials based on their abilities to differentiate *P. triticina* isolates under varying temperatures and light regimes. Three races that were found at high frequency were designated as Chinese leaf rust (CLR)1 to CLR3 (37). Annual surveys of race and virulence of *P. triticina* using this differential set with some complementary wheat genotypes have been conducted throughout the major wheat-growing areas by the Chinese National Wheat Rust Collaborative Group

since 1977 (6,14,23,36,40). The major virulence patterns were designated as CLR races with sequential numbers based on their chronological detection. By 1996, there were a total of 48 CLR races of *P. triticina* described in China.

Starting in 1989, a set of near-isogenic Thatcher lines developed by Dyck and Samborski (9) was introduced in China and has been used for surveying virulence of *P. triticina* from different wheat-growing areas of China (3,4,14,39,41). The races of *P. triticina* were designated using the North American *Prt* code system (23).

Over 60 officially designated genes for resistance to leaf rust (*Lr* genes), and many other temporarily designated *Lr* genes, have been described in wheat (27). Most of the genes condition race-specific resistance in a gene-for-gene relationship with *P. triticina*, and some have been used widely (26,27). However, when resistance to an obligate pathogen such as rust is determined by a single gene, it is common to observe within a few years the evolution of a new pathogenic race capable of overcoming the resistance. Annual surveys of *P. triticina* virulence frequencies have provided valuable data for wheat-breeding programs and also helped to track virulence evolution over time. In general, changes in races and their virulences have corresponded to changes in wheat cultivars deployed in commercial wheat production. Similar surveys have been conducted in Canada since 1931 (15), the United States since 1926 (16,19,20), Australia since 1920 (38), and Mexico since 1988 (32). The objectives of this study were to determine the races and virulence dynamics of the *P. triticina* population in China during 2000 to 2006, and to compare the data with those of previous surveys.

Materials and Methods

Collection of leaf rust isolates. Leaf rust samples were usually collected in the spring and summer months when the fungus was most common. Each year, samples were taken from widely grown cultivars along a field survey route by researchers from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, and cooperators from regional Plant Protection Stations located in different epidemic zones throughout China (Fig. 1). Some samples were collected from trap nurseries established at 29 locations in 16 provinces for monitoring effectiveness of resistance to stripe rust, leaf rust, stem rust, and powdery mildew (5). Samples with visible *P. triticina* uredinia were temporarily stored at 4°C in a refrigerator until multiplication. All samples were sourced

Corresponding author: W. Q. Chen, E-mail: wqchen@ippcaas.cn

Accepted for publication 22 May 2012.

from field collections under natural infection. A collection consisted of one to several leaves with uredinia from a single plant or cultivar. The leaves were air dried and stored at 4°C until spores were collected for inoculation and increase. Collections from inoculated nurseries were not included in the study.

Multiplication of single-pustule isolates and harvest. The dried leaves were put on wet filter paper in covered petri dishes at room temperature overnight. Uredinia were collected with a scalpel and inoculated onto 7- to 8-day-old seedling leaves of susceptible ‘Zhengzhou 5389’ planted in pots with low-nutrient soils. After inoculation, wheat plants were then put in a dew chamber at 18 to 24°C in darkness for 18 to 24 h. The inoculated wheat seedlings were then moved into a greenhouse with supplemental fluorescent light at 20 to 28°C in the daytime and 15 to 18°C at night. Each inoculated plant was placed in a glass cylinder with four layers of gauze on top to reduce cross-contamination. Using the same procedure, single-pustule isolates were increased on susceptible seedling plants. Isolates were collected by tapping or shaking the glass cylinder with an iron rod, and the urediniospores were put into tubes in desiccators.

Race and virulence identification. To determine races and virulences of *P. triticina*, the following set of near-isogenic Thatcher lines, each bearing a single resistance gene, was employed: *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3*, *Lr3bg*, *Lr3ka*, *Lr9*, *Lr10*, *Lr14a*, *Lr14b*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr19*, *Lr23*, *Lr24*, *Lr26*, *Lr29*, *Lr30*, *Lr32*, and *Lr33*. Also used were the lines Hussar, Thew, Transec(Awned), Gatcher, and CM2D-2M, which carried *Lr11*, *Lr20*, *Lr25*, *Lr27+31*, and *Lr28*, respectively. ‘Thatcher’ was used as a susceptible check. All of the tested wheat lines were grown together in 60-by-40-by-20-cm plastic trays. Each line was represented by 5 to 10 seedlings. Urediniospores of each isolate were inoculated onto 7-day-old seedlings of the 28 wheat lines in an automatic inoculation tower using urediniospores at 5 to 10 mg/ml and 0.25 ml of Soltrol 170 (Phillips 66 Company). After drying for 1 h, the seedlings were put into a metal cylinder with a

plastic cover for incubation at 18 to 24°C for 24 h at 100% relative humidity, and then placed on a bench in a greenhouse at 15 to 28°C with 8 h of supplemental fluorescent lighting.

IT scoring. The ITs on the first leaves were scored at 12 to 16 days post inoculation, when uredinia on Thatcher were fully developed. A 0-to-4 scale (24,31) was used for ITs: 0 = no visible sign of infection; semicolon (;) = no uredinia but small hypersensitive necrotic or chlorotic flecks of varying size; 1 = small uredinia surrounded by necrosis; 2 = small to medium uredinia surrounded by chlorosis, a green island that may be surrounded by chlorotic border; X = random distribution of variable-sized uredinia and flecks on single leaf with a pure culture; 3 = medium-sized uredinia that may be associated with chlorosis; 4 = large uredinia without chlorosis; a plus symbol (+) = slightly larger uredinia than expected for the IT; minus symbol (−) = slightly smaller uredinia than expected for the IT; and c = more chlorosis than expected for the IT. Where different ITs were produced on the same line, the most common IT was listed first. A three-letter code based on the low or high ITs of each isolate to the 12 differential Thatcher lines was employed to describe races of *P. triticina* (18,24).

Race or virulence diversity measurement. Diversity of race or virulence of *P. triticina* within areas and between years was determined with the Chao-1 estimator (S^*I) (2,8) and the calculator is provided courtesy of Niklaus J. Grünwald: $S^*I = S_{obs} + (a^2/2b)$, where S_{obs} is the number of races observed, a is the number of races observed just once, and b is the number of races observed just twice. Chao-1 is a nonparametric estimator which is not affected by sample size. In addition, a standard deviation (Sd) is computed using $Sd = b[(a/[4b])^4 + (ab)^3 + (a/[2b])^2]$. The program prints “−1” to indicate that $b = 0$ and the Chao estimator cannot be computed.

The rarefaction method allows comparison of the number of species or, in this case, races found in two regions when the sampling effort differed. One would expect that greater sampling effort would yield a larger sample and more races. Rarefaction uses the

Fig. 1. Agroecological areas for *Puccinia triticina* in China where leaf rust samples were collected: area 1, mixed wheat types but primarily winter wheat; areas 2 and 3, mixed wheat types but primarily facultative habit (winter or spring); area 4, mostly northern-adapted facultative wheat and southern-adapted spring wheat; and area 5, mixed wheat types but primarily winter habit. Blue triangles show trap nursery sites and red dots indicate the provincial capitals. Area 1, North China: BJ = Beijing city, HE = Hebei province, SX = Shanxi province; area 2, Huang-Huai-Hai regions: SD = Shandong province, HA = Henan province; area 3, Yangtze River valley: HB = Hubei province, JS = Jiangsu province, AH = Anhui province; area 4, Southwest China: SC = Sichuan province, GZ = Guizhou province, YN = Yunnan province, CQ = Chongqing city; and area 5, Northwest China: SN = Shaanxi province, GS = Gansu province, QH = Qinghai province, NX = Ningxia autonomous region, XJ = Xinjiang Uygur Autonomous Region.

data from the larger sample to answer the question, “How many races would have been found in a smaller sample from that region?”. If n individuals composed the sample in the less-sampled region, rarefaction takes hypothetical subsamples of n individuals from the more-sampled region and calculates the average number of races in such subsamples. This average can be compared with the number of races actually found in the less-sampled region. (The method computes a variance and standard deviation to facilitate interpretation of significant differences.)

Because sample sizes varied greatly, from 18 in 2002 to 158 in 2006, the diversity in a subsample of 18 isolates (Table 1, Sub₁₈) and that in a subsample of 35 isolates (Table 2, Sub₃₅) was recalculated by the rarefaction calculator to allow comparison of diversity in Chinese collections from different years at the same scale. In theory, any size of subsample could be chosen (i.e., $n = 18, 35, 126$, and so on) but, normally, the smallest sample size of all the collections is used. In 2005, 126 Chinese isolates were tested for virulence profiles and 30 races were identified. In the United States in 2005, 797 *Lr* single-uredinial isolates were tested and 72 virulence phenotypes (given in five letters, such as “BBBDB”) of *P. tritricina* were found. The same 12 Thatcher lines were used for race identification in China and the United States; thus, considering only the first three letters, such as “BBB,” 46 virulence phenotypes were detected in 2005 in the United States. The Sub₁₂₆ variable was computed to allow valid comparison of *P. tritricina* virulence diversity in the sample of 126 isolates in China and the sample of 797 isolates in the United States.

The Statistical Analysis System (SAS edition 8.10e; SAS Institute) was used for calculating the χ^2 statistic.

Results

Dynamics of races. In total, 613 single-uredinial isolates from the different wheat-growing areas of 16 provinces were tested for virulence, with 56 isolates in 2000, 59 in 2001, 18 in 2002, 53 in 2003, 143 in 2004, 126 in 2005, and 158 in 2006. In total, 79 races

of *P. tritricina* were detected in China among the 613 single-uredinial isolates. Predominant races were PHT at 23.7%, THT at 14.7%, PHJ at 11.4%, THJ at 4.2%, PCG at 4.1%, and seven other races for which virulence frequencies were between 1.6 and 2.6% (Table 3). The virulence formulae of the 12 races with frequency greater than 1%, averaging across areas, are listed in Table 3. The change in frequency of five dominant races with frequencies more than 4% is shown in Figure 2. The most common races, PHT and THT, decreased in frequency but also fluctuated from year to year (Fig. 2). The frequency of PHT was 25.0% in 2000, increased to 33.3% in 2002, declined to 24.5% in 2004, increased to 34.9% in 2005, and dramatically decreased to 10.8% in 2006. The frequency of race THT dropped from 41.1% in 2000 to 3.2% in 2006 but fluctuated in between. The frequency of PCG stood at 2 to 7%, except when it was not detected in 2001 and 2002.

Distribution of races. Five races (PHT, THT, PHJ, THJ, and PCG) were detected in all five regions and, together, they made up 58.1% of all isolates during 2000 to 2006 (Table 3). In north China (area 1), 20 races were found among the 74 single-uredinial isolates tested. Races PHT (21.6%), THT (20.3%), and PHJ (14.9%) were the three most common races in this area. In the Huang-Huai-Hai regions (area 2), five races accounted for 120 of 177 isolates from this area: PHT (23.2%), THT (20.3%), PHJ (9.6%), THJ (8.5%), and THD (6.2%). In the Yangtze River Valley (area 3), 11 races were found among the 35 isolates tested; PHT (31.4%), THT (25.7%), PHJ (11.4%), and PHD (8.6%) were the 4 most common races. In the southwest provinces (area 4), PHT (19.3%), THT (7.2%), PHJ (13.0%), PCG (6.3%), PCR (4.8%), and DHJ (4.8%) accounted for more than 50% of the isolates tested. In the northwest provinces (area 5), 36 races were found among 120 single-uredinial isolates tested; PHT (30.8%), THT (12.5%), PHJ (9.2%), and FHT (8.3%) were the 4 dominant races. Among regions, χ^2 tests indicated significant differences in the distribution of *P. tritricina* races ($\chi^2 = 459.7$, DF = 312, $P < 0.001$), especially for PHT, THJ, and PHJ ($\chi^2 = 16.5$, DF = 8, $P = 0.0361$).

Table 1. Chao-1 estimator (S^*I) measures the phenotypic diversity and richness for *Puccinia tritricina* populations in China from 2000 to 2006

Population ^b	Year (number of isolates) ^a							
	2000 ($n = 56$)	2001 ($n = 59$)	2002 ($n = 18$)	2003 ($n = 53$)	2004 ($n = 143$)	2005-C ($n = 126$)	2005-U ($n = 797$)	2006 ($n = 158$)
S^*I	46.2	19.0	15.3	61.3	37.3	58.1	46.4	68.8
S_d	16.2	8.3	5.9	24.2	5.7	15.1	0.7	12.4
Sub ₁₈	7.8	6.7	9.0	9.3	9.9	9.6	11.5	13.0
S_{d18}	1.6	1.2	0	1.5	1.7	1.7	1.6	1.6
Sub ₁₂₆	...	...	...	...	...	30	29.4	...
S_{d126}	...	...	...	...	...	0.0	2.4	...

^a In 2005, 2005-C indicates isolates in China and 2005-U indicates isolates in the United States.

^b The Chao-1 estimator of phenotypic diversity is calculated as $S^*I = S_{obs} + (a^2/2b)$, where S_{obs} is the number of races observed, a is the number of races observed just once, and b is the number of races observed just twice. S_d (standard deviation of S^*I) = $b[(a/[4b])^4 + (a/b)^3 + (a/[2b])^2]$. Sub₁₈ means the average number of races in annual collections when sample sizes >18 were recalculated at the 18-isolate scale by rarefaction. Sub₁₂₆ allows comparison of the richness of *P. tritricina* races among years. S_{d18} = standard deviation of races. Sub₁₂₆ = recalculation by rarefaction of the number of U.S. and Chinese races in 2005 as if sample sizes in both countries had been 126. S_{d126} = standard deviation of races.

Table 2. Chao-1 estimator (S^*I) of the phenotypic diversity and richness of *Puccinia tritricina* populations from five geographical areas in China from 2000 to 2006

Population ^b	Area (number of races) ^a				
	1 ($n = 20$)	2 ($n = 33$)	3 ($n = 11$)	4 ($n = 36$)	5 ($n = 50$)
S^*I	80.5	105.3	29.0	67.5	92.3
S_d	37.1	36.1	15.1	14.4	17.3
Sub ₃₅	13.4	14.1	11	16.2	18.3
S_{d35}	1.6	2.0	0	2.2	2.2

^a Area 1: BJ = Beijing city, HE = Hebei province, SX = Shanxi province; area 2: SD = Shandong province, HA = Henan province; area 3: HB = Hubei province, JS = Jiangsu province, AH = Anhui province; area 4: SC = Sichuan province, GZ = Guizhou province, YN = Yunnan province, CQ = Chongqing city; area 5: SN = Shaanxi province, GS = Gansu province, QH = Qinghai province, NX = Ningxia autonomous region, XJ = Xinjiang Uygur Autonomous Region. Total number of races = 79.

^b The Chao-1 estimator of phenotypic diversity is calculated as $S^*I = S_{obs} + (a^2/2b)$, where S_{obs} is the number of races observed, a is the number of races observed just once, and b is the number of races observed just twice. S_d (standard deviation of S^*I) = $b[(a/[4b])^4 + (a/b)^3 + (a/[2b])^2]$. Sub₃₅ = average number of races in regional collections with sample size >35, recalculated at the 35-isolate scale by rarefaction. Sub₃₅ allows comparison of the richness of *P. tritricina* races among regions. S_{d35} = standard deviation of races.

Frequencies of virulence genes. The 613 isolates of *P. trititica* were tested for virulence to 28 known *Lr* genes or gene combinations (Table 4). Frequencies of virulence differed among the regional populations of *P. trititica* in China during the past 7 years. Less than 30% of all isolates were virulent to *Lr2a*, *Lr9*, *Lr15*, *Lr19*, *Lr24*, *Lr25*, *Lr28*, or *Lr29*. Less than 3% of all isolates were virulent to *Lr9*, *Lr19*, *Lr24*, *Lr25*, *Lr28*, or *Lr29*, whereas virulence to *Lr2c*, *Lr3*, *Lr14a*, *Lr26*, or *Lr33* was common throughout the Chinese areas surveyed (frequencies of more than 90%). Virulences to *Lr3ka* and *Lr20* were also found with frequency of 55.8 and 36.2%, respectively, in the total population. The weighted average frequency across years of virulence to *Lr2b*, *Lr3bg*, *Lr10*, *Lr14b*, *Lr18*, *Lr23*, *Lr27+31,10*, *Lr30*, and *Lr32* was high (62.0 to 89.5%). The results suggest that the corresponding leaf rust resistance genes *Lr9*, *Lr19*, *Lr24*, *Lr25*, *Lr28*, and *Lr29* are effective against the wheat leaf rust population in China at present, which is basically consistent with the results of 1997 to 1999, except for the decreasing frequency of virulence to *Lr25* (30). Virulence to *Lr2a*, *Lr3ka*, *Lr11*, and *Lr30* is still common, even though it may have declined in recent years (Table 4). The frequency of virulence to *Lr1*, *Lr2c*, *Lr3*, *Lr16*, *Lr17*, and *Lr26* was high (73.6 to 100%) during 2000 to 2006 but fluctuated from year to year, which indicated that wheat cultivars with those genes were widely planted in the Chinese wheat-growing area. The weighted average frequency of virulence to *Lr1*, *Lr2c*, *Lr3*, *Lr16*, *Lr17*, and *Lr26* was 67.0, 94.4, 74.0, 96.6, and 98.2%, respectively during 1992 to 1996 in China (3) and 87.1, 98.0, 91.0, 82.1, and 85.0%, respectively, during 2000 to 2006. Among the 613 isolates tested, virulence to *Lr9* was only found once in Yunnan province in 2005. Nine isolates were virulent to *Lr19* (one in Henan in 2007, two in Shandong in 2003 and 2007, one in Hubei in 2007, two in Gansu in 2007, two in Yunnan in 2003 and 2007, and one in Sichuan province in 2007). Two isolates showed virulence to *Lr24*; both were found in 2002, one in Henan and the other in Xinjiang. In all, 2, 5, and 13 isolates had virulence to *Lr25*, *Lr28*, and *Lr29*, respectively. Combined virulence to *Lr24* and *Lr29* was found only once (in Emin county, Xinjiang Uygur Autonomous Region in 2002).

Diversity of races and virulence. The *P. trititica* population in China was highly diverse, with 79 races being detected in samples collected from 2000 to 2006. In general, *P. trititica* populations sampled from 2000 to 2006 showed a tendency toward increasing

race diversity (Fig. 3; Table 1), which was compared at the same scale of 18 samples by rarefaction calculator (11). The Chao-1 estimator fluctuated between years that showed different diversities (Fig. 3; Table 1). The *P. trititica* population in Huang-Huai-Hai regions of China (area 2) was the most diverse of the population regions according to the Chao-1 estimator, whereas those in northwest China (area 5) and in north China (area 1) displayed less diversity. The diversity in the Yangtze River Valley was lowest compared with other regions (Table 2).

Discussion

The *P. trititica* populations in China appeared to be somewhat stable among regions but changed on an annual basis with respect to virulence. The frequency of PHT remained basically stable from 2000 to 2005 but dropped in 2006. The frequency of THT displayed a decreasing tendency from 2000 to 2006. Both races are virulent to *Lr1*, *Lr2c*, *Lr3*, *Lr11*, *Lr16*, *Lr17*, *Lr26*, *Lr3ka*, or *Lr30* and are avirulent to *Lr9* and *Lr24*. In 1997 to 1999 (30), THT was detected at higher average frequency (22.1%) than from 2000 to 2006 (14.7%) whereas the frequency of PHT appeared fairly stable between the two sampling periods (20.8 to 23.7%). These two races were distributed widely throughout China, and were the most common races of *P. trititica* in China during and prior to the pe-

Fig. 2. Frequency changes of predominant races of *Puccinia trititica* on wheat in China during 2000 to 2006.

Table 3. Distribution and frequencies of predominant races of *Puccinia trititica* from samples collected in Chinese wheat fields in 2000 to 2006^a

Races	Avirulence/virulence formulae	North China ^b		Huang-Huai-Hai regions ^c		Yangtze River valley ^d		Southwest China ^e		Northwest China ^f		Total ^g	
		N	%	N	%	N	%	N	%	N	%	N	%
PHT	2a,9,24/1,2c,3,16,26,3ka,11,17,30	16	21.6	41	23.2	11	31.4	40	19.3	37	30.8	145	23.7
THT	9,24/1,2a,2c,3,16,26,3ka,11,17,30	15	20.3	36	20.3	9	25.7	15	7.3	15	12.5	90	14.7
PHJ	2a,3ka,9,24,30/1,2c,3,16,26,11,17	11	14.9	17	9.6	4	11.4	27	13.0	11	9.2	70	11.4
THJ	9,24,3ka,30/1,2a,2c,3,16,26,11,17	5	6.8	15	8.5	1	2.9	3	1.5	2	1.7	26	4.2
PCG	2a,9,16,24,3ka,17,30/1,2c,3,11,26	3	4.1	6	3.4	0	0.0	13	6.3	3	2.5	25	4.1
PCR	2a,9,16,17,24/1,2c,3,26,3ka,11,30	1	1.4	3	1.7	1	2.9	10	4.8	1	0.8	16	2.6
PHD	2a,3ka,9,11,24,30/1,2c,3,16,17,26	1	1.4	6	3.4	3	8.6	5	2.4	1	0.8	16	2.6
THD	3ka,11,9,24,30/1,2a,2c,3,16,26,17	3	4.1	11	6.2	0	0.0	0	0.0	2	1.7	16	2.6
FHT	1,2a,9,24/2c,3,3ka,11,16,17,26,30	0	0.0	3	1.7	0	0.0	3	1.5	10	8.3	16	2.6
DHJ	1,2a,3,9,24,3ka,30/2c,16,17,11,26	0	0.0	0	0.0	0	0.0	10	4.8	2	1.7	12	2.0
THP	9,24,11/1,2a,2c,3,16,26,3ka,17,30	0	0.0	5	2.8	1	2.9	1	0.5	3	2.5	10	1.6
NHJ	2a,3,9,24,3ka,30/1,2c,16,17,11,26	0	0.0	1	0.6	0	0.0	8	3.9	1	0.8	10	1.6
Other ^h	...	19	25.7	33	18.6	5	14.3	72	34.8	32	26.7	161	26.3
Total ⁱ	...	74	...	177	...	35	...	207	...	120	...	613	...

^a Data are numbers and percentages of isolates.

^b North China (area 1): BJ = Beijing city, HE = Hebei province, SX = Shanxi province.

^c Huang-Huai-Hai regions (area 2): SD = Shandong province, HA = Henan province.

^d Yangtze River valley (area 3): HB = Hubei province, JS = Jiangsu province, AH = Anhui province.

^e Southwest China (area 4): SC = Sichuan province, GZ = Guizhou province, YN = Yunnan province, CQ = Chongqing city.

^f Northwest China (area 5): SN = Shaanxi province, GS = Gansu province, QH = Qinghai province, NX = Ningxia autonomous region, XJ = Xinjiang Uygur Autonomous Region.

^g Weighted average frequencies during 2000 to 2006.

^h Races were detected no more than six times (<1% frequency) within the 7 years.

ⁱ Total isolates for test.

riod of this study. PHT and THT occurred at very similar frequency (between 20.3 and 23.2%) in north China (area 1) and the Huang-Huai-Hai region (area 2) but occurred at variable frequency in the other wheat-growing areas; for example, 30.8 to 12.5% in north-west China (area 5), 19.3 to 7.3% in southwest China (area 4), and 31.4 to 25.7% in the Yangtze River Valley (area 3) during 2000 to 2006 (Table 3). This probably reflects the selective effects of wheat cultivars with different leaf rust resistance genes in the different wheat-growing areas.

The Chinese *P. tritricina* population exhibited great diversity with a complex virulence structure, which could be mostly ascribed to the diversity of wheat genotypes planted in various wheat-growing regions. In total, 79 races were detected during the period 2000 to 2006. Since the 1990s, wheat breeding for rust resistance, with emphasis on the development of slow-rusting or durably resistant cultivars, has been a priority in parts of China such as Sichuan, Gansu, Henan, Shaanxi, and Yunnan provinces (12). Cultivars with different resistance genes or gene combinations would be expected to select different matching virulences in the pathogen. However, despite a large number of *P. tritricina* races being detected across the country over the 7-year period, only a few races such as PHT, THT, and PHJ appeared with relatively high frequencies, suggesting that the situation of the most common Chinese wheat cultivars having simple resistance to leaf rust has remained unchanged (21,33).

In previous reports (21,42), 16 named genes (*Lr1*, *Lr2a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr13*, *Lr14a*, *Lr16*, *Lr17a*, *Lr18*, *Lr20*, *Lr23*, *Lr24*, *Lr26*, *Lr34*, and *LrZH84*) were postulated to be present in 265 Chinese wheat cultivars but *Lr26* and *Lr16* were most frequently detected, with frequencies of 47.2 and 20.4%, respectively. Most of the 16 genes were ineffective against the Chinese *P. tritricina* population. The high frequency of virulence to *Lr26* was due to the widespread use of the 1BL/1RS translocation bearing *Yr9* and *Lr26* in Chinese breeding programs since the 1970s (21,33). For example, 'Aikang 58' (bearing *Lr26*) was a popular commercial cultivar in the Huang-Huai-Hai region, planted on more than 0.5 million ha after its release in 2005. 'Zhengmai 9023', which carried *Lr16* and an unknown *Lr* gene, was released at Henan and in the Yangtze

River Valley (at Hubei in 2001 and Jiangsu and Anhui in 2002). Since 2003, Zhengmai 9023 has been planted every year and has become the predominant commercial cultivar in the Huang-Huai-Hai region, with a cropped area of more than 2 million ha in 2006. Since the 1980s, 'Fan 6' and its derivatives with the resistance gene *Lr16* have become the leading cultivars in the southwestern regions and the mid-valley of the Yangtze River. Those cultivars provided favorable conditions for the directional selection of virulence in Chinese *P. tritricina* populations, and at least partially accounted for the high frequency of virulence to *Lr26* and *Lr16*.

The frequency of *Lr1* in 265 wheat cultivars or lines tested was about 7.6% (21,33), and 33.9% by polymerase chain reaction (PCR) detection using *Lr1*-specific primers in 116 Chinese-released cultivars (unpublished data), which probably accounts for the high frequency of virulence to *Lr1* in Chinese leaf rust populations. Other *Lr* genes such as *Lr9*, *Lr19*, and *Lr24* are infrequently present in Chinese wheat cultivars, as detected by gene postulation and molecular methods (21,33), which could explain the low virulence frequency of the matching virulences in the Chinese *P. tritricina* population. Consequently, diversity of planted wheat cultivars

Fig. 3. Diversity and richness of the *Puccinia tritricina* population in China from 2000 to 2006 indicated by the Chao-1 estimator (S^*I) and Sub_{18} . See the text for calculation of the Chao-1 estimator. Sub_{18} indicates the average richness of *P. tritricina* races among samples with ≥ 18 isolates calculated by rarefaction and using subsamples of 18.

Table 4. Frequency of virulence to wheat leaf rust resistance genes among 613 *Puccinia tritricina* isolates collected in China during 2000 to 2006

Testers	<i>Lr</i> genes	Frequency (%)							Fx ^a
		2000	2001	2002	2003	2004	2005	2006	
TcLr1	<i>Lr1</i>	85.7	93.2	83.3	73.6	92.3	89.7	83.5	87.1
TcLr2a	<i>Lr2a</i>	53.6	50.9	27.8	30.2	13.3	31.8	25.3	29.4
TcLr2b	<i>Lr2b</i>	69.1	93.2	81.3	88.7	90.9	76.2	88.6	85.1
TcLr2c	<i>Lr2c</i>	98.2	98.3	100.0	94.3	99.3	97.6	98.1	98.0
TcLr3	<i>Lr3</i>	85.7	100.0	100.0	98.1	96.5	92.7	79.8	91.0
TcLr3bg	<i>Lr3bg</i>	66.1	98.3	88.9	94.3	96.5	93.6	79.1	88.4
TcLr3ka	<i>Lr3ka</i>	75.0	86.4	66.7	64.2	42.7	74.6	30.3	55.8
TcLr9	<i>Lr9</i>	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.2
TcLr10	<i>Lr10</i>	82.1	100.0	94.1	96.2	95.1	93.7	77.2	89.5
Hussar(W976)	<i>Lr11</i>	89.3	100.0	94.4	90.6	82.5	81.0	62.7	80.4
TcLr14a	<i>Lr14a</i>	100.0	100.0	100.0	98.1	98.6	97.6	100.0	99.0
TcLr14b	<i>Lr14b</i>	92.7	98.3	100.0	86.5	84.6	82.5	89.2	88.1
TcLr15	<i>Lr15</i>	23.6	10.2	33.3	24.5	43.4	19.8	25.3	27.0
TcLr16	<i>Lr16</i>	91.1	86.4	94.4	84.9	78.3	91.3	70.9	82.1
TcLr17	<i>Lr17</i>	92.9	89.8	100.0	88.7	81.1	93.7	74.1	85.0
TcLr18	<i>Lr18</i>	68.5	94.9	66.7	60.4	48.3	76.2	48.7	62.0
TcLr19	<i>Lr19</i>	0.0	0.0	0.0	3.8	0.7	0.0	3.8	1.5
Thew(W203)	<i>Lr20</i>	41.8	56.9	60.0	48.1	26.6	20.6	41.8	36.2
TcLr23	<i>Lr23</i>	53.6	89.7	77.8	60.4	75.5	90.5	20.3	62.4
TcLr24	<i>Lr24</i>	0.0	0.0	11.1	0.0	0.0	0.0	0.0	0.3
Transec(Awned)	<i>Lr25</i>	0.0	1.7	0.0	1.9	0.0	0.0	0.0	0.3
TcLr26	<i>Lr26</i>	89.3	96.6	100.0	94.3	98.6	98.4	95.6	96.4
Gatcher	<i>Lr27+ 31,10</i>	30.4	81.0	92.9	85.7	88.1	97.6	93.6	85.4
CM2D-2M	<i>Lr28</i>	3.6	0.0	0.0	0.0	0.0	0.8	1.3	0.8
TcLr29	<i>Lr29</i>	3.6	3.4	16.7	9.4	0.0	0.8	0.6	2.3
TcLr30	<i>Lr30</i>	71.4	86.4	66.7	67.9	42.7	73.0	31.0	55.6
TcLr32	<i>Lr32</i>	58.9	100.0	83.3	71.7	54.6	76.2	48.7	64.6
TcLr33	<i>Lr33</i>	85.7	93.2	83.3	73.6	92.3	89.7	83.5	87.1

^a Weighted average in the 7 years.

with different *Lr* genes probably is the main reason that the Chinese *P. trititica* population has maintained great diversity and relative stability for races and virulences.

Distribution patterns of *P. trititica* races and virulences and the diversity of populations differ among regions of China, suggesting that Chinese *P. trititica* populations may be isolated from other global populations of the fungus. Comparison of *Chao-1* estimators within China and between China and the United States indicated that the *P. trititica* populations in the two countries showed substantial differences in virulence and diversity. However, the richness or evenness of races seemed similarly distributed in both countries by *Sub*₁₂₆ (See Materials and Methods and Table 1). There were 30 races in China in 2005, with 29.4 ± 2.4 races for *Sub*₁₂₆. In contrast, races TDB, MLD, MFP, MCB, TNR, and TFB were the most common in the United States in 2005 and 2007 (19,20). Moreover, the frequencies of virulence to *Lr* genes differed between the two countries. Virulence to *Lr3ka*, *Lr11*, *Lr16*, *Lr18*, *Lr26*, and *Lr30* was, in each case, over 55.6% in China but below 34.6% in the United States; in particular, the frequency of virulence to *Lr16* was only 8.7% in the United States in 2006 but reached 82.1% in China during the period 2000 to 2006. Differences in virulence to *Lr9*, *Lr24*, and *Lr28* were also found, with frequencies of <1% in China; whereas, in the United States, virulence to *Lr9* was 15.3 and 12.9%, to *Lr24* was 56.1 and 72.4%, and to *Lr28* was 70.3 and 66.5% in 2005 and 2007, respectively.

In Mexico, races TBD/TM (virulent to *Lr10*, *Lr17*, and *Lr27+31* but avirulent to *Lr23* and *Lr26*) and TCB/TD (virulent to *Lr23* and *Lr26* but avirulent on *Lr10*, *Lr17*, and *Lr27+31*) were the most common before 1994 (32). A new race, BBG/BN, with high virulence to durum wheat, was detected in northwestern Mexico in 2001 (34). An annual survey of *P. trititica* races in South America indicated that race MCP-10, with additional virulence on *Lr10*, and races MDR-10, MDR-20, and MFP-20 were present in high frequencies during 2004 to 2007 (10). Mesterházy et al. (28) conducted a European virulence survey for *P. trititica*, which included France, Germany, Italy, Czech Republic, Slovakia, Great Britain, Spain, Hungary, Poland, Bulgaria, Romania, and Switzerland, and identified 105 races in a total of 2,608 isolates using 15 near-isogenic Thatcher lines. Virulence to *Lr12*, *Lr13*, *Lr22a*, and *Lr37* varied among locations but no virulence was found to *Lr9*, *Lr19*, and *Lr35*. One leaf rust survey in Iran from 2002 to 2004 indicated virulence to *Lr24*, and no virulence to *Lr18*, *Lr34*, *Lr35*, *Lr36*, or *Lr37* was detected (1). Races CBHN (9.2%), DDPR (10.8%), FNMQ (7.5%), and JNDC (10.8%) were detected to be most common in Tunisia during 2005 to 2007 (29). Differing climatic conditions and wheat planting systems, particularly differences in wheat genotypes planted in various areas, are the main reasons for the geographic differences in *P. trititica*.

Acknowledgments

Financial support was provided by the Ministry of Science and Technology, P. R. China (grant number 2011CB100403 and 2010CB951503); Ministry of Agriculture, P. R. China (grant number 200903035); and National Natural Scientific Foundation of China (number 30800709). We thank X. Chen, United States Department of Agriculture–Agricultural Research Service and Washington State University, and R. F. Park, Plant Breeding Institute at University of Sydney, for their critical and conscientious review of the previous version of this article prior to submission; and anonymous reviewers and the senior editor for their efforts, and for the improvements made possible by their critical reviews.

Literature Cited

1. Afshari, F., Torabi, M., Kia, S., Dadrezaei, S., Safavi, S., Chaichi, M., Khiavi, H. K., Zakeri, A., Kamangar, S. B., and Nasrollahi, M. 2006. Monitoring of virulence factors of *Puccinia trititica* Eriksson, the causal agent of wheat leaf rust in Iran during 2002–2004. *Seed Plant Improv. J.* 21:485–496.
2. Chao, A. 1984. Nonparametric estimation of the number of classes in a population. *Scand. J. Stat.* 11:265–270.
3. Chen, W. Q., Hu, C. C., and Zhang, S. X. 1993. Analysis of virulence genes of *Puccinia recondita* f. sp. *tritici* population in China. *Sci. Agric. Sin.* 26:17–23. (In Chinese with English abstract)
4. Chen, W. Q., Qin, Q. M., Chen, Y. L., and Yan, S. B. 1998. Virulence dynamics of *Puccinia recondita* f. sp. *tritici* in China during 1992–1996. *Acta Phytopathol. Sin.* 28:101–106. (In Chinese with English abstract)
5. Chen, W. Q., Wu, L. R., Liu, T. G., Xu, S. C., Jin, S. L., Peng, Y. L., and Wang, B. T. 2009. Race dynamics, diversity, and virulence evolution in *Puccinia striiformis* f. sp. *tritici*, the causal agent of wheat stripe rust in China from 2003 to 2007. *Plant Dis.* 93:1093–1101.
6. Chen, W. Q., Yan, S. B., Hu, C. C., and Xie, S. X. 1994. Physiologic race and pathogenicity of *Puccinia recondita* f. sp. *tritici* in China during 1990–1993. *Acta Phytophylac. Sin.* 21:289–295. (In Chinese with English abstract.)
7. Chen, X. M. 2005. Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. *Can. J. Plant Pathol.* 27:314–337.
8. Colwell, R. K., and Coddington, J. A. 1994. Estimating terrestrial biodiversity through extrapolation. *Phil. Trans. R. Soc. Lond. (Ser. B)* 345:101–118.
9. Dyck, P. L., and Samborski, D. J. 1968. Genetics of resistance to leaf rust in the common wheat varieties Webster, Loros, Brevit, Carina, Malakof and Centenario. *Can. J. Genet. Cytol.* 10:7–17.
10. Germán, S., Barcellos, A., Chaves, M., Kohli, M., Campos, P., and de Viedma, L. 2007. The situation of common wheat rusts in the Southern Cone of America and perspectives for control. *Aust. J. Agric. Res.* 58:620–630.
11. Grünwald, N. J., Goodwin, S. B., Milgroom, M. G., and Fry, W. E. 2003. Analysis of genotypic diversity data for populations of microorganisms. *Phytopathology* 93:738–746.
12. He, Z. H., Rajaram, S., Xin, Z. Y., and Huang, G. Z. 2001. A History of Wheat Breeding in China. CIMMYT, Mexico, D.F.
13. Hu, C. C., and Roelfs, A. P. 1985. The wheat rusts in the People's Republic of China. *Cereal Rust Bull.* 13:1–28.
14. Hu, C. C., and Roelfs, A. P. 1989. Races and virulence of *Puccinia recondita* f. sp. *tritici* in China in 1986. *Plant Dis.* 73:499–501.
15. Johnston, T. 1956. Physiologic races of leaf rust of wheat in Canada 1931 to 1955. *Can. J. Agric. Sci.* 36:323–332.
16. Johnston, C. O., Caldwell, R. M., Compton, L. E., and Browder, L. E. 1968. Physiologic races of *Puccinia recondita* f. sp. *tritici* in the United States from 1926 through 1960. *U. S. Dep. Agric. Tech. Bull.* 1393:1–18.
17. Johnston, C. O., and Mains, E. E. 1932. Studies on physiologic specialization in *Puccinia trititica*. *U. S. Dep. Agric. Tech. Bull.* 313:1–22.
18. Kolmer, J. A. 1997. Virulence in *Puccinia recondita* f. sp. *tritici* isolates from Canada to genes for adult-plant resistance to wheat leaf rust. *Plant Dis.* 81:267–271.
19. Kolmer, J. A., Long, D. L., and Hughes, M. E. 2007. Physiologic specialization of *Puccinia trititica* on wheat in the United States in 2005. *Plant Dis.* 91:979–984.
20. Kolmer, J. A., Long, D. L., Hughes, M. E. 2009. Physiologic specialization of *Puccinia trititica* on wheat in the United States in 2007. *Plant Dis.* 93:538–544.
21. Li, Z. F., Xia, X. C., He, Z. H., Li, X., Zhang, L. J., Wang, H. Y., Meng, Q. F., Yang, W. X., Li, G. Q., and Liu, D. Q. 2010. Seedling and slow rusting resistance to leaf rust in Chinese wheat cultivars. *Plant Dis.* 94:45–53.
22. Line, R. F., and Chen, X. M. 1995. Successes in breeding for and managing durable resistance to wheat rusts. *Plant Dis.* 79:1254–1255.
23. Liu, Q. C., Tang, Y. L., and Liu, Q. J. 1995. Physiological races and virulent genes analysis of *Puccinia recondita* f. sp. *tritici* in Shandong province. *Shandong Agric. Sci.* 4:35–38. (In Chinese with English abstract.)
24. Long, D. L., and Kolmer, J. A. 1989. A north American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. *Phytopathology* 79:525–529.
25. McIntosh, R. A., and Brown, G. N. 1997. Anticipatory breeding for resistance to rust diseases in wheat. *Annu. Rev. Phytopathol.* 35:311–326.
26. McIntosh, R. A., Wellings, C. R., and Park, R. 1995. Wheat Rusts: An Atlas of Resistance Genes. Kluwer Academic Publishers, Dordrecht, The Netherlands and CSIRO Australia.
27. McIntosh, R. A., Yamazaki, Y., Devos, K. M., Dubcovsky, J., Rogers, J., and Appels, R. 2003. Catalogue of gene symbols for wheat. In: Tenth Int. Wheat Genet. Symp. N. E. Pogna, N. Romano, E. Pogna, and G. Galterio, eds. Instituto Sperimentale per la Cerealicoltura, Rome.
28. Mesterházy, Á., Bartoš, P., Goyeau, H., Niks, R. E., Csösz, M., Andersen, O., Casulli, F., Ittu, M., Jones, E., Manisterski, J., Manninger, K., Pasquini, M., Rubiales, D., Schachermayr, G., Strzembicha, A., Szunics, L., Todorova, M., Unger, O., Vanco, B., Vida, G., and Walther, U. 2000. European virulence survey for leaf rust in wheat. *Agronomie* 20:793–804.
29. Mosbah, M., Yahyaoui, A., Sayar, R., Hajlaoui, M. R., Sakkouhi, L., and Daaloul, A. 2009. Virulence analysis of *Puccinia recondita* in Tunisia during the period 2005–2007. *Tunis. J. Plant Prot.* 4:111–120.
30. Qin, Q. M., and Chen, W. Q. 2000. Races and virulence of *Puccinia recondita* f. sp. *tritici* in China during 1997–1999. Page 214 in: Proc. 1st Asian Conf. Plant Pathol. G. H. Zhou and H. F. Li, eds. China Agriculture Science Technology Press, Beijing.
31. Roelfs, A. P., Singh, R. P., and Saari, E. E. 1992. Rust Diseases of Wheat: Concepts and Methods of Disease Management. CIMMYT, Mexico, D.F.
32. Singh, R. P. 1991. Pathogenicity variations of *Puccinia recondita* f. sp. *tritici* and *P. graminis* f. sp. *tritici* in wheat-growing areas of Mexico during 1988 and 1989. *Plant Dis.* 75:790–794.
33. Singh, R. P., Chen, W. Q., and He, Z. H. 1999. Leaf rust resistance of spring, facultative, and winter wheat cultivars from China. *Plant Dis.* 83:644–651.
34. Singh, R. P., Huerta-Espino, J., Pfeiffer, W., and Figueroa-Lopez, P. 2004. Occurrence and impact of a new leaf rust race on durum wheat in north-

- western Mexico from 2001 to 2003. *Plant Dis.* 88:703-708.
35. Wang, H. R. 1940. Notes on physiologic specialization in leaf rust of wheat in China. *Phytopathology* 37:680-681.
 36. Wang, H. R., Yuan, J. S., Zhu, Z. Y., Wang, Y. X., and Zhao, L. B. 1987. Studies on physiological race of *Puccinia recondita* f. sp. *tritici* during 1973-1986. *J. Hebei Agric. Univ.* 10:158-171. (In Chinese with English abstract)
 37. Wang, K. N., and Hong, X. W. 1982. Studies on physiological specialization of *Puccinia recondita* f. sp. *tritici*. *Acta Phytopathol. Sin.* 12:13-20. (In Chinese with English abstract)
 38. Waterhouse, W. L. 1952. Australian rust studies. IX. Physiologic race determination and surveys of cereal rusts. *Proc. Linn. Soc. N. S. W.* 77:209-258.
 39. Yang, W. X., Meng, Q. F., Feng, S. D., and Liu, D. Q. 2004. Virulence of *Puccinia triticina* on wheat in China in 1999. *Acta Phytophylac. Sin.* 31:45-50. (In Chinese with English abstract)
 40. Yuan, J. S., and Zhu, Z. Y. 1995. The study on growth and decline of physiological race of *Puccinia recondita* f. sp. *tritici* from 1993 to 1995. *J. Hebei Agric. Univ.* 18:134, 129. (In Chinese with English abstract)
 41. Yuan, Z. Y., Wu, Y. P., Li, Y. F., and Zeng, W. F. 2005. Study on virulence genes of population of *Puccinia recondita* f. sp. *tritici* in different wheat ecological areas of Shanxi. *Chin. J. Eco-Agric.* 13:144-146.
 42. Zhao, X., Zheng, T., Xia, X., He, Z., Liu, D., Yang, W., Yin, G., and Li, Z. 2008. Molecular mapping of leaf rust resistance gene *LrZH84* in Chinese wheat line Zhou 8425B. *Theor. Appl. Genet.* 117:1069-1075.