Molecular Characterization of International Collections of the Wheat Stripe Rust Pathogen *Puccinia striiformis* f. sp. *tritici* Reveals High Diversity and Intercontinental Migration

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ABSTRACT

Puccinia striiformis f. sp. *tritici* causes stripe rust (yellow rust), one of the most important wheat diseases worldwide. To understand the genetic variation of the pathogen in a global scale, 283 *P. striiformis* f. sp. *tritici* isolates collected from 16 countries in eight geographic regions were genotyped using 24 codominant simple sequence repeat markers. The overall collection had a high level of genetic diversity, and the diversity levels in the Asian populations were generally higher than those of the other regions. Heterozygosity of isolates ranged from 0 to 75%, with an average of 46%. Mean heterozygosity in individual countries ranged from 34 to 59%. A total of 265 multilocus genotypes (MLGs) were detected, which were classified into eight molecular groups. Some of the molecular groups were present in all geographic regions. Moreover, many isolates from different regions were found to

be identical or very closely related MLGs. Analysis of molecular variance revealed high variation within countries and intermediate variation between countries, but it revealed low and insignificant variation among geographic regions. Pairwise comparisons of regional populations detected considerable effective migrants and only low to moderate levels of differentiation. The molecular genotypes had a moderate level of correlation with the virulence phenotypes, and some of the molecular/virulence groups contained isolates from different continents. The results indicate tremendous migrations of *P. striiformis* f. sp. *tritici* and warrant the development of management strategies considering the global pathogen population.

Keywords: ecology and epidemiology, population biology

Stripe rust (yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss., is one of the most damaging diseases of wheat (*Triticum* spp.) in most wheat-growing countries throughout the world. Major stripe rust-vulnerable regions include the Arabian Peninsula, the Mediterranean, Central Asia, the Caucasus, South Asia, East Asia, East Africa, northwestern Europe, Oceania, and Pacific regions along North and South America (Chen and Kang 2017; Stubbs 1985; Wellings 2011). Stubbs (1988) classified potential stripe rust regions into different epidemiological zones at an international scale based on the similarity of virulence factors or races in combination with geographical features and potentials of urediniospore dispersal. Similarly, stripe rust epidemiological

regions have been defined at a national level: for example, in China (Zeng and Luo 2006) and the United States (Chen 2005; Chen et al. 2010).

The populations of *P. striiformis* f. sp. *tritici* in different countries have traditionally been characterized through virulence testing on wheat genotypes used to differentiate races. These studies were mostly conducted in Australia, China, European countries, India, and the United States (Chen et al. 2009b; Line and Qayoum 1992; Stubbs 1985, 1988; Wan and Chen 2014; Wan et al. 2004; Wellings 2007). Because the pathogen is an obligate biotrophic fungus that requires host plants for growth and reproduction, virulence testing requires dew chambers and greenhouse facilities. Also, because *P. striiformis* f. sp. *tritici* requires relatively low temperatures for growth and survival, special facilities are needed to store and work with the pathogen. Because of the limitation of research conditions, the *P. striiformis* f. sp. *tritici* populations in many countries with the serious stripe rust problem have not been studied. Through international collaborations, we have received *P. striiformis* f. sp. *tritici* isolates from 16 countries in 2006 to 2010 and characterized most of the isolates through virulence testing (Sharma-Poudyal et al. 2013). Comparison of virulence phenotypes of the isolates from different countries revealed possible migration of the pathogen between countries or even between different continents. However, because virulence phenotypes are strongly under selection of resistance genes in wheat cultivars, virulence relatedness may not reflect the genetic relationships.

Understanding of the genetic variability of plant pathogens is crucial for developing effective management strategies (Ali et al. 2014; Ordoñez and Kolmer 2007). Neutral molecular markers are useful for studying genetic diversity and population structure (McDonald and Linde 2002). Because *P. striiformis* f. sp. *tritici* urediniospores are dikaryotic, codominant markers, such as single-nucleotide repeat (simple sequence repeat [SSR]) markers, are

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needed for distinguishing homokaryotic from heterokaryotic isolates. SSR markers have been developed and widely used to study genetic characteristics of *P. striiformis* f. sp. *tritici* populations in different parts of the world (Bahri et al. 2009a, b, 2011; Chen et al. 2009a; Cheng and Chen 2014; Cheng et al. 2012; Duan et al. 2010; Enjalbert et al. 2002, 2005; Mboup et al. 2009). Most previous studies on *P. striiformis* f. sp. *tritici* focused on populations from a single country or a relatively small region in a country, and only a few studies included *P. striiformis* f. sp. *tritici* samples from different countries (Ali et al. 2010, 2014; Hovmøller et al. 2008).

Characterization of *P. striiformis* f. sp. *tritici* samples from different countries is useful for understanding the pathogen variation in the global scale. Because *P. striiformis* f. sp. *tritici* urediniospores are airborne, the pathogen is capable of dispersal over long distance; knowing virulence phenotypes and molecular genotypes in different countries is essential for assessing the risk of a particular race or genotype to wheat production in various regions of the world and for selecting genes to develop cultivars with effective resistance to the pathogen populations in other regions as well as in the region. In a previous study, a large number of virulence phenotypes were identified from *P. striiformis* f. sp. *tritici* isolates from 13 countries through testing on *Yr* single-gene lines and wheat genotypes used to differentiate *P. striiformis* f. sp. *tritici* races in the United States (Sharma-Poudyal et al. 2013). The virulence data provided useful information for both effective and ineffective resistance genes and suggested possible migration of the pathogen between countries. The objectives of this study were to identify multilocus genotypes (MLGs) from these collections using SSR markers and use the marker data to determine the possibility of intercontinental migration.

MATERIALS AND METHODS

***P. striiformis* f. sp. *tritici* samples.** A total of 283 *P. striiformis* f. sp. *tritici* isolates from 16 countries were used in

TABLE 1. Number of isolates of *Puccinia striiformis* f. sp. *tritici* and their collected geographic regions and countries

Geographic region and country	No. of isolates	Sampling year
East Asia		
China	63	2006–2007
South Asia		
Nepal	24	2007–2008
Pakistan	46	2006–2009
Total	70	2006–2009
Central Asia		
Kyrgyzstan	4	2008
Tajikistan	1	2008
Turkmenistan	4	2008
Uzbekistan	17	2009–2010
Russia ^a	9	2006
Total	35	2006–2010
Mediterranean		
Algeria	1	2009
Spain	2	2008–2009
Turkey	56	2009–2010
Total	59	2008–2010
East Africa		
Kenya	4	2006
North America		
Mexico	4	2006
U.S.A.	28	2006–2010
Total	32	2006–2010
South America		
Chile	15	2007
Oceania		
Australia	5	2006
Total		
All 16 countries	283	2006–2010

^a All samples from Russia were from the Asian part of Russia.

this study (Table 1), of which 224 were included in the previous study and characterized for virulence spectra (Sharma-Poudyal et al. 2013), and the remaining isolates were from either received dead urediniospores or DNA samples. In addition, 28 U.S. isolates were selected to represent different regions and races during the same period of the international collections for the purpose of comparison. Urediniospores for each isolate were increased on seedlings of stripe rust-susceptible wheat cultivar Nugaines following the standard procedure as previously described (Chen et al. 2010; Sharma-Poudyal et al. 2013). Increased urediniospores were dried and kept in a desiccator at 4°C for later use.

DNA extraction. About 20 mg of dried urediniospores were used to extract genomic DNA from each isolate. Twenty micrograms of sterilized 0.7-mm Zirconia beads (BioSpec Products Inc.) and 800 µl of DNA extraction lysis buffer (2% cetyl trimethylammonium bromide, 1.4 M NaCl, 100 mM Tris [pH 8.0], 20 mM EDTA [pH 8.0], and 1% β-mercaptoethanol) were added to a 1.5-ml centrifuge tube containing urediniospores. The mixture was shaken for 3 min in a mini beater (BioSpec Products Inc.) and incubated at 65°C for 1 h, during which the tube was inverted 10 times at every 15-min interval. After incubation, 500 µl of 24:1 (vol/vol) chloroform/isoamyl alcohol was added to the tube, mixed well, and centrifuged for 15 min at 13,000 rpm. Clean supernatant of about 700 µl was transferred into another 1.5-ml centrifuge tube. Cold isopropyl alcohol (approximately 500 µl) was added into the tube, and the tube was incubated at –20°C for at least 20 min. Nucleic acid was precipitated as a pellet by centrifuging at 12,000 rpm for 15 min. Nucleic acid was rinsed with 700 µl of 75% ethanol (stored at –20°C) by centrifuging at 13,000 rpm for 5 min. After centrifugation, ethanol supernatant was discarded, and DNA in the tube was air dried at room temperature. The dried DNA was dissolved with 100 µl of TE buffer (10 mM Tris-HCl and 1 mM EDTA [pH 8.0]) containing RNase A (20 µg/ml). After incubating at 4°C overnight for digesting RNA, about 60 µl of cold isopropyl alcohol was added into the tube. After keeping at –20°C for at least 20 min, DNA in the tube was precipitated as a pellet at 12,000 rpm for 15 min and washed with 700 µl of 75% cold ethanol at 13,000 rpm for 5 min. The purified DNA was air dried, dissolved with 100 µl of TE buffer, and quantified using the mini-gel method and a spectrophotometer (Nano drop ND-1000; Bio-Rad). Stock DNA was stored at –20°C, and a working solution was obtained by diluting the stock DNA solution to 1 ng/µl with deionized sterilized water and stored at 4°C.

PCR amplification. The *P. striiformis* f. sp. *tritici* isolates were genotyped using 24 codominant SSR markers (Supplementary Table S1). The PCR mix consisted of 1.2 µl of 10× standard *Taq* polymerase buffer, 1.2 µl of 25 mM MgCl₂, 0.96 µl of 2.5 mM each of the dNTP mixture (Promega), 0.12 µl of 5 µM forward primer, 0.24 µl of 10 µM M13 universal primer, 0.6 µl of 5 µM reverse primer, 0.12 µl of 5 U/µl *Taq* polymerase (Promega), 2 ng of DNA, and 5.56 µl of sterilized distilled deionized H₂O (ddH₂O) in a final volume of 12 µl. Each forward primer had an M13 tail (CACGACGTTGTAACGAC) at the 5' end. The M13 universal primer was labeled with one of four fluorescent reporter dyes: FAM, VIC, NED, and PET (Applied Biosystems). Amplification of DNA was done with a Peltier thermal cycler (BIO-RAD) using a temperature profile of 94°C for 5 min for initial denaturation; 40 cycles of 94°C for 30 s, 45 to 54°C for 30 s depending on SSR primers, and 72°C for 1 min; and 7 min of final extension at 72°C. Four PCR products with four different fluorescent reporter dyes were pooled together (Symonds and Lloyd 2004) with a final volume of 25 µl. The PCR product mix was prepared by mixing 3 µl of PCR product with FAM, 3 µl of PCR product with VIC, 4 µl of PCR product with NED, and 6 µl of PCR product with PET, and it was diluted with 9 µl of ddH₂O using Biomek NXP Laboratory Automation Workstation (Beckman Coulter). To prepare for a loading sample, 3 µl of the above PCR product mix was mixed again with 9 µl of Hi-Di Formamide (Applied Biosystems) and 1 µl of

DNA ladder (445-LIZ; Applied Biosystems). After denaturing at 95°C for 5 min, the loading samples were subject to capillary electrophoresis using an ABI3730 DNA Analyzer (Applied Biosystems).

Determination of diversity and heterozygosity. Software GenAlEx v6.503 (Peakall and Smouse 2006) was used to calculate mean genetic variations across the SSR loci based on observed alleles (N_o), effective alleles (N_e), and Shannon's information index (I) (Nei 1978) for each country, each geographic region, and all isolates used in this study. The software was also used to compute percentage of polymorphic loci and number of private alleles. Genotypic diversity based on the Shannon–Wiener index (Shannon 1948) was estimated using the “poppr” function in the poppr R package version 2.8.3 (Kamvar et al. 2014). Because *P. striiformis* f. sp. *tritici* is a dikaryotic fungus at the uredinal stage and DNA was extracted from urediniospores, each isolate was scored for homozygous or heterozygous at each SSR locus using software GeneMarkerV1.5 (Softgenetics). Heterozygosity in percentage was calculated for each isolate based on the number of loci with different alleles among the 24 SSR markers. Mean heterozygosity was calculated for each country and region.

Identification of MLGs and cluster analysis. MLGs were identified based on the alleles for each of the 24 SSR loci. The homozygous and heterozygous alleles were considered different for each locus. Isolates with the identical allele pattern across the 24 SSR loci were considered as the same MLG.

To determine the genetic relationships among the *P. striiformis* f. sp. *tritici* isolates, a dendrogram was constructed through the hierarchical cluster analysis using dissimilarities and the “ward.D2” method in the R stats 3.5.3 program with the “hclust” function (Murtagh and Legendre 2014). Ward's is an agglomerative clustering method based on a classical sum-of-square criterion, producing groups that minimize within-group dispersion at each binary fusion (Murtagh 1985; Murtagh and Legendre 2014). The hierarchical cluster analysis separated the isolates into molecular groups (MGs). To assess the robustness of the grouping, the bootstrap analysis of clustering was further performed using the “Pvclust” function in the R-based Ape program with 1,000 bootstrap repeats (Efron et al. 1996; Paradis et al. 2004; Suzuki and Shimodaira 2006). To further investigate the population differentiation at the regional level, neighbor-joining trees were generated based on Nei's genetic distance for the eight geographic regions, and bootstrap probability was calculated using the same programs also with 1,000 bootstrap repeats.

Analysis of molecular variance and pairwise comparison of *P. striiformis* f. sp. *tritici* populations between regions. Analysis of molecular variance (AMOVA), which allows the hierarchical partitioning of genetic variation within and between regions (Excoffier et al. 1992), was conducted by defining the *P. striiformis* f. sp. *tritici* isolates based on country of origin and grouped into eight regions based on country proximity and epidemiological regions using function “poppr.amova” in the poppr R program version 2.8.3 (Kamvar et al. 2014; Meirmans and Liu 2018). Pairwise comparisons among regional collections of *P. striiformis* f. sp. *tritici* were conducted for Nei's genetic distance and differentiation (F_{ST}) values (Nei 1972). Nei's genetic distance, number of effective migrants (N_m), and F_{ST} were estimated using software GenAlEx 6.5 (Peakall and Smouse 2006). The probability (P) value of F_{ST} was determined based on 999 permutations. The F_{ST} range from 0 to 0.05 indicates little genetic differentiation, the F_{ST} range from 0.05 to 0.15 indicates moderate genetic differentiation, the F_{ST} range from 0.15 to 0.25 indicates great genetic differentiation, and the F_{ST} range >0.25 indicates very great genetic differentiation (Wright 1978).

Determine correlation between the molecular and virulence data. The SSR data of 224 isolates that were previously studied for their virulence (Sharma-Poudyal et al. 2013) were analyzed together with their virulence data for correlation using NTSYSpc v2.21 (Rohlf 2008). Similarity matrices based on simple matching

coefficient were computed with the SIMQUAL module using the SSR and virulence data separately. The association between these similarity matrices was determined using the Mantel test in the NTSYS program (Rohlf 2008).

RESULTS

SSR loci. The 24 codominant SSR primer pairs produced a total of 87 alleles in the 283 *P. striiformis* f. sp. *tritici* isolates. The alleles of the 283 isolates at the 24 loci are provided in Supplementary Table S2, and the sizes of alleles of the 24 SSR loci are provided in Supplementary Table S3. The number of alleles and the number of allelic genotypes at each of the 24 SSR loci are given in Supplementary Table S1. The number of alleles per locus ranged from 2 to 7, with an average of 3.6. The number of allelic genotypes per locus ranged from 3 to 12, with an average of 6.0. All 24 SSR loci were polymorphic among the *P. striiformis* f. sp. *tritici* isolates and therefore, suitable for studying the genetic relationships of the isolates.

Population diversity and heterozygosity. The diversity of the *P. striiformis* f. sp. *tritici* population and subpopulations was estimated using several parameters. The mean numbers of observed and effective alleles per locus were calculated for each country and geographic region. The mean observed number of alleles ranged from 1.71 in Turkmenistan to 2.83 in Pakistan, and the mean number of effective alleles ranged from 1.57 in Turkmenistan to 2.10 in Nepal (Table 2). The grand mean of effective alleles per locus was 1.79, which was slightly lower than the grand mean of observed alleles (2.27) across all countries. Comparing the different regions, South Asia had the highest mean of effective alleles (2.08) followed by East Asia (1.87) and the Mediterranean (1.84), whereas East Africa and Oceania had the lowest effective alleles (both 1.61). The highest Shannon's information index was observed in Nepal (0.80), whereas the lowest was in Turkmenistan and Kenya (both 0.44), with the grand mean of 0.61. Similarly, South Asia had the highest Shannon's information index (0.79) followed by East Asia (0.67) and the Mediterranean (0.64), whereas East Africa had the lowest index (0.44).

The frequency of polymorphic loci was determined based on the number of loci of the 24 SSR loci showing differences among the isolates in a country or geographic region. The highest frequency of polymorphic loci (100%) was found in Nepal, Pakistan, and the United States; the frequencies in China, Uzbekistan, Russia, Turkey, Chile, and Australia were also above 90%, whereas Kenya had the lowest frequency (67%) (Table 2). Among the eight geographic regions, six regions (East Asia, South Asia, the Mediterranean, North America, South America, and Oceania) had 90% or higher frequencies of polymorphic loci, whereas the remaining two regions (Central Asia and East Africa) had frequencies below 90% (Table 2).

Private alleles contributed to the population diversity in a country or a region. A total of 18 private alleles in country collections were observed at 15 SSR loci. Private alleles and loci were detected in East Asia (China), South Asia (Nepal and Pakistan), Central Asia (Kyrgyzstan and Russia), the Mediterranean (Turkey), and South America (Chile) (Table 2). No private alleles or loci were observed in East Africa, North America, and Oceania. The private alleles were found from 33 isolates, of which 12 were from East Asia (China), 12 were from South Asia, 4 were from South America, 3 were from the Mediterranean, and 2 were from Central Asia.

The ratio of MLGs to isolates can be used to assess population diversity. From the 283 isolates, 265 MLGs were identified (Table 2 and Supplementary Table S2). The MLG to isolate ratio ranged from 0.75 (Kenya) to 1.00 (Algeria and Tajikistan), with a mean of 0.94 in all 16 countries (Table 2) (almost one genotype per isolate). Among the geographic regions, all regions had the ratios above 0.95 except East Africa (0.75) and the Mediterranean (0.88), with ratios below 0.90.

The genotypic diversity of the global *P. striiformis* f. sp. *tritici* population was high (5.53) (Table 2). The diversity index values varied greatly among countries, with the lowest (1.04) in Kenya and the highest (4.12) in China. Regarding the eight geographic regions, the highest (4.23) was in South Asia followed by East Asia (4.12), the Mediterranean (3.89), Central Asia (3.56), and North America (3.42), whereas East Africa, South America, and Oceania had diversity values below 3.0.

The frequencies of homozygous (homokaryotic) and heterozygous (heterokaryotic) alleles of the 24 SSR loci are provided in Supplementary Table S3. The frequencies of heterozygous alleles ranged from 4.24 (CPS14) to 75.97% (PstP006), with a mean of 44.02%. For country-wise comparison, the mean heterozygous allele frequencies ranged from 34 in Australia to 59% in Kenya (Table 2). China (0 to 71%), Pakistan (4 to 75%), and Turkey (4 to 71%) had the largest variations in heterozygous loci among their isolates. Regarding the geographic regions, the highest mean heterozygous allele frequency was observed in East Africa (59%) followed by East Asia (47%), North America (46%), the Mediterranean (45%), South America (45%), Central Asia (42%), and South Asia (41%), and the lowest frequency was observed in Oceania (34%). The results indicated that the *P. striiformis* f. sp. *tritici* populations were highly heterozygous (i.e., the two nuclei in most of the isolates were different).

MGs. The hierarchical cluster analysis based on the dissimilarities clustered the 283 isolates into eight MGs (Fig. 1). MG1 consisted of 48 isolates (17.0%) from six countries (China,

Pakistan, Uzbekistan, Turkey, Spain, and the United States). MG2 consisted of 23 isolates (8.1%) from five countries (China, Nepal, Pakistan, Turkey, and Chile). MG3 had 44 isolates (15.5%) from five countries (China, Nepal, Uzbekistan, Turkey, and Chile). MG4 was the smallest group, consisting of nine isolates (3.2%) from only East Asia (China). MG5 was the largest group, consisting of 87 isolates (30.7%) from all countries except Kyrgyzstan, Tajikistan, and Turkmenistan. MG6 consisted of 18 isolates (6.4%) from six countries (Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, Russia, and Turkey). MG7 had 17 isolates (6.0%) from three countries (Nepal, Pakistan, and Russia). MG8 had 37 isolates (13.1%) from seven countries (Nepal, Pakistan, Russia, Mexico, the United States, Chile, and Australia) (Fig. 1). Among the MGs, MG2, MG3, and MG4 were more closely related to each other. M5 and MG6 were more close to each other, and MG7 and MG8 were more close to each other than to other MGs, whereas MG1 was distant from the others (Fig. 1). The clusters of MG4, MG7, and MG8 were more robust, whereas the separations of other MGs were less robust.

It was worthy of note that many isolates from different countries had identical or similar MLGs. Isolate TR10-17 collected from Turkey in 2010 was molecularly identical to isolate UZ10-4 from Uzbekistan in 2010 and isolate PK09-6 collected from Pakistan in 2009 was identical to isolates TR10-5 and TR10-43 from Turkey in 2010 in MG1 (Fig. 1). MG5 included several groups of identical MLGs consisting of isolates from different countries or continents. One Pakistani isolate (PK09-5), three Turkey isolates (TR09-4,

TABLE 2. Mean observed and effective alleles; Shannon's information index; frequencies of polymorphic marker loci; numbers of private marker loci, alleles, and isolates; frequencies of heterozygous loci; and numbers and ratios of multilocus genotypes (MLGs) of *Puccinia striiformis* f. sp. *tritici* collections in various countries and geographic regions

Origin of isolate: Geographic region and country	Observed alleles (N_a)	Effective alleles (N_e) ^a	Shannon's information index (I) ^b	Polym. loci (%) ^c	Private alleles ^d			MLGs		Genotypic diversity (H) ^f	Heterozygous frequency (%) ^g
					No. of loci	No. of alleles	No. of isolates	No.	Ratio ^e		
East Asia											
China	2.67	1.87	0.67	96	3	3	12	62	0.98	4.12	47 (0–71)
South Asia											
Nepal	2.75	2.10	0.80	100	2	3	6	24	1.00	3.18	51 (8–67)
Pakistan	2.83	2.07	0.79	100	3	3	6	45	0.98	3.80	36 (4–75)
Total (mean)	2.79	2.08	0.79	100	5	6	12	69	0.99	4.23	41 (4–75)
Central Asia											
Kyrgyzstan	1.88	1.76	0.53	75	1	1	1	4	1.00	1.39	53 (43–58)
Turkmenistan	1.71	1.57	0.44	71	0	0	0	4	1.00	1.39	49 (42–57)
Uzbekistan	2.46	1.83	0.68	96	0	0	0	17	1.00	2.83	38 (4–63)
Russia	2.25	1.72	0.59	96	1	1	1	9	1.00	2.20	39 (13–54)
Total (mean)	2.07	1.72	0.56	85	2	2	2	35	1.00	3.56	42 (4–63)
East Africa											
Kenya	1.67	1.61	0.44	67	0	0	0	3	0.75	1.04	59 (54–67)
Mediterranean											
Spain	2.00	1.74	0.58	88	0	0	0	2	1.00	0.69	38 (17–58)
Turkey	2.71	1.93	0.71	96	3	3	3	49	0.88	3.82	44 (4–71)
Total (mean)	2.35	1.84	0.64	92	3	3	3	54	0.88	3.89	45 (4–71)
North America											
Mexico	1.96	1.59	0.49	83	0	0	0	4	1.00	1.39	43 (33–58)
U.S.A.	2.33	1.89	0.67	100	0	0	0	27	0.96	3.28	46 (4–67)
Total (mean)	2.15	1.74	0.58	92	0	0	0	31	0.97	3.42	46 (4–67)
South America											
Chile	2.46	1.77	0.63	96	2	4	4	15	1.00	2.71	45 (29–67)
Oceania											
Australia	2.08	1.61	0.52	92	0	0	0	5	1.00	1.61	34 (13–58)
Grand total (mean)	2.27	1.79	0.61	82	15	18	33	265 ^h	0.94	5.53	46 (0–75)

^a Number of effective alleles = $1/(1 - H_e)$.

^b Shannon's information index = $-\sum p_i(\ln p_i)$, where $\ln$ is the natural logarithm and p_i is the frequency of the i th allele.

^c Percentage of polymorphic (Polym.) loci was determined based on the number of loci of the 24 simple sequence repeat marker loci showing differences among the isolates in each country or geographic region.

^d Private alleles are those found in one or more isolates in the individual country but not found in any other country.

^e The ratio was calculated by dividing the number of MLGs by the number of isolates (Table 1).

^f H = Shannon–Wiener genotypic diversity index.

^g Mean of heterozygous loci of 24 simple sequence repeats; values in the parentheses are ranges.

^h The total number of 265 MLGs includes two more MLGs: one from Algeria and one from Tajikistan. The diversities of these countries were not calculated because they each had only one sample.

TR0923, and TR0974), one Mexican isolate (MX06-1), and two U.S. isolates (US07-187 and US08-264) were found to be molecularly identical. Isolate CL07-6 collected from Chile in 2007 and US09-122-14sp2 from the United States in 2009 were identical. Isolate PK09-13 from Pakistan and isolate TR09-11 from Turkey were the same MLG. TR09-85 and US07-132 were identical. Two Kenya isolates (KE06-1 and KE06-2), four Turkey isolates (TR09-37, TR09-40, TR10-32, and TR10-34), one Nepal isolate (NP05-8), and one Pakistani isolate (PK08-6) were identical. To a lesser extent, many isolates from different countries or continents were found to be genetically very close to each other; for example, Chile isolate CL07-5 and Chinese isolate CN07-10 in MG3, Pakistani isolate PK09-27 and U.S. isolate US07-064 in MG5, Turkey isolate TR09-55 and U.S. isolate US01-169 in MG5, Australian isolate AU06-5 and Chile isolate CL07-3 in MG8, Chile isolate CL07-8 and U.S. isolate US03-391 in MG8, and Chile isolate CL07-9 and Nepal isolate 07-10 in MG8 were similar

(Fig. 1). These results indicated that *P. striiformis* f. sp. *tritici* urediniospores migrated among different continents.

The distributions of the MGs in the geographic regions are shown in Figure 2. MG1 had a significant proportion in East Asia, Central Asia, and the Mediterranean, and it was also presented in North America. MG2 was significant in South Asia and present in the Mediterranean. MG3 was predominant in East Asia and present in South Asia, Central Asia, and the Mediterranean. MG4 was present only in East Asia. MG5 was predominant in the Mediterranean, North America, East Africa; it was significant in South Asia and South America, and it was also present in East Asia and Central Asia. MG6 was predominant in Central Asia and also present in the Mediterranean. MG7 had a significant proportion in South Asia and was also present in Central Asia. MG8 was predominant in South America and also had a significant proportion in North America and South Asia (Fig. 2). The presence of the same MGs in different continents also suggested intercontinental migrations of the stripe rust pathogen.

Fig. 1. Dendrogram of eight molecular groups (MGs) of *Puccinia striiformis* f. sp. *tritici* collections based on dissimilarities assessed with 24 simple sequence repeat markers using the hierarchical cluster analysis. The first two letters denote the country of the isolate. The isolates starting with a number were from the United States. Au, Australia; CL, Chile; CN, China; DZ, Algeria; ES, Spain; KE, Kenya; KG, Kyrgyzstan; MX, Mexico; NP, Nepal; PK, Pakistan; RU, Russia; TJ, Tajikistan; TM, Turkmenistan; TR, Turkey; US, United States; and UZ, Uzbekistan.

Genetic relationships of regional *P. striiformis* f. sp. *tritici* populations. To determine the genetic relationships among the populations of different regions, the phylogenetic analysis was conducted based on MLGs using Nei's genetic distance. The relationships among the eight geographic regions are shown in Figure 3. Among the eight regional populations, the Asian populations, including East Asia, South Asia, the Mediterranean, and Central Asia, were more closely related to each other than to those in other continents; those of North America, South America, and Oceania (Australia) were more closely related to each other than to those of Asia, and the East African population was between the two groups.

Partition and differentiation of genetic variation. The AMOVA results revealed that the genetic variation was mostly within countries or among isolates rather than among geographic regions or among countries within regions. Genetic variations within countries accounted for 81.14% ($P = 0.001$), and genetic variations among countries within regions accounted for 13.42%, which was also significant ($P = 0.001$). However, the regional difference was only 5.44% and insignificant ($P = 0.153$).

Regional pairwise comparisons of genetic distance, number of effective migrants, and genetic differentiation based on F_{ST} are given in Table 3. Nei's genetic distances ranged from 0.038 (South America to North America) to 0.274 (East Asia to South America). Longer Nei's genetic distances were found between East Asia and other regions outside Asia than within Asia. The correlation coefficient between the F_{ST} values and the Nei's genetic distances was high ($r = 0.99$), indicating a close association between genetic differentiations based on the F_{ST} and Nei's genetic distance values. The number of effective migrants ranged from 0.08 (between East Asia and South America) to 8.18 (between North America and South America), and it was inversely correlated with the Nei's genetic distance ($r = -0.81$) and F_{ST} values ($r = -0.84$). Among the 15 region pairs with significant genetic differentiations, 8 pairs (Central Asia to the Mediterranean, Central Asia to North America,

East Asia to the Mediterranean, the Mediterranean to North America, North America to South America, South Asia to Central Asia, South Asia to the Mediterranean, and South Asia to North America) had little differentiation ($0 < F_{ST} \leq 0.05$), and 7 pairs (Central Asia to South America, East Asia to Central Asia, East Asia to North America, East Asia to South America, the

Fig. 3. Neighbor-joining trees based on the Nei's genetic distances among eight geographic regions.

Fig. 2. Distributions of molecular groups (MGs) of *Puccinia striiformis* f. sp. *tritici* in different geographic regions. The circle size represents the relative numbers of isolates.

Mediterranean to South America, South Asia to East Asia, and South Asia to South America) had moderate differentiation ($0.05 < F_{ST} \leq 0.15$) (Table 3). None of pairwise regions had great differentiations. The results indicated that tremendous *P. striiformis* f. sp. *tritici* migration events occurred across the different regions or continents.

Correlation between molecular and virulence variations.

Correlation analysis was conducted using the marker data obtained from this study and virulence data from the previous study (Sharma-Poudyal et al. 2013). The MGs and virulence groups (VGs) of the isolates are provided in Supplementary Table S4. The overall correlation coefficient was moderate and significant ($r = 0.41$; $P < 0.0001$). The numbers of isolates across the MGs and VGs from different countries are given in Table 4. Isolates in MG1 and MG2 were mostly present in VG1. Isolates in MG3 were in VG1 and VG5 but not in other VGs. Isolates of MG4 (from China) were only present in VG5. The majority of the MG5 isolates were in VG5, but a small number of isolates were in VG1, VG2, and VG6 to VG8. The two isolates of MG6 were in VG5 and VG6. The MG7 isolates were mostly in VG5. The isolates of MG8 were distributed in 8 of 11 VGs. VG5 was the largest group, with >50% of the isolates present in all eight MGs, whereas VG2 isolates were only from MG5, VG4 isolates were only from MG1, and VG9 and VG11 isolates were only from MG8. The results showed that, although isolates in the same MG could be in different VGs and vice versa, correspondence existed between molecular characterization and virulence phenotypes. Because many isolates from countries in different continents were identified as the same MG/VG groups, both molecular and virulence data strongly supported *P. striiformis* f. sp. *tritici* migration among continents.

DISCUSSION

Stripe rust is continually threatening wheat production worldwide. In this study, we genotyped *P. striiformis* f. sp. *tritici* collections from 16 countries belonging to eight geographic regions using codominant SSR markers. The *P. striiformis* f. sp. *tritici* populations were highly heterozygous, indicating high diversity within isolates and within country or regional populations. The different regions have different levels of population diversity; however, population differentiation between the countries or regions was generally low, indicating *P. striiformis* f. sp. *tritici* migrations between countries and between different continental regions. From the 283 isolates, we identified 265 MLGs and clustered them into eight MGs. By correlating the molecular genotypes with the previously reported virulence phenotypes of a subset of the isolates (Sharma-Poudyal

et al. 2013), we found a moderate correlation between the SSR and virulence data. The presence of isolates from different continents in the same MG/VG groups further confirmed the pathogen intercontinental migrations.

A high level of diversity was observed for the entire *P. striiformis* f. sp. *tritici* collection, and different countries or geographic regions had different levels of diversity. In general, the Asian population, including East Asia, South Asia, the Mediterranean, and Central Asia, had higher genotypic diversities than other continents. Except East Africa, our genotypic diversity values were slightly higher than those reported by Ali et al. (2014), especially for China, Nepal, and Pakistan, from which both studies had similar numbers of samples. These results indicate that the *P. striiformis* f. sp. *tritici* fungus is more diverse in Asia, and the high diversity may be partially related to possible sexual reproduction in this continent (Ali et al. 2014; Duan et al. 2010; Jin et al. 2010; Mboup et al. 2009; Zhao et al. 2013, 2016). In this study, we found a high level of genotypic diversity (3.82) for the Turkey population, similar to that of the Pakistan population (3.80). Because we included Turkey in the Mediterranean region, the genotypic diversity of this region was also high (3.89), suggesting a center of origin for *P. striiformis* f. sp. *tritici* as postulated by Stubbs (1985). In 2014, the Himalayan region was proposed as a *P. striiformis* f. sp. *tritici* origin (Ali et al. 2014). The whole Mediterranean to Asia region could be the big center of origin for the stripe rust fungus. This modified hypothesis is related to the long history of cereal cultivation and stripe rust epidemics in this big geographic region (Chen and Kang 2017; Stubbs 1985). No matter where is the origin, stripe rust is a global problem, affecting all major wheat-growing regions.

The presence of private alleles in some country collections may contribute to the overall diversity. Private alleles were found in collections from China, Pakistan, Nepal, Turkey, Kyrgyzstan, Russia, and Chile. Ali et al. (2014) also found relatively high numbers of private alleles in Pakistan, China, and Nepal. The private alleles are likely the result of mutations or genetic recombination, which might have occurred recently, and isolates with the alleles have not migrated outside the country. However, the private alleles need to be further monitored in the global *P. striiformis* f. sp. *tritici* survey because they may be related to specific virulence factors or other traits involved in the pathogen adaptation.

Heterozygosity is important for understanding evolution of plant pathogens that are either diploid or dikaryotic, like *P. striiformis* f. sp. *tritici*. In this study, we found that up to 76% of the isolates were heterozygous at some of the marker loci. As previously discussed (Liu et al. 2012), the heterozygous variations are likely aroused

TABLE 3. Nei's genetic distance, number of effective migrants (N_m), and genetic differentiation (F_{ST}) between geographic region populations of *Puccinia striiformis* f. sp. *tritici* based on 24 simple sequence repeat markers

Geographic regions ^a	Nei's genetic distance	N_m	Genetic differentiation	
			F_{ST}	P value ^b
Central Asia–Mediterranean	0.04	6.67	0.02	0.009
Central Asia–North America	0.07	3.61	0.04	<0.001
Central Asia–South America	0.14	1.82	0.08	<0.001
East Asia–Central Asia	0.11	2.11	0.06	<0.001
East Asia–Mediterranean	0.08	2.70	0.05	<0.001
East Asia–North America	0.19	1.15	0.10	<0.001
East Asia–South America	0.27	0.80	0.15	<0.001
Mediterranean–North America	0.06	4.17	0.04	0.003
Mediterranean–South America	0.15	1.54	0.09	<0.001
North America–South America	0.04	8.18	0.03	0.054
South Asia–Central Asia	0.07	4.46	0.03	<0.001
South Asia–East Asia	0.12	1.98	0.06	<0.001
South Asia–Mediterranean	0.05	5.47	0.03	<0.001
South Asia–North America	0.05	5.58	0.03	<0.001
South Asia–South America	0.10	2.84	0.06	<0.001

^a East Africa and Oceania regions were excluded from these analyses because their isolate numbers were <10.

^b Probability values based on 999 permutations.

through mutation. Genetic recombination, either sexually or asexually, may also produce heterozygous isolates. A high level of heterozygosity has been reported in other rust pathogens, including *Puccinia triticina* (Ordoñez and Kolmer 2007), *Puccinia graminis* f. sp. *tritici* (Burdon and Roelfs 1985), *Uromyces appendiculatus* (Groth et al. 1995), and *Melampsora lini* (Barrett et al. 2008). In *P. striiformis*, previous studies through genome sequencing also reported high heterozygosity within isolates (Ali et al. 2014; Cantu et al. 2013; Xia et al. 2016, 2018; Zheng et al. 2013). In a similar study, Ali et al. (2014) also studied heterozygosity of *P. striiformis* f. sp. *tritici* populations in different countries and reported heterozygosity values of about 0.28 in China, 0.40 in Nepal, and 0.35 in Pakistan. Our results were remarkably similar for Pakistan (0.36) but much higher for China (0.47) and Nepal (0.51). The difference could be owing to different years and areas of samples as well as different SSR markers used. It has been hypothesized that high genomic variations within dikaryotic urediniospores provide raw genetic materials for the rapid genome evolution of *P. striiformis* (Xia et al. 2018). Heterozygous isolates may have advantages in the pathogen evolution because of the heterosis effect that may be involved in adaptation to different environments and on host plants with different resistance genes.

In this study, we identified 265 MLGs from the international *P. striiformis* f. sp. *tritici* collections using 24 SSR markers and clustered them into eight MGs. The overall ratio of MLGs to isolates was >0.9; almost one isolate was a different genotype. Although the high number could be as a result of collections from different countries, the high MLG number indicated a high level of diversity of the global population. In a similar study, Ali et al. (2014) identified about 170 MLGs from 409 *P. striiformis* f. sp. *tritici* isolates using 20 SSR markers and clustered them into six genetic groups. Their ratio of MLGs to isolates was about 0.4, lower than

that of this study. The difference could be owing to different samples and different SSR markers. Among the eight MGs identified in this study, MG5 was the biggest genetic group, containing isolates from all eight geographic regions. This group may be related to the aggressive strains widely distributed in the world as reported in previous studies (Ali et al. 2014; Bahri et al. 2009b, 2011; Hovmøller et al. 2008). Some smaller MGs were specific to a country or geographic region. For example, MG4 was specifically from China. MG3 consisted of isolates mostly from China, but some isolates were from Nepal, Uzbekistan, Turkey, and Chile. Isolates in MG7 were mostly from Pakistan, with a few from Nepal. Although the relatively specific genetic groups are problems to the specific country or region, they need to be included in the future global *P. striiformis* f. sp. *tritici* monitoring for possible dispersal to other countries or regions.

The airborne urediniospores of *P. striiformis* f. sp. *tritici* can be dispersed mainly by wind thousands of kilometers during a crop-growing season or between seasons. The air movement of spores is the major process for increasing the regional population diversity and reducing distinctions of regional populations. However, natural barriers, like mountains, oceans, and deserts, can limit or reduce spore dispersal. With the addition of different cropping system effects, mainly from cultivars with or without resistance genes, these different factors or forces can make the global *P. striiformis* f. sp. *tritici* population complex (Chen 2017; Stubbs 1985). In this study, we found that the overall genetic variation between regional populations was only 5.44% of the total variation, which was not significant at the 0.05 *P* value level. However, when we conducted pairwise comparison between the geographic regions (excluding the East Africa and Oceania regions because of small numbers of isolates), we found that differentiations between some regions were significant but at small to moderate levels (Table 3). In general,

TABLE 4. Presence of molecular groups (MGs) and virulence groups (VGs) of *Puccinia striiformis* f. sp. *tritici* in different countries

VG ^a	Country ^b (no. of isolates)								Total
	MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8	
VG1	CN (12), ES (1), PK (4), TR (10), UZ (9)	CN (1), NP (3), PK (6), TR (5)	CN (6), NP (2), UZ (2)		KE (1), PK (2), UZ (1)			AU (1), NP (1), PK (2)	
Subtotal	36	15	10		4			4	69
VG2					TR (1), KE (1)				
Subtotal					2				2
VG3	CN (3), TR (2)							CL (1)	
Subtotal	5							1	6
VG4	CN (1), TR (1)								
Subtotal	2								2
VG5	CN (1), TR (1)	NP (7)	CL (1), CN (24), NP (2), TR (1), UZ (3)	CN (9)	AU (1), CL (3), CN (2), DZ (1), KE (1), NP (1), PK (10), RU (1), TR (28)	TR (1)	PK (12), RU (1)	AU (1), CL (1), NP (1)	
Subtotal	2	7	31	9	48	1	13	3	114
VG6		PK (1)			CL (1), PK (3), UZ (1)	TR (1)	PK (1)		
Subtotal		1			5	1	1		8
VG7					KE (1), PK (1)			NP (1)	
Subtotal					2			1	3
VG8					CL (1), TR (4)			CL (5), NP (1), PK (1)	
Subtotal					5			7	12
VG9								AU (2)	
Subtotal								2	2
VG10		CL (1)						CL (2), PK (1)	
Subtotal		1						3	4
VG11								PK (2)	
Subtotal								2	2
Total	45	24	41	9	66	2	14	23	224

^a The data on VGs are from Sharma-Poudyal et al. (2013).

^b AU, Australia; CL, Chile; CN, China; DZ, Algeria; ES, Spain; KE, Kenya; NP, Nepal; PK, Pakistan; RU, Russia; TR, Turkey; and UZ, Uzbekistan.

more significantly differentiated regions are more geographically distant as expected.

The identical or similar genotypes detected in different countries or continents indicate long-distance migration of *P. striiformis* f. sp. *tritici*. A considerable number of isolates from different countries or continents were identified as the same or very closely related MLGs as shown in Figure 1 and listed in Supplementary Table S2. These identical or similar MLGs clearly showed migrations between different continents. The global distribution of some of the MGs, especially MG5, may indicate that the migration of *P. striiformis* f. sp. *tritici* populations across different continents has occurred at several occasions in time. The introduction of rust pathogen isolates or populations through long-distance migrations of urediniospores by wind or human activity has been well documented (Hodson 2011; Hovmøller et al. 2002; Kolmer 2005; Kolmer et al. 2019). Stripe rust of wheat in Australia in 1979 was considered to be caused by urediniospores carried on clothing, probably from a source in Europe (Wellings 2007). Single clonal migration of *P. striiformis* f. sp. *tritici* through urediniospores was reported between the United Kingdom, France, Germany, and Denmark (Hovmøller et al. 2002). Similar strains of *P. striiformis* f. sp. *tritici* have rapidly spread in North America, Australia, and Europe (Hovmøller et al. 2008). In 2011, Bahri et al. (2011) observed *P. striiformis* f. sp. *tritici* isolates representing lineages in the western Mediterranean, northern Europe, Central Asia, and also, Pakistan. This study adds more evidence to the global migration of *P. striiformis* f. sp. *tritici*.

Most isolates in this study were previously tested for their virulence phenotypes (Sharma-Poudyal et al. 2013), which allowed us to compare the molecular and virulence information. The isolates detected as identical MLGs as discussed above generally did not have identical virulence phenotypes (Sharma-Poudyal et al. 2013), and thus, they were unlikely contaminants. Only two groups of identical MLGs were also identical in their virulence patterns. Isolates PK09-5 (from Pakistan) and TR10-43 (from Turkey) that were an identical MLG also had identical virulence phenotype. Because these isolates were obtained in different years, they could not be cross-contaminants. Isolates CN07-37 and CN07-57 had identical SSR and virulence patterns, which were reasonable because both isolates originated from China. The moderate overall mean correlation coefficient (0.41) was comparable with the value of 0.39 reported between virulence phenotypes and molecular genotypes in a U.S. *P. striiformis* f. sp. *tritici* population (Cheng and Chen 2014). The moderate coefficient value was reflected by the correspondence of MGs to VGs (Table 4). The majority of the isolates in MG1 were also the majority of the VG1 isolates. This MG1-VG1 group was detected in Asia and the Mediterranean countries. Isolates in the MG1-VG1 group were commonly virulent to resistance genes *Yr1*, *YrA*, *Yr6*, *Yr9*, *Yr21*, and *Yr31*, but they were virulent or avirulent to other genes or differentials, except avirulent to *Yr5* and *Yr15*, to which no any isolates in the study was virulent (Sharma-Poudyal et al. 2013). The majority isolates of MG5 were also the majority isolates in VG5. This is the most virulent group, and its isolates had virulence genes to all *Yr* single-gene lines and differentials tested except *Yr5* and *Yr15* (Sharma-Poudyal et al. 2013). The presence of MG5 isolates in the other five VGs indicates that this genetic group has diverse virulence patterns distributed throughout all eight geographic regions. However, VG5 also had isolates in the other seven MGs, suggesting that VG5 isolates might have been evolved from other genetic groups in addition to the majority from MG5. No matter how many genetic groups from which the isolates in the VG5 group have evolved, the wide distribution of the highly virulent group warrants the emphasis of using nonrace-specific resistance in breeding programs throughout the world.

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