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Analysis of population structure of *Puccinia striiformis* in Yunnan Province of China by using AFLP

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Abstract Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most destructive wheat diseases in China. Yunnan Province, located in south-western China, possesses unique features of geography, climate, wheat growth and stripe rust epidemics, different from main epidemic regions in China. The isolates of this pathogen were collected from nine counties in Yunnan Province during February to May of 2008. Used as a comparison, isolates were also collected from five counties of Gansu Province, the province important in inter-regional stripe rust epidemics in China. Amplified fragment length polymorphism (AFLP) method was applied to study the population genetics of the pathogen among different populations in these two provinces. Forty one AFLP genotypes were obtained from 150 isolates and the genotype qj3 showed the highest frequency in Yunnan Province. While 22 genotypes were detected from 40 isolates, no genotype showing as predominant was identified in Gansu Province. Genotypic diversity in Gansu Province was higher than that in Yunnan Province. A free recombination signature was detected in Gansu Province but not in Yunnan Province. We concluded that the population of *P. striiformis* in Yunnan Province can be considered as a clonal population.

Keywords Wheat stripe rust · Population genetic structure · Molecular epidemiology

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Introduction

Wheat stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most destructive diseases of wheat in China (Li and Zeng 2002). As a pathogen possessing long-distance dispersal features (Brown and Hovmøller 2002) with prevailing winds, *P. striiformis* can affect more than 20 million ha of wheat in China by causing inter-regional epidemics (Zeng and Luo 2006). Annual race surveys demonstrated a high-degree of variation of pathogenicity among races and frequency changes in races among the populations occurred in different regions in nearly 60 years (Wan et al. 2004).

Intensive analysis of pandemics of stripe rust classified the whole epidemic area into 15 regions in China according to the unique features and the relationships among these regions in disease development and pathogen life cycle (Zeng and Luo 2006). Four oversummering areas were identified and partially characterized (Zeng 2000; Zeng and Luo 2006, 2008). The specific features of pathogen oversummering in the specific ecological zones and geographic areas of southern and eastern Gansu Province had been clearly defined (Li and Zeng 2002; Zeng and

Luo 2006). The south-western region of China including Yunnan and Guizhou Provinces is considered as another potential important area in China where the pathogen could oversummer. However, the information about how these areas could provide oversummering inoculum to affect the disease epidemics in vast northern, north-central and eastern regions of wheat-growing areas of China is still unclear. Since it is difficult to determine the relationship in epidemics of *P. striiformis* among different geographic regions, the information on pathogen population structures may provide clues to infer the possible population exchanges due to spore dispersal.

Yunnan Province is located in south-western China where about 600,000 ha of winter wheat are grown (Li 2004). This province demonstrates a huge variation of climate, vegetation and agriculture. Variations of wheat planting systems, maturity and cultivars of wheat are unique in this province compared with other provinces in China. The Yunnan-Guizhou plateau crosses Yunnan Province from west to east. In these areas, wheat is grown from lowland in the valleys to highland with overlapping of growth stages along with the altitude which provides year-round host to *P. striiformis*.

Annual race surveys demonstrated that the frequencies of several races in Yunnan Province were different from those in main epidemic regions (Wu et al. 1993; Yang and Wu 1990). Before answering the question about whether the populations of *P. striiformis* in Yunnan Province could relate to those of other regions, it is important to better understand the pathogen population structures within the province. Thus, the status of the population structure among different sub-regions or geographical areas in Yunnan Province should be determined.

Various molecular marker systems were used to study the population structures of this pathogen at various levels in Europe, Australia and New Zealand. Restricted clonal populations in these regions were found (Hovmøller et al. 2002; Hovmøller and Justesen 2007; Enjalbert et al. 2005; Steele et al. 2001). A recombination signature in *P. striiformis* has been suggested in Gansu Province (Mboup et al. 2009; Duan et al. 2010). Amplified fragment length polymorphism (AFLP) markers for population studies on *P. striiformis* were generated (Justesen et al. 2002) and the set of these markers was intensively used to study the population genetics of this pathogen in Europe (Justesen et al. 2002) and North America (Markell and Milus 2008).

Since Gansu Province shows high genetic diversity of the *P. striiformis* populations (Mboup et al. 2009; Duan et al. 2010) and is the major area of China where the pathogen can oversummer to complete its life cycle, that affects the whole regional epidemics in China (Zeng and Luo 2006), we also collected isolates from this province as a reference for comparison in population structure between Yunnan and Gansu Provinces. In this study, we used AFLP analysis to test the degrees of genetic differentiation among populations at different geographic regions in Yunnan Province. We also compared the genetic and genotypic diversities between Yunnan and Gansu Provinces. The possibilities of recombination in the pathogen populations in Yunnan and Gansu Provinces were also studied.

Materials and methods

Sampling strategy

Wheat leaves showing sporulation of *P. striiformis* were collected from nine counties of Yunnan Province during February and May of 2008. All the isolates sampled from a county were defined as a population. Nine populations were obtained in north, northeast, west and centre of Yunnan Province (Table 1, Fig. 1), respectively. Infected leaves were sampled in farm fields. Ten to twenty sampling sites with distances of at least 50 m apart from each other were chosen and approximately 30–50 lesions were collected in each field. Whenever possible, leaves were sampled from known wheat cultivars and at different altitudes, in order to minimize the effect of clonality. In this study, the longest distance between any two counties (Qiaojia and Shidian counties) was no less than 450 km, while the shortest distance between any two counties (Yuxi and Jiangchuan counties) was 28 km (Table 1). Forty isolates were also collected from five counties of Gansu Province (Fig. 1) during November 2008 and January 2009 (Table 1) with the same method described above. These isolates were combined to represent a population in Gansu Province to compare with those of Yunnan Province in this study.

The single-lesion isolates were obtained as described below. The urediniospores of *P. striiformis* of each sample were reproduced on seedlings of the susceptible cultivar Mingxian169. Six 10-day-old seedlings in a pot with 9-cm in diameter were rubbed

Table 1 Information of the samples of *Puccinia striiformis* collected from different counties in Yunnan and Gansu Provinces, China

Region in Gansu	County/Code	N[a]	Latitude/Longitude	Location	Cultivar	Altitude (m)
North	Qiaojia(8[b])/qj	46	27°03′N /102°54′E	Jiuying	Gogo mai	1824
				Qiaojiaying	Guangtou mai	1417
				Liming	Xifu #7	720
				Yeya	Xifu #7	994
	Yongren(3)/yr	33	26°07′N /101°50′E	Take	Panmai #7	1855
				Yijiu	Yunmai #1	1710
				Yangjiuzha	V43	1450
				Lianchi	Panmai #7	1560
				Nuoda	Xihu #4	1640
East-north	Fuyuan(9)/fy	4	25°57′N /104°24′E	Dahe	Guangtou mai	1750
Centre	Eshan(5)/esh	13	24°16′N /102°38′E	Bajiewo	Yunmai #42	1530
				Dayutang	Mianyang#20	1550
				Baoshan	Mianyang#20	1850
				Baoshan	Yunmai #47	1800
	Yimen(4)/ym	14	24°57′N /102°15′E	Wugongli	Yunmai #42	1764
				Xishan	Yunmai #50	2031
				Yongjingshao	Yunmai #42	2010
				Dengtang	Yunmai #42	2320
				Zengsuo	Yunmai #47	1560
				Agu	Yunmai #47	1402
	Jiangchuan (7)	5	24°27′N /102°53′E	Liaojiaying	Yunmai #42	1740
				Liaojiaying	unknown	1740
	Yuxi(6)	8	24°35′N /102°52′E	Beichengzhen	Yunmai #42	1680
West	Baoshan (1)/bsh	13	25°10′N /99°10′E	Longyang	Yunza #5	1620
				Longyang	Deguodunmai	1700
				Longyang	Deguodunmai	1715
				Longyang	Yunza #6	1650
				Longyang	Yunza #5	1630
	Shidian(2)/sd	30	24°49′N /99°15′E	Shuichang	186	1718
				Renhe	Yunmai #11	1460
				Renhe	Jinmai #11	1460
				Dianyang	Deguodunmai	1785
				Shuichang	Deguodunmai	1844
				Dianyang	186	1632
				Dianyang	Qingniu #55	1608
				Shuichang	Yangnongpi #2	1501
Region in Gansu	County/Code	N[a]	Latitude/Longitude	Location	Cultivar	Altitude (m)
Center-south	Gangu/gg	14	34°46′N /105°21′E	Daping	unknown	1450
			34°39′N /105°21′E	Dongsanshipu	Mingxian169	1733
			34°39′N /105°21′E	Dongsanshipu	Longjian9811	1733
			34°39′N /105°21′E	Dongsanshipu	Jingshuang#16	1733
			34°48′N /105°22′E	Chunshuxian	unknown	1722
			34°48′N /105°27′E	Xiadianzi	unknown	1828
	Kangle/kl	2	35°36′N /103°40′E	Majiazuicun	475	2109

Table 1 (continued)

Region in Gansu	County/Code	N[a]	Latitude/Longitude	Location	Cultivar	Altitude (m)
			35°30′N /103°48′E	Chalu	210B39	2244
			35°33′N /103°55′E	Gaolouzicun	92362	2160
			35°59′N/103°41′E	Wuhuxiang	92362	2204
	Tongwei/tw	12	35°21′N /105°27′E	Caopo	Lantian16	1750
			35°13′N /105°43′E	Sihe	Zhongliang#5	2000
			35°24′N /105°40′E	Guwan	Zhongliang#14	2100
			35°28′N /105°34′E	Qingyang	7210	1950
			35°14′N /105°40′E	Leicha	Zhuanglang#10	1900
	Longxi/lx	8	34°35′N /104°35′E	Xianjiamen	unknown	2100
			34°52′N /104°52′E	Caotan	unknown	2180
			34°52′N /104°52′E	Jianshan	unknown	2200
	Zhuanglang/zhl	4	34°46′N /105°21′E	Liuliang	Lantian#18	1875
			34°46′N /105°21′E	Liuliang	Zhongliang#25	1865

[a] number of isolates sampled. [b] The corresponding numbers displaying in the map of Fig. 1. Code: The first two letters refer to the abbreviation of county

to remove wax on the leaves and sprayed with 0.5% Tween20 water on leaf surface. The seedlings were inoculated by rubbing the wheat leaf with mini-inoculating needles carrying spores from a single-lesion. The inoculated seedlings were incubated in a dew chamber at 8°C for 24 h and then in an incubator at 17°C/14°C (day/night) with a 16 h photoperiod day. After 10 days, the successful infection sites could be observed. One leaf with a single-lesion per pot was chosen and the remaining leaves were removed. The pots of plants were then transferred into the incubator. After 7 days, spores were harvested and dried in desiccators at 4°C for 1 day before being used for inoculation. If no isolate was successfully obtained from a location, the additional sporulating leaves from this location were re-sampled to obtain the new isolate by using the method described above. These spores were then used to make spore suspensions to inoculate healthy leaves in order to produce enough spores for DNA extraction. To prevent cross contamination, inoculated plants were kept separate from each other in individual glass chambers (8.5 cm wide and 20 cm high) during the spore reproduction process.

DNA extraction and the AFLP analysis

Pathogen genomic DNA was extracted from fresh urediniospores (about 10 mg) by using a modified CTAB procedure (Enjalbert et al. 2002). The DNA concentration of each sample was determined using a spectrophotometer. The following AFLP procedures generated by Justesen et al. (2002) were performed. *Pst*I and *Mse*I were used to digest about 100 ng genomic DNA. A pre-selective amplification was performed by using primers *Pst*I0 and *Mse*I0 (Table 2) under the following condition for 20 cycles: 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s. The pre-amplification products were diluted 50 times with sterile ddH_2O before selective amplification. For selective amplification, the primer combinations M12/P11, M16/P12 and M26/P12 (Table 2) were chosen based on a primer screening. The selective amplifications were carried out in 20 μl of PCR buffer containing 5 μl of pre-amplified DNA, 4 μl of each selective primer (50 pmol μl^{-1}), 0.4 U of Taq DNA polymerase (Takara) and 11 μl of sterile ddH_2O. The amplification was performed with the following protocol: 13 cycles at 94°C for 30 s, 65°C for 30 s with a 0.7°C decrement per cycle, and 72°C for 1 min, and then 23 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min. All AFLP reactions were performed in a Thermal Cycler (Eppendorf AG, Germany).

Final amplification products were separated on a 6% polyacrylamide denaturing gel at constant power of 80 W and then visualized by using silver nitrate stain (Chalhoub et al. 1997).

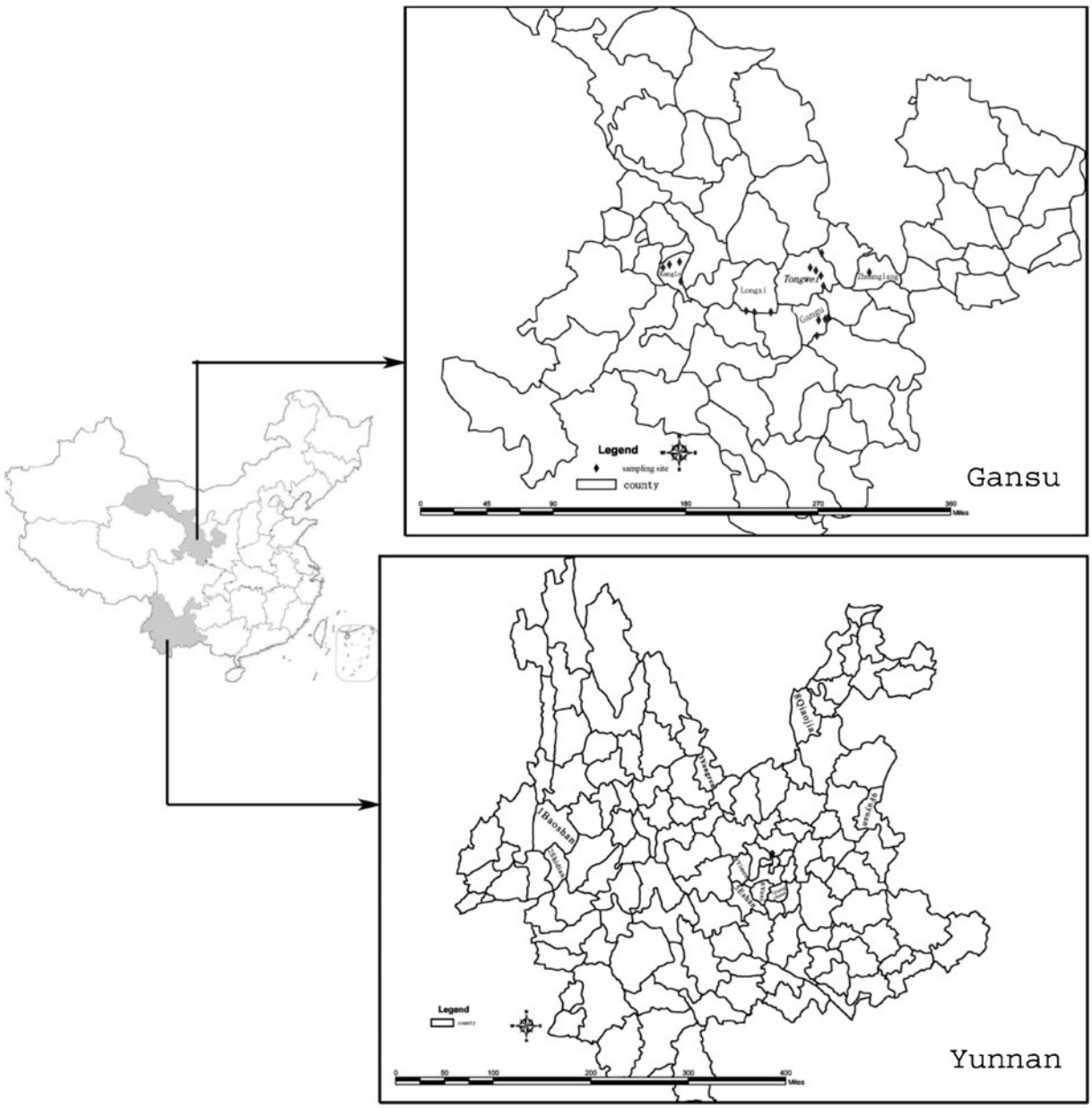

Fig. 1 Geographic locations of nine counties in Yunnan Province and five counties in Gansu Province of China, where the diseased leaves of wheat stripe rust showing sporulation of *Puccinia striiformis* were sampled and used in analysis of pathogen population structure in this study

Scoring

Each AFLP amplification band was assumed to represent a single locus and only clearly repeatable markers between 100 and 500 bp in size were used for analysis. For each primer combination, the presence (1) or absence (0) of bands was recorded and confirmed independently by two experienced individuals.

Data analysis

The numbers of different AFLP genotypes within each county and among overall isolates were calculated with the MULTILOCUS 1.3 program (Agapow and Burt 2001), then the degree of genotypic diversity was assessed. A clone-corrected dataset was used in order to minimize the effects of clonal reproduction

Table 2 Primers and corresponding sequences used for AFLP analysis in this study

Primer	Sequence
*Pst*I-adapter	5′-tgtacgcagtctac-3′
	3′-acgtacatgcgtcagatgctc- 5′
*Mse*I-adapter	5′ –tactcaggactcat-3′
	3′-gagtcctgagtagcag-5′
*Pst*I0	5′-gtagactgcgtacatgcag-3′
*Mse*I0	5′-gacgatgagtcctgagtaa-3′
M12/P11	*Mse*I0+AC/ *Pst*I0+AA
M16/P12	*Mse*I0+CC/ *Pst*I0+AC
M26/P12	*Mse*I0+TT/ *Pst*I0+AC

Abbreviations of the primers are given

on estimation of genetic diversity and population differentiation. Isolates that had a unique genotype (i.e. every different genotype was represented only once) were selected for further analysis.

To examine genetic diversity within populations, Nei's diversity index DG (Nei 1972) was calculated using the following equation: $DG=\frac{n}{n-1}\left(1-\sum_i P_i^2\right)$, where p_i is the frequency of the ith genotype and n is the total number of unique genotypes. This value is 0 if all the isolates are the same, and 1 if all isolates are different from each other. Nei's unbiased diversity index, Shannon's information index and the percentage of polymorphic loci were also used to evaluate the genetic diversity within each population. These parameters can be calculated for any two isolates in a population and the average value was used to represent the genetic diversity in the population. The Shannon's information index gives more weight to the presence than to the absence of bands. Nei's diversity index gives more weight to the bands present in both individuals, emphasizing the similarity between individuals rather than their dissimilarity. Nei's unbiased diversity index is similar to Nei's diversity index, but for a small sample size.

To examine hierarchical partition of molecular variation among populations, two parameters for measuring the population genetic structure, *F*st and φ*pt* (analogous to *F*st for binary data), were computed to estimate population genetic differentiation using the analysis of molecular variance (AMOVA) option with GENALEX 6.0 software (Peakall and Smouse 2005). The observed φ*pt* value was tested against the null hypothesis of no genetic difference among populations. If the observed φ*pt* value differed significantly from zero, the null hypothesis was rejected. Level of genetic differentiation among geographic regions was also estimated using Weir and Cockerham's θ (Weir and Cockerham 1984). The difference from zero of each calculated θ was tested with 999 permutations using MULTILOCUS 1.3 (Agapow and Burt 2001).

To test the degree of multi-locus linkage disequilibrium, two analyses were performed on the AFLP data. First, $\bar{r}_d$, a modified version of the index of association (I_A), was used. I_A is the traditional measure of multilocus linkage disequilibrium, but it depends on the number of loci used, which makes comparisons among studies difficult (Maynard et al. 1993; Haubold et al. 1998). $\bar{r}_d$ is identical to I_A when estimating the degree of recombination of populations but overcomes the deficiency of I_A. $\bar{r}_d$ was estimated and the null hypothesis was tested by 999 permutations using MULTILOCUS 1.3 (Agapow and Burt 2001). Second, the parsimony tree length permutation test (PTLPT) was used with PAUP 4.0 (Sinauer Associates, Inc. Sunderland, Massachusetts). The length of the most parsimonious tree of the observed dataset was compared with those of the most parsimonious trees estimated from 1000 randomized dataset for both Yunnan and Gansu populations.

Results

Genotypic diversity

A total of 41 AFLP genotypes were obtained from 150 isolates in Yunnan Province with genotypic diversity=0.27 (Fig. 2a, Table 3). The predominant genotype qj3 occurred in six of nine counties and had a frequency of 0.4 across the province (Fig. 2a). Three genotypes (bsh1, bsh2 and yr3) had frequencies between 0.04 and 0.07 and 14 genotypes (qj2, qj4, qj7, ym2, esh2, sd2, sd5, yr1, yr4, yr7, yr8, yr9, yr10 and yr11) had frequencies of 0.01. The frequencies of all the rest genotypes were less than 0.01 (Fig. 2a). Fourteen genotypes were re-sampled from two to five times and 23 genotypes were observed only once from all the samples in this study (Fig. 2a, Table 3).

The genotypic diversity varied among counties in Yunnan Province (Table 3). A higher genotypic

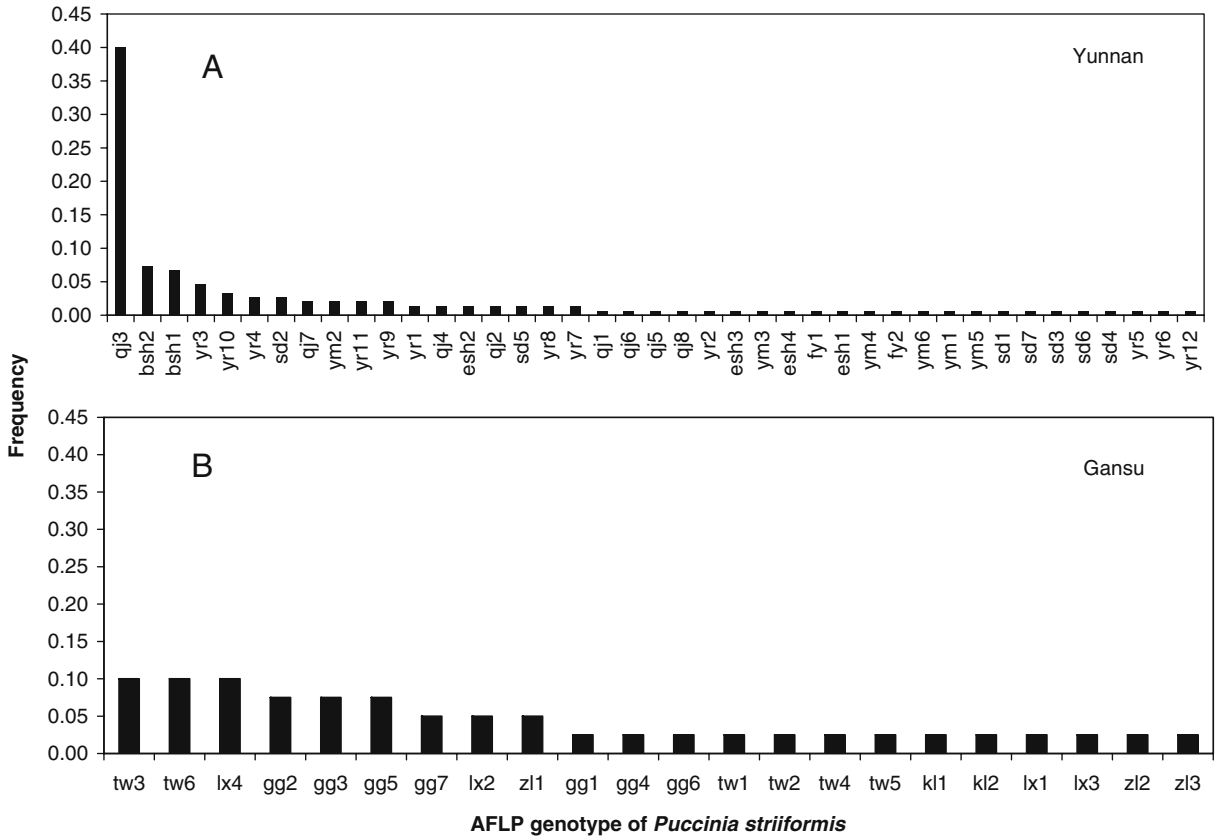

Fig. 2 The amplified fragment length polymorphism (AFLP) genotypes and the corresponding frequencies obtained from nine counties of Yunnan Province (A) and five counties of Gansu Province (B) of China in this study

diversity was observed in Eshan and Yimen Counties (0.64 and 0.50, respectively). The genotypic diversity was 0.40 in Yongren and Shidian Counties. The lowest genotypic diversity was observed in Yuxi (0.13) and Baoshan (0.15) Counties (Table 3).

In comparison with the result from Yunnan Province, a higher genotypic diversity (0.55) was detected in Gansu Province, showing 22 genotypes out of 40 isolates used in this study (Table 3). Among these 22 genotypes, 13 genotypes were unique while nine were re-sampled from two to four times. No predominant genotype was identified and all frequencies of genotypes were equal to or less than 0.1 (Fig. 2b). Genotypes with the highest frequency as 0.1 occurred in Tongwei County (tw3 and tw6) and Longxi County (lx4), respectively. Genotypes with frequency between 0.075 and 0.05 were identified in Gangu County (gg2, gg3, gg5, gg7) and Longxi County (lx2). The frequencies of all the other 13 genotypes were 0.025 (Fig. 2b).

There were seven common AFLP genotypes shared in both Yunnan and Gansu Provinces. For example, the genotypes of Yunnan Province qj5, ym4, sd7, yr1, yr4, yr5 and sd4 were identical to the corresponding genotypes of Gansu Province gg3, gg4, gg5, kl2, tw5, tw6 and kl1, respectively.

Genetic diversity within and among counties of Yunnan province

Genetic diversities within each population and among seven counties were determined by several parameters. Populations collected from Jiangchuan and Yuxi counties of Yunnan Province were not taken into account in this analysis due to only one genotype, qj3, being present. Despite the slight discrepancy among the coefficients, consistent order of genetic diversity among seven counties was obtained (Table 4). The results demonstrated that the highest genetic diversity was observed in Yongren County. The order of this

Table 3 Information on isolates of *Puccinia striiformis* used in this study and AFLP genotypes obtained from different counties in Yunnan and Gansu Provinces, China

Province	County	No. of isolates free of missing data	No. of genotypes	Genotypic diversity	Genotypes observed in each county (No. of isolates of the corresponding genotype)	No. of distinct genotype in entire province	Genotypic diversity in entire population	AFLP genotypes obtained in entire province (total number of isolates of the corresponding genotype)
Yunnan	Qiaojia	41	8	0.20	qj1(1)[a], qj2(2), qj3 (30), qj4(2), qj5(1), qj6(1), qj7(3), qj8(1)	8	0.20	qj1(1), qj2(2), qj3(60), qj4(2), qj5(1), qj6(1), qj7(3), qj8(1), yr1(2), yr2(1), yr3(7), yr4(4), yr5(1), yr6(1), yr7(2), yr8(2), yr9(3), yr10(5), yr11(3), yr12 (1), fy1(1), fy2(1), esh1(1), esh2(2), esh3(1),esh4(1), ym1 (1), ym2(3), ym3(1), ym4(1), ym5(1), ym6(1), bsh1(10), bsh2(11), sd1(1), sd2(4), sd3 (1), sd4(1), sd5(2), sd6(1), sd7 (1)
	Yongren	30	12	0.40	yr1(2), yr2(1), yr3(6), yr4(4), yr5(1), yr6 (1), yr7(2), yr8(1), yr9(3), yr10(5), yr11 (3), yr12(1)	12	0.40	
	Fuyuan	4	3	0.75	fy1(1), fy2(1), bsh2(2)	2	0.50	
	Eshan	11	7	0.64	esh1(1),esh2(2),esh3 (1),esh4(1),yr8(1), bsh1(1),qj3(4)	4	0.36	
	Yimen	14	7	0.50	ym1(1), ym2(3), ym3 (1), ym4(1), ym5(1), ym6(1),qj3(6)	6	0.43	
	Jiangchuan	4	1	0.25	qj3(4)	0	0	
	Yuxi	8	1	0.13	qj3(8)	0	0	
	Baoshan	13	2	0.15	bsh1(4), bsh2(9)	2	0.15	
	Shidian	25	10	0.40	sd1(1), sd2(4), sd3(1), sd4(1), sd5(2), sd6 (1), sd7(1), bsh1(5), yr3(1),qj3(8)	7	0.28	
Gansu		40	–	–	–	22	0.55	tw1(1), tw2(1), tw3(4), tw4(1), tw5(1),tw6(4), gg1(1), gg2(3), gg3(3), gg4(1), gg5(3), gg6(1), gg7(2),lx1(1), lx2(2), lx3(1), lx4(4),kl1(1),kl2(1),zl1(2), zl2 (1), zl3(1)

[a] The first two letters refer to the abbreviation of county

diversity among these counties were: Yongren> Yimen>Qiaojia>Eshan>Shidian>Baoshan=Fuyuan (Table 4). Compared to the results of Yunnan Province, the four parameters for Gansu Province showed higher diversity than those for any counties of Yunnan Province (Table 4).

Analysis of genetic differentiation assessed for pair-wise comparisons among seven populations of Yunnan Province demonstrated that the value of φ*pt* greater than 0.25 indicated a significant genetic differentiation between two populations (Hartl and Clark 1997). The Qiaojia-Fuyuan, Qiaojia-Shidian, Qiaojia-Yongren, Baoshan-Shidian, Fuyuan-Yongren, Fuyuan-Shidian and Eshan-Shidian populations were significantly different ($P<0.05$) according to *Fst* and φ*pt* (Tables 5 and 6). The two pair-wise comparisons of populations with both measures of genetic differentiation for Eshan-Yimen and Qiaojia-Baoshan were not significant ($P>0.05$) (Table 6). The φ*pt* statistics for Fuyuan-Yimen genetic differentiation was not significant, while the pair-wise comparison between the two populations was significant at $P=0.046$ based on *Fst* (Tables 5 and 6).

AMOVA hierarchically partitioned molecular variation among populations of Yunnan Province and revealed that 68% molecular variation was attributable to the difference within populations and 32% of it was attributable to that among populations ($P=0.001$)

Table 4 Within-population genetic diversity indices of *Puccinia striiformis* for seven counties of Yunnan Province and for Gansu Province determined with AFLP

Province	County	I[b]	H[c]	Uh[d]	%[e]
Yunnan	Qiaojia	0.391±0.045[a]	0.247±0.032	0.282±0.037	85.00%
Yunnan	Fuyuan	0.139±0.064	0.100±0.046	0.200±0.092	20.00%
Yunnan	Eshan	0.314±0.073	0.219±0.051	0.292±0.068	50.00%
Yunnan	Yimen	0.420±0.066	0.289±0.047	0.347±0.056	70.00%
Yunnan	Baoshan	0.139±0.064	0.100±0.046	0.200±0.092	20.00%
Yunnan	Shidian	0.325±0.051	0.204±0.034	0.238±0.039	70.00%
Yunnan	Yongren	0.535±0.040	0.363±0.032	0.395±0.034	95.00%
Total of Yunnan		0.323±0.024	0.217±0.017	0.279±0.024	58.57%±11.27%
Gansu		0.693±0	0.500±0	0.524±0	100.00±0

[a] mean±SE for all values of I, H, and Uh

[b] Shannon's information index

[c] Nei's diversity index

[d] Nei's unbiased diversity

[e] Percentage of polymorphic loci

(Table 7). Similarly, genetic differentiation among geographic regions was also estimated by using θ, and the result, θ=0.319, was significantly different from zero ($P<0.001$). The test led to rejection of the null hypothesis of no genetic difference among geographic populations and confirmed the conclusion about molecular differentiation among the seven populations.

Test for recombination

When considering an overall population in Yunnan Province, the associated index $\bar{r}_d$ for the entire Yunnan population was different from zero, expected for a panmictic population ($\bar{r}_d=0.034$, $P<0.001$). Similarly, the most parsimonious tree length estimated from the observed AFLP dataset was 106 steps, which was significantly shorter ($P=0.001$) than those in the distribution of the tree length generated from a randomized dataset (Fig. 3a). Both tests led to rejection of the null hypothesis of free recombination, which confirms the expected clonality in Yunnan population.

Comparatively, the PTLPT used in the Gansu population showed that the most parsimonious tree length of the observed AFLP dataset (94 steps) was located within the distribution of tree length generated from a randomized dataset with $P=0.01$ (Fig. 3b). The result implies the existence of a certain level of recombination of this pathogen in the Gansu population. This result is consistent to the conclusions drawn from the previous studies regarding the population genetics of this pathogen in Gansu Province (Mboup et al. 2009; Duan et al. 2010).

Discussion

No correlation between races and AFLP genotype was observed in this study (unpublished data),

Table 5 Pair-wise matrix of Nei's genetic distance among *Puccinia striiformis* populations sampled from seven counties in Yunnan Province of China

County	Qiaojia	Fuyuan	Eshan	Yimen	Baoshan	Shidian	Yongren
Qiaojia							
Fuyuan	0.381						
Eshan	0.190	0.163					
Yimen	0.181	0.122	0.093				
Baoshan	0.119	0.588	0.294	0.302			
Shidian	0.452	0.484	0.534	0.362	0.632		
Yongren	0.298	0.549	0.374	0.361	0.343	0.294	

Table 6 Pair-wise *Fst* values (upper-right part) and φ*pt* values (lower-left part) for *Puccinia striiformis* populations sampled from seven counties in Yunnan Province of China

County	Qiaojia	Fuyuan	Eshan	Yimen	Baoshan	Shidian	Yongren
Qiaojia		0.020	0.011	0.002	0.376	0.001	0.001
Fuyuan	0.397		0.277	0.580	0.332	0.028	0.017
Eshan	0.218	0.092		0.403	0.137	0.008	0.004
Yimen	0.199	−0.033	−0.033		0.031	0.001	0.001
Baoshan	0.037	0.600	0.299	0.248		0.028	0.062
Shidian	0.485	0.520	0.519	0.392	0.581		0.001
Yongren	0.291	0.368	0.302	0.293	0.241	0.302	

consistent with previously published results (Keiper et al. 2006). It is difficult to determine the relationship between AFLP genotypes and races for the *P. striiformis* populations. Furthermore, the correspondence of AFLP genotypes to virulence was also not found in this study. For example, five races were determined in Gansu Province (Tianshui, Lintao, Longnan and Gangu counties) and Sichuan Province that were each identified as race Suwon11-14 by using 17 Chinese differential varieties. However, the AFLP genotypes were found to cross among the five races such that no clear distinction among races by AFLP genotypes was found. Further study should focus on determination of virulent genotypes based on AFLP markers in the studied populations. The potential objective should answer the question about whether the shift in the virulent genotypes could relate to the geographic, climatic and other crop growing features, such as the evidence found in Europe and North America suggests (Milus et al. 2009)

One of the aims of the present study was to estimate the genetic relationship among populations of *P. striiformis* in different regions of Yunnan Province. A low level of genetic diversity among populations was observed when analyzed by AMOVA, showing that 68% of the total genetic differences were within populations (P=0.001). Gansu population was compared with five populations of Yunnan Province (Qiaojia, Eshan, Yimen, Shidian and Yongren counties), and 82% genetic differences within populations was found by AMOVA analysis (Φpt=0.184, P<0.001). *P. striiformis* causes epidemics most frequently in north and central-west of Yunnan Province (Li 2004).

Although most genetic variation was found within populations, a small part of the variation was from among populations. Except for the predominant genotype qj3, 23 genotypes occurred once and 17 genotypes were found at frequencies varying from 2 to 11 times (Table 3, Fig. 2a). The level of differentiation among pair-wise populations is not only based on genetic variations but also affected by the population size. The small populations usually could reflect higher genetic drift, thus, increasing *Fst* values. For instance in this study, only 4 isolates were collected from Fuyuan County, the *Fst* values between Fuyuan and Yimen were the highest (*Fst*=0.58) compared with those between Fuyuan and Shidian (n=25) and between Fuyuan and Yongren (n=30) (Table 6).

Several hypotheses could explain why marked molecular divergence in Yunnan population was observed. One is due to the diversity of host cultivars in Yunnan Province. Host selection would favour the most fit pathogen individuals during the epidemic outbreak and lead to an increase in their frequency (McDonald and Linde 2002). Goyeau et al. (2007) reported a strong selection exerted by host cultivars on *Puccinia triticina* population in France.

Table 7 Results of analysis of molecular variance (AMOVA) for populations of *Puccinia striiformis* sampled from seven counties in Yunnan Province of China

Source	df	Sum of squares	Mean square	Est. Var.	%
Among Pops	6	69.357	11.559	1.514	32%
Within Pops	34	107.619	3.165	3.165	68%
Total	40	176.976		4.679	100%

Φpt=0.324, P=0.001

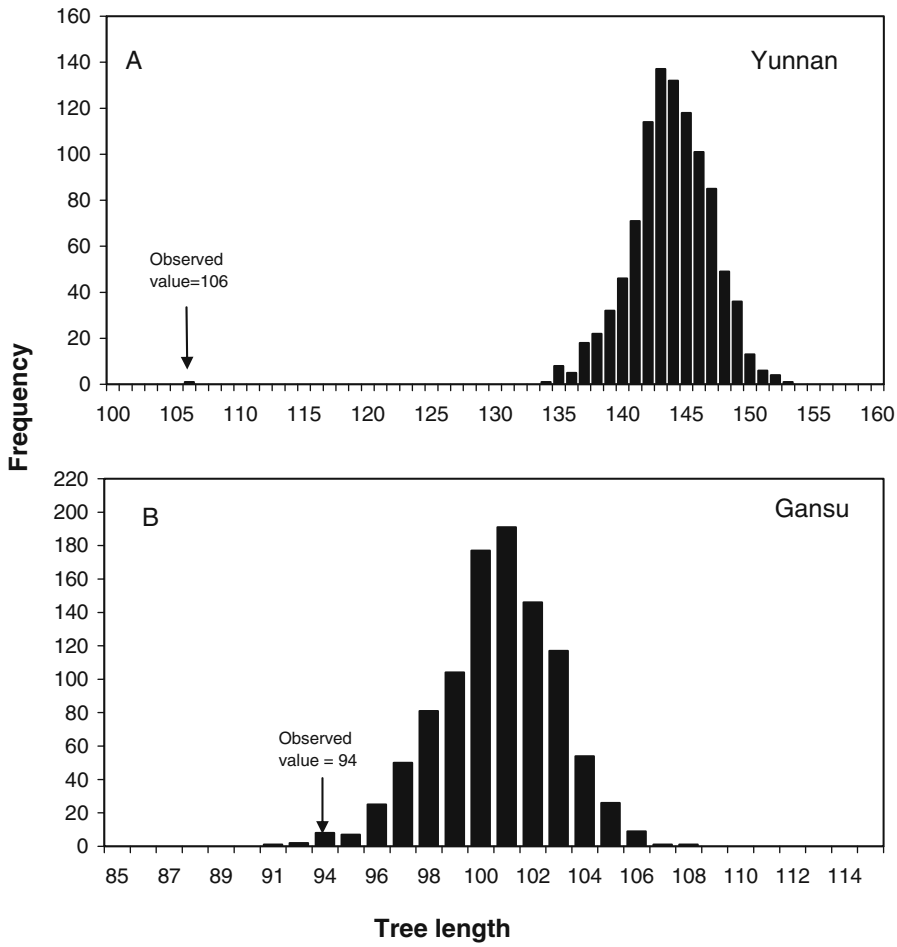

Fig. 3 Randomized distribution and observed values of the length of the most parsimonious tree estimated from AFLP dataset for Yunnan population (A) and Gansu population (B) of China. The histogram represents the expected tree length distribution under the hypothesis of linkage equilibrium between markers. Arrows indicate the observed values of the tree length for the corresponding populations

In Yunnan Province, although the overall occurrence frequency of pathotypes Hybrid46+YrSu was over 90%, the frequencies of individual pathotypes in specific locations were quite different. For instance, in central Yunnan (Kunming area), a large number of cultivars including Yumai, Jimai, Fengmai, Jintai, Funo wheat were planted, possessing various and a large number of resistance genes. In this region, 12 races were detected in 2008 (Li et al. 2009), demonstrating a large genetic diversity in the pathogen population. However, in western Yunnan such as in Baoshan County, the pathotype YrSu, a predominant race in other regions, had not been detected. But the frequency of the pathotype Hybrid46 was 92.3% (Li et al. 2009). In this area was planted a significantly low number of cultivars, including Dunmai and Yunza lines, possessing relatively fewer resistance genes. In our study, we obtained only two AFLP genotypes from 13 isolates in this county, demonstrating the relatively low level of pathogen genetic diversity (Table 3). Obviously, these cultivars strongly selected Hybrid46 rather than YrSu. Thus, selection by host plays an important role in the pathogen population structure indicated by *Fst* values.

Another hypothesis regarding the molecular divergence of the pathogen population may also relate to the environmental factors. Yunnan-Guizhou plateau crosses Yunnan Province and results in the climatic difference between northern and south-central Yunnan Province, this may influence the dynamics of population structure of the pathogen. The survey results of *P. striiformis* races based on 72 samples from 54 wheat cultivars in 12 geographical regions in Yunnan Province in 2008 (Li et al. 2009) indicated the huge genetic diversity of the pathogen populations. Eighteen races and pathotypes were detected, including CY17, CY18, CY21, lovren10-6, CY31, CY32, Hybrid46 pathotypes (Hy25, Hy26, Hy27, Hy28), and Suwon 11 pathotypes (Su21, Su23, Su24, Su25, Su210, Su211, Su212, Su214) (Wan et al. 2004). Five predominant races were CY32, Su214, Su24, Hy28 and Su211 with the

corresponding frequencies of 44.4%, 16.7%, 5.6%, 4.2% and 4.1%, respectively. The survey also demonstrated that the frequencies of the samples in the pathogen population that were virulent to seven host resistance genesYr1, Yr3, Yr3b4b, Yr6, Yr9, YrA and YrSu were 93.0%, 91.7%, 55.5%, 61.1%, 77.8%, 90.2%, and 93.1%, respectively. Thus, based on the above information, the cultivars with these genes might lose resistance when leaves were infected by these pathogen populations in Yunnan Province.

The reproduction phase of a pathogen is an important factor considered in studies of pathogen genetic structure (Urbanelli et al. 2003). Clonal reproduction allows the most fit individuals to efficiently reproduce quickly and occupy the host. Resampling of identical multilocus genotypes is often the most robust and the significant evidence of clonal reproduction, especially when the identical genotypes reappear in excess in various localities (Tibayrenc et al. 1991). Our results agree with this criterion. A predominant genotype qj3 was detected from different cultivars and different counties in Yunnan population, representing about 40% of the AFLP genotypes. In Yuxi and Jiangchuan counties, the qj3 genotype was the only one genotype detected. Nonsexual genetic exchange with hyphal fusion among genetically different individuals has been demonstrated in several species. Populations of *Puccinia recondita* in Morocco showed both sexual and asexual reproduction (Farida et al. 2010). It seems that the probability of recombination in Yunnan population of *P. striiformis* could be very small, since $\overline{r}_d$ was significantly different from a panmictic population ($\overline{r}_d$=0.034, P<0.001). Additionally, The PTLPT approach also demonstrated the very small probability of recombination of the Yunnan population (Fig. 3a). Thus, it can be considered as a clonal population.

A recombination signature in *P. striiformis* has been suggested in Tianshui County, Gansu Province (Mboup et al. 2009). In the present study, isolates collected from five counties of Gansu Province (Table 1) were estimated by the PTLPT approach. The most parsimonious trees length demonstrated a probability of free recombination of the *P. striiformis* populations in Gansu Province (Fig. 3b) that is consistent with the results from Mboup et al. (2009).

Further studies should focus on investigation of the population genetic structures in other adjoining provinces to determine the relationship among the pathogen populations. The information will be useful to determine the possible role of the Yunnan population in the pandemics of wheat stripe rust in China, and to answer the question about whether the epidemics of stripe rust and inoculum generation in Yunnan Province could relate to those of other epidemic regions in China. The information will be useful to predict the regional epidemics and design the regional disease management strategies on deployment of various resistant varieties to reduce the frequency of virulent races or genotypes.

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