

Spatial genetic diversity and interregional spread of *Puccinia striiformis* f. sp. *tritici* in Northwest China

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Abstract In China, wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most destructive diseases of wheat. The Longnan and Linxia regions in Gansu Province and Qinghai Province are the major over-summering regions for the pathogen and key epidemiological zones in Northwest China. Population genetic diversity and interregional long-distance spread of the wheat stripe rust pathogen in Northwest China were studied using SSR markers. The genetic diversity in the Longnan population was much higher than those in the Linxia and Qinghai populations. Therefore, the molecular data confirmed that the Longnan region is a center of genetic diversity for *P. striiformis* f. sp. *tritici* in Northwest China. The low genetic differentiation ($G_{st}=0.15$) and the extensive gene flow ($N_m=1.37$) were found among the three regions in Northwest China. The most important

conclusion of this study is that the stripe rust inoculum in Qinghai can come from both Longnan and Linxia, but mainly from Longnan directly in the spring.

Keywords Gene flow · Genetic differentiation · *Puccinia striiformis* f. sp. *tritici* · Spread of pathogen · Stripe rust · *Triticum aestivum*

Introduction

Stripe rust of wheat, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is one of the most damaging diseases of wheat in many areas around the world (Stubbs 1985; Chen 2005). In terms of the area that can be affected by the disease, China is the largest epidemic region for stripe rust of wheat in the world (Wan et al. 2007). The epidemics of 1950, 1964, 1990 and 2002 caused yield losses up to 6.0, 3.0, 1.8 and 1.3 million metric tons, respectively (Wan et al. 2004, 2007).

Northwest China, including Gansu and Qinghai provinces, is one of the largest and the most important over-summering areas for the wheat stripe rust pathogen in China. The region provides inoculum to the major wheat-growing regions to the east. Almost all Chinese races of *P. striiformis* f. sp. *tritici* were first detected in the Longnan region of Gansu (Li and Zeng 2000). In the Longnan region (about 42,892 km²), both winter and spring wheats are grown in valleys and mountains ranging from 1,000 to 2,000 m above sea level (a.s.l.). Winter wheat is

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planted in August to October and harvested in June to August and spring wheat is planted in March to May and harvested in September and October. Various stages of wheat crops in this relatively small geographic region all year long provide host plants for the pathogen to infect. The relatively high precipitation (1,000 mm annual) in the growing season provide good moisture conditions for stripe rust infection and development. In addition, the mild summer in the high elevation areas and mild winter in the low elevation areas in the region are ideal conditions for the stripe rust pathogen to survive both summer and winter. Thus, stripe rust epidemics are more frequent in the Longnan region than many other regions of China and this region often provides inoculum to infect wheat crops in other regions (Li and Zeng 2000).

In contrast to the historically frequent occurrence of stripe rust in the Longnan region of Gansu, stripe rust was less severe and less frequent in Linxia of Gansu province and Qinghai province before the year 2002. However, the disease has become more frequent and severe since 2002. The Linxia region (about 8,169 km²) covers high plateau areas with elevations ranging from 1,900 to 2,100 m a.s.l. In this region, winter wheat is planted in October and harvested in July the next year. This region is dryer than the Longana region (about 537 mm annual precipitation and falling mostly during summer and autumn). The Qinghai region is in even higher plateau areas for wheat production with elevations ranging from 2,100 to 2,900 m a.s.l. The winter is too cold for the stripe rust fungus to survive winter (Li et al. 1997). Although the severe epidemics in the recent years can be attributed to growing susceptible cultivars and favourable summer weather conditions, a big question is where the inoculum has come from. During our disease surveys in the recent years (Kang et al. unpublished data), we have found that stripe rust can be easily found in November just before winter in the Longnan region. In the spring, active stripe rust uredia were often first found in March and developed to epidemics in May and continuously to August in this region. In contrast, no stripe rust was observed by ourselves or reported by others in October and November before the winter in the regions of Linxia and Qinghai. Stripe rust could not be found until May and developed to epidemics in June in the Linxia region and until July, and developed to epidemics in August in Qinghai. Based on these observations, our hypothesis is that urediniospores of *P. striiformis* f. sp.

tritici disperse from the Longan region to Linxia and Qinghai and cause severe damage of wheat crop in these regions. The objective of this study was to test the hypothesis through characterization of the pathogen populations in these regions using SSR markers. Knowledge of interregional relationships of the stripe rust pathogen populations should provide a scientific basis for developing strategies for effective control of the disease.

Materials and methods

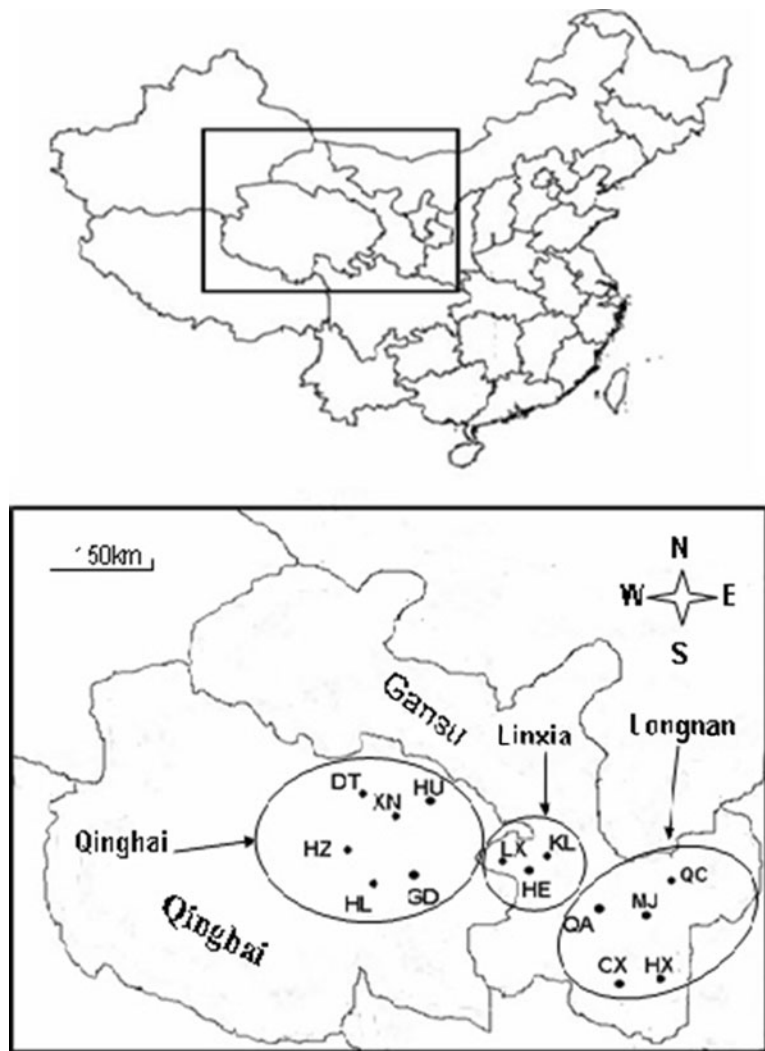
Sample collection

In this study, the Longnan and Linxia regions of Gansu province and Qinghai province were considered to be three epidemic regions (Fig. 1) (Table 1). Stripe rust surveys were conducted in these regions from March to August. During the surveys, leaves with uredia of *P. striiformis* f. sp. *tritici* were sampled in wheat fields. Samples were collected from 15 locations in five areas in Longnan, 12 locations in three areas in Linxia, and 15 locations in six areas in Qinghai. Each area covered a county or a city-controlled agricultural region. Three to five samples were collected from a single field and the distance between samples was at least 10 m. The samples were designated by the nearest village in sequential order, and were considered to represent the population for each location. Any two locations within an area were at least 5 km apart. The samples were selected from leaves with low disease severities as detached leaf segments each containing one or more separate stripes of uredia. Leaves were kept in plastic bags in a cooler during the surveys and immediately put in a desiccator at 4°C when brought to the laboratory.

Isolation and multiplication of urediniospores

Isolation and multiplication of urediniospores were done in the greenhouse at the Taibai Experimental Station of Northwest A&F University in Shaanxi province. About 10 seeds of winter wheat cultivar Mingxian 169, highly susceptible to all races of *P. striiformis* f. sp. *tritici* identified so far in China, were sown in a 7×7×7 cm pot, and the seedlings were grown in the greenhouse under daylight conditions with temperatures set at 18±2°C (day)/

Fig. 1 Regions and locations of *Puccinia striiformis* f. sp. *tritici* isolates in the southeast of Gansu Province and the east of Qinghai Province, China. The two capital letters indicate the names of the sampling locations (see Table 1). Eleven populations were made from multiple fields in following locations, *FH* Fenghuang Mountain; *GL* Gaolu Mountain; *HC* Hongchuan; *HL* Hengling Mountain; *JL* Jiangluo; *MC* Micang Mountain; *PN* Pingnan; *QS* Qishou Mountain; *WN* Weinan; *YS* Yun Mountain and *ZT* Zhongtan



10±2°C (night) from May to September, 2007. Seven- to 12-day-old seedlings, mostly when the first leaves were fully expanded, were inoculated with stripe rust samples. Before inoculation, leaves of stripe rust samples were washed with distilled water, taped in a Petri dish with the sporulating side upwards, and placed in a dew chamber at 100% rh at 12°C for 12–16 h. When fresh spores emerged, urediniospores from a single uredium were used to inoculate the seedlings of Mingxian 169. The inoculated seedlings were incubated in a dew chamber at 100% rh at 12°C for 24 h and then transferred to a greenhouse with light and temperature conditions as described as above. In order to prevent cross-contamination in the greenhouse, each pot was covered with a cellophane paper. Fifteen days

after inoculation, uredia could be observed. One leaf bearing uredia was kept in each pot for each sample, and the remaining leaves were removed. About 18–20 days after inoculation, urediniospores were collected in a 2.2 ml microtube, dried in a desiccator at 4°C for 3 days, and then stored at –80°C.

DNA extraction

DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) procedure as described by Chen et al. (2008). For each isolate, 5 mg of urediniospores were transferred into a 1.5 ml tube, 100 µl extraction buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 100 mM EDTA) was added and homogenized with a plastic pestle attached to an

Table 1 Regions and locations, codes, elevations, and number of samples of *Puccinia striiformis* f.sp. *tritici* populations from the Northwest of China used in this study

| Region | Population location | Number of isolation | Elevation (m) | Code |
|-----------|---------------------|---------------------|---------------|------|
| Longnan | Maiji | 111 | 1200–1500 | MJ |
| | Qincheng | 49 | 1400–1650 | QC |
| | Qing'an | 30 | 1700–1850 | QA |
| | Chengxian | 30 | 1100–1200 | CX |
| | Huixian | 38 | 1100–1200 | HX |
| Sub-total | | 258 | | |
| Linxia | Linxia | 67 | 1900–2000 | LX |
| | Hezheng | 65 | 1900–2100 | HE |
| | Kangle | 74 | 1900–2100 | KL |
| Sub-total | | 206 | | |
| Qinghai | Xining | 47 | 2100–2230 | XN |
| | Huangzhong | 33 | 2700–2850 | HZ |
| | Guide | 41 | 2300–2420 | GD |
| | Hualong | 37 | 2600–2758 | HL |
| | Datong | 31 | 2650–2797 | DT |
| | Huzhu | 32 | 2500–2674 | HU |
| Sub-total | | 221 | | |
| Total | | 685 | | |

electric drill. Then, 5- μ l proteinase K (1 mg ml⁻¹) was added and the suspension was filled up to 500 μ l with extraction buffer. After incubation for 30 min at 65°C, the mixture was extracted with phenol/chloroform/isoamylalcohol (25/24/1, pH=8.0) and chloroform. The top aqueous phase was transferred to a clean tube, and an equal volume of cold isopropanol was added. After 1 h of incubation at -20°C, the solution was centrifuged for 20 min at 12,000 rpm at 4°C to precipitate the nucleic acid. The pellet was rinsed twice with cold 70% ethanol, dried and dissolved in 0.1 ml of TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). 1 μ l of ribonuclease at 10 mg/ml was added (final concentration 20 μ g/ml) and kept at 4°C overnight to completely digest RNA. DNA was re-precipitated, rinsed with cold 70% ethanol, dried and dissolved in 50 μ l of TE. DNA was quantified using a spectrophotometer (Eppendorf AG22331, Germany).

SSR markers

In this study, 11 pairs of SSR primers were used (Table 2), of which Cps08, Cps09, Cps10, Cps15,

Cps34 and Cps36 were developed in our lab (Chen et al. 2008) and the others were developed by Enjalbert et al. (2002). These primers were synthesized by Shanghai Shengong Biological Engineering Technology and Service Co., Ltd. (Shanghai, China). Amplification was carried out in a volume of 10 μ l. Each reaction contained 1.0 μ l 10 \times reaction buffer [750 mM Tris-HCl, 200 mM (NH₄)₂SO₄, 0.1% Tween-20], 0.8 μ l 25 mM MgCl₂, 1.0 μ l 2.5 mM dNTPs (dATP, dCTP, dGTP, dTTP), 0.5 μ l 10 μ M TP-M13 (Li-Cor, Lincoln, NE, USA), 0.5 μ l 10 μ M reverse primer, 0.5 μ l 10 pmole/ml IR-labeled M13 primer, 0.15 μ l *Taq* polymerase (5 U/ μ l, Applied Biosystems), 1.0 μ l 50 ng/ μ l DNA and 4.55 μ l ddH₂O.

The PCR reaction was done in a thermal cycler programmed for an initial denaturation at 94°C for 4 min, followed by ten cycles of denaturation at 94°C for 45 s, annealing at 64°C for 45 s and extension at 72°C for 45 s, the annealing temperature being reduced by 1°C from the previous cycle. This procedure was followed by 25 cycles of denaturation at 94°C for 45 s, annealing at 54°C for 45 s and extension at 72°C for 45 s, and a final extension at 72°C for 10 min. The PCR products were kept at 4°C in the cycler until moved into a refrigerator at 4°C until use. PCR products were added to 3.0 μ l of the loading buffer (98% deionized formamide, 2 mM EDTA, 0.05% bromophenol-blue and 0.05% xylene-cyanol) and heated for 5 min at 94°C, 0.8 μ l of the denatured sample was loaded on a 6% denaturing acrylamide gel and electrophoresed using a LI-COR 4300 IR2 automated DNA analysis system. Gels were run in a 64-well format at 1,500 V, 40 mA, for a maximum of 3 h, depending on the size of the PCR products.

Data analyses

A polymorphic band among the isolates was scored as presence (1) or absence (0) for each isolate. The data of SSR markers were used to determine diversity of all samples and those in each region. The gene diversity (*Ht*) in population is the sum of average gene diversity among the regional populations (*Dst*) and average gene diversity within the regional populations (*Hs*) (Yeh et al. 1997). The genetic differentiation (*Gst*) among the regional populations was computed as $Gst = Dst/Ht$. Gene flow was

Table 2 Sequences of simple sequence repeat (SSR) and M13 primers used in this study and repeat motifs for the primers to amplify and annealing temperatures (T_m) used in polymerase chain reaction

| Primer | Primer sequence (5'-3') | Repeat motif | T_m (°C) |
|-------------------------|--|----------------------|------------|
| IR-labeled M13 | CACGACGTTGTAAAACGAC | | |
| TP-M13 | CACGACGTTGTAAAACGAC | | |
| CPS08F TP-M13 CPS08R | TP-M13-GATAAGAAACAAGGGACAGC CAGTGAACCCAATTACTCAG | (CAG) ₁₄ | 55 |
| TP-M13-CPS09F CPS09R | TP-M13-CGGGAGAAGACCTGAGC AGAAAACGGAATGTAATGTG | (GTT) ₉ | 58 |
| TP-M13-CPS10F CPS10R | TP-M13-TCTACTGGGCAGACTGGTC CGGTTTGTTCGTTTC | (TAG) ₈ | 56 |
| TP-M13CPS15F CPS15R | TP-M13-GATGGGGAA AAGTAAGAAGT GGTGGGGGATGTAAGTATGTA | (TTC) ₄ | 55 |
| TP-M13CPS34 F CPS34R | TP-M13-GTTGGCTACGAGTGGTCATC TAACACTACACA AAAGGGGTC | (TC) ₉ | 55 |
| TP-M13CPS36F CPS36R | TP-M13-TCCAGGCAGTAAATCAGACGC ATCAGCAGGTGTAGCCCCATC | (GAC) ₆ | 55 |
| TP-M13RJ27F RJ27R | TP-M13-CGTCCCGACTAATCTGGTCC ATGAGTTAGTTTAGATCAGGTCGAC | (TC) ₁₀ | 52 |
| TP-M13RJ18F RJ18R | TP-M13-CTGCCCATTGCTCTTCGTC GATGAAGTGGGTGCTGCTG | (TGT) ₅ | 52 |
| TP-M13RJ20F RJ20R | TP-M13-AGAAGATCGACGCACCCG CCTCCGATTGGCTTAGGC | (CAG) ₄ | 52 |
| TP-M13RJ21F RJ21R | TP-M13-TTCCTGGATTGAATTCGTCG CAGTTCTCACTCGGACCCAG | (GTT) ₆ | 52 |
| TP-M13RJ24F RJ24R | TP-M13-TTGCTGAGTAGTTTGCAGGTGAG CTCAAGCCCATCCTCCAACC | (GTT) ₅₊₉ | 52 |

estimated from F_{ST} , which, for multiple allelic loci, is equivalent to G_{ST} according to Nei (1973), as $Nm = 0.25(1 - F_{ST})/F_{ST}$. Genetic distances between populations were estimated using Nei's unbiased genetic distance coefficient. The mean genetic similarity between-regional populations was obtained by averaging individual G_{ST} estimates using the whole set of isolates belonging to the regional populations being compared. All calculations and analyses were conducted using the software POPGENE version 1.31 (Yeh et al. 1997). Analyses of genotypic diversity among different areas and regions were done using software DCFA 1.1 (Zhang and Ge 2002).

To statistically assess genetic variation within and among populations of the areas and regions, we performed an analysis of molecular variance (Excoffier et al. 1992) with the software package Arlequin, version 2000 (Schneider et al. 2000). An unrooted tree of the isolates was estimated from the SSR data. The set of most parsimonious trees (where the criterion was to find the tree with the minimum number of changes) was found using the MIX program in

Phylyp (Felsenstein 1989). A majority rule consensus tree, which consists of all groups that occur in more than 50% of the trees investigated, was selected using the CONSENSE program.

To determine the migration of the pathogen among different regions in the Northwest of China using SSR markers, the null hypothesis that there is no migration of the wheat stripe rust pathogen in the three regions of Northwest China was tested. If the pathogen migration is restricted, isolates from different regions should have different genotypes, and high genetic differentiation and low gene flow among regions should be expected. Otherwise, the data should support our hypothesis that there have been substantial migrations of *P. striiformis* f. sp. *tritici* among the three regions.

Results

A total of 685 samples of *P. striiformis* f. sp. *tritici* were obtained, of which 258 were collected from the Longnan region, 206 from the Linxia regions and 221

from the Qinghai region (Table 1). The numbers and distributions of the isolates demonstrated that they represented the *P. striiformis* f. sp. *tritici* population for the three regions and were suitable for determining the population structures in each region and for comparisons among regions.

The 11 primer pairs produced 40 SSR markers, of which 38 were polymorphic. Eight of the polymorphic markers were selected because they were more informative. These markers revealed 17 genotype groups. Figure 2 shows the genetic relationships of these genotype groups (A–G), and Fig. 3 shows frequencies of the genotype groups in the three epidemic regions. All 17 genotype groups (A–Q) were detected in the Longnan region. In contrast, only 13 of them (A, B, C, D, F, I, J, K, L, M, N, O, P) were detected in the Linxia region and 12 (A, B, C, D, F, G, I, J, L, M, N, O) in the Qinghai region. The Linxia and Qinghai regions shared genotype groups A, B, C, D, F, I, J, L, M, N and O. However, genotypes K and P were presented in the Linxia region, but not detected in the Qinghai region. Similarly, genotypes G was presented in the Qinghai region, but not detected in the Linxia region. In general, genotypes with frequencies more than 5% in the Longnan region were also presented in the Linxia and Qinghai regions, while those with frequencies less than 5% in the Longnan region were detected in: both Linxia and Qinghai regions (genotype groups F, M and O); only

one of the two regions (G, K and P); or none of the two regions (E, H and Q). The results showed that the Linxia and Qinghai populations were part of the Longnan population and that the Linxia and Qinghai populations consisted of common and different populations.

The overall genetic diversity of the Northwest population was 0.24. There were differences among and within the regional populations. The genetic diversities in the Longnan population ($H=0.26$) was significantly higher than those of the Linxia ($H=0.23$) and Qinghai ($H=0.22$) populations. For the Northwest population, the genetic differentiation was low ($Gst=0.15$), suggesting extensive gene exchange among the regions in the Northwest of China (Table 3).

The high value of gene flow ($Nm=1.37$) indicated the low genetic differentiation among the three regions. Substantial gene flow was found between regions: from the highest, between the Longnan and Linxia regions (2.88); moderate, between the Longnan and Qinghai regions (2.35); to the lowest, between the Linxia and Qinghai regions (1.69) (Table 3). The data suggested that the stripe rust inoculum to start the Qinghai population was more likely or mainly to originate from the Longnan region than from Linxia.

The analysis of molecular variation partitioned the total genetic variation. The most (80.39%) of the

Fig. 2 Unrooted tree of *Puccinia striiformis* f. sp. *tritici* based on SSR data for isolates sharing genotype with determined virulence in the Northwest of China. The isolates were classified into 17 (A to Q) genotype groups. Two capital letters representing a population code (Table 1). The length of the bar indicates one marker-polymorphism

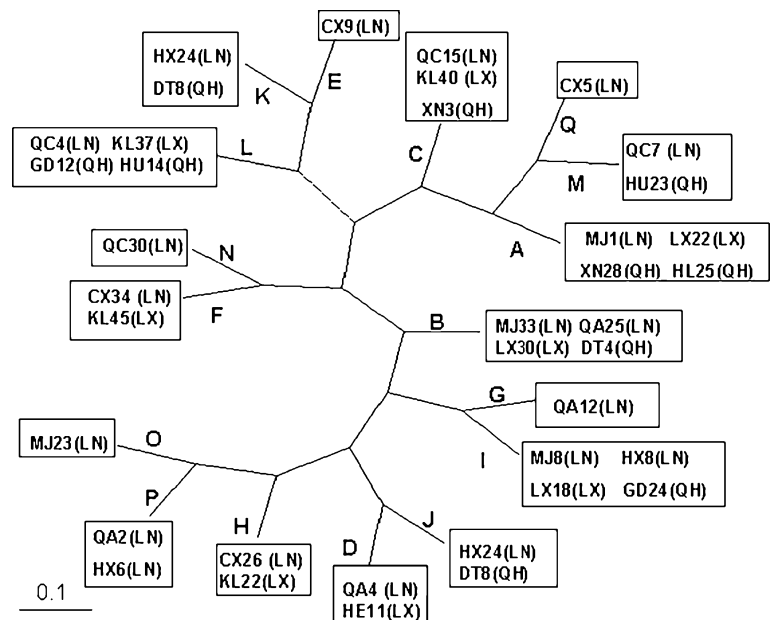
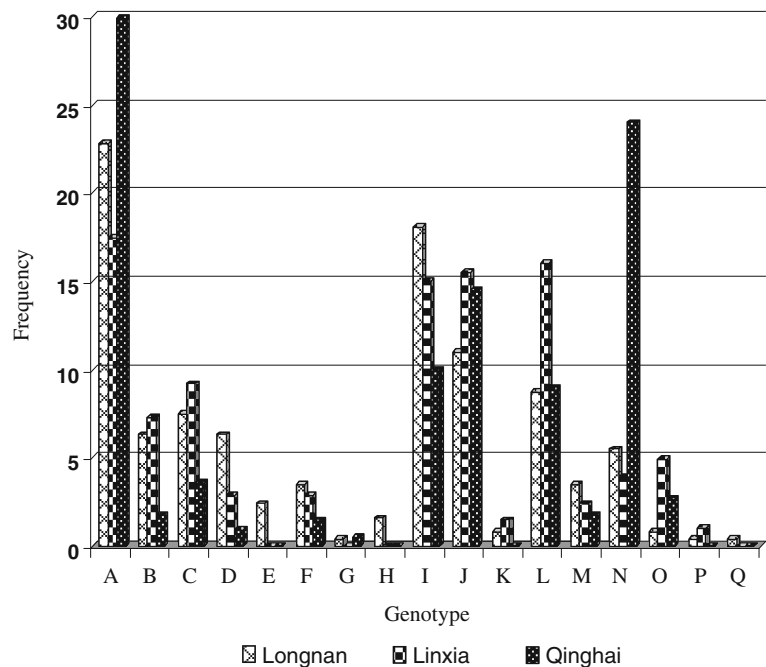


Fig. 3 Genotype and frequencies of SSR genotypes of *P. striiformis* f. sp. *tritici* in the Longnan (LN), Linxia (LX), and Qinghai (QH) regions in the Northwest of China



variation was presented within populations of locations, 17.63% among populations of areas within regions, and only 1.98% among regions (Table 4). These data supported the finding of considerable gene flow and insignificant separation among the regional populations.

Table 3 Gene diversity, genetic differentiation and gene flow of *P. striiformis* f.sp. *tritici* within and among geographical regions in the Northwest of China

| Regions | <i>Ht</i> ^a | <i>Hs</i> ^b | <i>Dst</i> ^c | <i>Gst</i> ^d | <i>Nm</i> ^e |
|-----------------|------------------------|------------------------|-------------------------|-------------------------|------------------------|
| Longnan | 0.26* | 0.22 | 0.03 | 0.13* | 1.63 |
| Linxia | 0.23 | 0.21 | 0.02 | 0.09 | 2.69 |
| Qinhai | 0.22 | 0.20 | 0.01 | 0.07 | 3.53 |
| Total | 0.24 | 0.20 | 0.04 | 0.15 | 1.37 |
| Longnan/Linxia | | | | | 2.88* |
| Longnan/Qinghai | | | | | 2.35* |
| Linxia/Qinghai | | | | | 1.69 |

^a *Ht* denotes total gene diversity

^b *Hs* denotes gene diversity within populations

^c *Dst* denotes gene diversity among populations

^d *Gst* denotes population genetic differentiation

^e *Nm* denotes gene flow

*denotes significant level ($0.01 < P < 0.05$)

Discussion

The important conclusion of this study is that the populations of *P. striiformis* f. sp. *tritici* possessed relatively high levels of genetic diversity but a low genetic differentiation in the Northwest of China. These results are in consistent with previous reports (Shan et al. 1998; Zheng et al. 2001). Different from our results, the levels of genetic diversity of *P. striiformis* f. sp. *tritici* were low in reports from other countries, especially in Europe. For example, Lorys et al. (2002) studied genetic variation of the stripe rust pathogen on a local scale in four fields located in northern France. In their study, 77% of the isolates had the same AFLP pattern, all other patterns being rare or unique. In the Northwest of China, the genetic diversities of the pathogen populations in the Longnan region were much higher than those of Linxia and Qinghai regions. The higher diversities in Longnan could be attributed to the cooler weather conditions favourable for the pathogen to over-winter and over-summer in the mountainous regions. More diverse wheat cultivars and spring and winter crops help the pathogen maintain a high diversity. Such weather and cropping conditions would enable the pathogen to generate mutation and to maintain genetic variation. Our SSR marker data suggest that urediniospores of *P. striiformis* f. sp. *tritici*

Table 4 Analysis of molecular variance (AMOVA) of *P. striiformis* f.sp. *tritici* based on SSR markers for 14 populations from the Northwest of China

| Source of variance | DF ^a | Sum of squares | Variance component | Percentage of variation (%) | F | P |
|----------------------------------|-----------------|----------------|--------------------|-----------------------------|-----------|--------|
| Among regions | 4 | 151.67 | 0.09 | 1.98 | FCT, 0.01 | 0.263 |
| Among populations within regions | 11 | 424.23 | 1.53 | 17.63 | FSC, 0.23 | <0.001 |
| Within populations | 669 | 2336.55 | 4.26 | 80.39 | FST, 0.21 | <0.001 |
| Total | 684 | 2912.45 | 5.88 | 100.00 | | – |

^a D.F. denotes degree of freedom

in Linxia and Qinghai are dispersed from Longnan. Therefore, the genetic diversities of the pathogen in the former regions are determined by the populations in the latter regions. The results suggest that the Longnan region is the most important centre of the stripe rust pathogen in the Northwest of China.

Recently, Mboup et al. (2009) reported the diversity of *P. striiformis* f. sp. *tritici* in the Tianshui county of about 2,500 km², which is just a part of the Longnan region of Gansu. They found 38% phenotypic diversity and 89% genotypic diversities. They did not find site ($F_{st}=0.004$) or altitude ($F_{st}=0.0098$) differences for the samples, which is reasonable for such small area. In the present study, we used 685 isolates that were collected from Longnan and Linxia of Gansu and Qinghai, covering all major wheat epidemic areas in the Northwest of China of about 135,000 km. The genetic differentiations ($G_{st}=0.15$ for all regions and $G_{st}=0.13$ for the Longnan region) were not significant among regions, but much higher than that found by Mboup et al. (2009). Our results demonstrate high gene flow or urediniospore dispersal over the area of about 135,000 km². We are currently conducting studies to determine genetic diversity and gene flow throughout China.

Our molecular data indicated that there was long-distance dispersal of the wheat stripe rust pathogen in the Northwest of China. The 13 genotypes in Linxia and 12 genotypes in Qinghai were all detected in the Longnan region, where a total of 17 genotypes were detected suggesting that the pathogen inoculum in the Linxia and Qinghai regions were related to Longnan. The long-distance migration of the pathogen among regions in Northwest China is also supported by the low genetic differentiation and high gene flow. The most important molecular evidence is the common SSR genotypes of isolates from different regions. The high genetic similar-

ities among isolates from different regions suggested that the isolates have the same origin. The genotype groups B, F, H, K and J commonly detected in the three regions provide direct evidence for the long-distance dispersal of urediniospores of the pathogen.

During our disease surveys from 2006 to 2008, stripe rust was observed in late March and the disease developed to epidemics in May in the Longnan region. The disease was not found until June and developed to epidemics in July in Linxia where both winter and spring wheat crops were grown. Stripe rust was found in July and developed to epidemics in August in Qinghai when the weather was favourable for the disease development. The observations of sequential occurrence appear to support a hypothesis that the pathogen first spreads from Longnan to Linxia, increases in Linxia, and then spreads to Qinghai. However, our molecular data showed that the interregional gene flow, as an indication of urediniospore dispersal, was higher between Qinghai and Longnan than that between Qinghai and Linxia. Genotype groups K and P that were detected in the Linxia region were not detected in the Qinghai. In contrast, genotype group G that was detected in the Qinghai region was not detected in Linxia. The molecular data support more another hypothesis that the major inoculum of stripe rust for infection of wheat crops in Qinghai is directly from the Longnan region.

The field observations of the earlier rust development in Linxia than in Qinghai could be due to the weather and crop season differences. The elevations in Qinghai are much higher than those in Linxia as shown in Table 1 and the wheat crop in Qinghai is later than that in Linxia. The low temperatures in the Qinghai region of high elevations make stripe rust development slow. The latent period of stripe rust infection is much longer and the disease development is much slow under relatively low temperatures than high temperatures

(Line 2002; Chen 2005). The prevailing winds in Northwest China are from the southeast to northwest in March to August. In June and July in the Longnan region, stripe rust continue producing urediniospores which can disperse to Qinghai and Linxia. Based on these discussions, the stripe rust inoculum in Qinghai can originate from both Longnan and Linxia, but mainly directly from Longnan.

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