SSR Genotypes of the Puccinia triticina in 15 Provinces of China Indicate Regional Migration in One Season from East to West and South to North

Zhe Xu[1], Shuang Xia[1], Bo Liu[1], Li Gao[1], Wanquan Chen[1], and Taiguo Liu[2]

[1]Chinese Academy of Agricultural Sciences Institute of Plant Protection
[2]Institute of Plant Protection, Chinese Academy of Agricultural Sciences

February 25, 2021

Abstract

Leaf rust of wheat caused by Puccinia triticina (Pt) is one of the most common and widespread fungal diseases which has a wide incidence area, especially in the southwest and northwest of China, the middle and lower reaches of the Yangtze river, and the southern part of the Huang-huai-hai river basin. To explore the relationship between the epidemic flora of leaf rust and transmission, a total of 709 isolates from Beijing, Hebei, Henan, Shandong, Shanxi, Shaanxi, Anhui, Jiangsu, Hubei, Yunnan, Sichuan, Gansu, Qinghai, Heilongjiang, Inner Mongolia 15 provinces was genotyped using 13 simple sequence repeat (SSR) markers. The Puccinia triticina populations from the 15 provinces were obviously divided into three predominant populations including the eastern Pt populations consisting of Pt samples from eight eastern provinces of Beijing, Hebei, Shandong, Anhui, Henan, Shanxi, Shaanxi and Heilongjiang; the four western Pt populations from Gansu, Sichuan, Qinghai and Inner Mongolia provinces; and the bridge Pt populations including Pt samples from Jiangsu, Hubei and Yunnan provinces which were communicated with the other two populations as a "bridge". And the pathogen source transmission of eastern Pt populations was more frequent than western Pt populations. The linkage disequilibrium test indicated that the whole Pt population was linkage disequilibrium. Beijing, Hebei, Shaanxi, Jiangsu, Henan and Heilongjiang provinces were showed obviously linkage equilibrium phenomena while the five provinces of Qinghai, Hubei, Anhui, Shandong and Inner Mongolia were supported clonal mode of reproductions. In addition, provinces of Shanxi, Yunnan, Gansu, and Sichuan showed weak linkage disequilibrium phenomena. We systematically revealed the genotypic diversities, population differentiation and reproduction of P. triticina in 15 wheat producing areas in China.

SSR Genotypes of the *Puccinia triticina* in 15 Provinces of China Indicate Regional Migration in One Season from East to West and South to North

Zhe Xu[1, 2+], Xiaoshuang Xia[1, 2+], Bo Liu[1], Li Gao[1, 3], Wanquan Chen[1, 3*], Taiguo Liu[1, 2, 3*]

[1] State Key Laboratory for the Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Science, Beijing 100193, China

[2] Key Laboratory of Biological Hazard Factors (Plant Source) Control of Agricultural Products Quality and Safety, Ministry of Agriculture and Rural Affairs, Beijing 100193, China

[3] National Agricultural Experimental Station for Plant Protection at Gangu, Ministry of Agriculture and Rural Affairs, Gansu 741200, China

+ These authors contributed equally to this work.

*** Correspondence:**

Wanquan Chen

wqchen@ippcaas.cn

Taiguo Liu

tgliu@ippcaas.cn

Abstract

Leaf rust of wheat caused by*Puccinia triticina* (*Pt*) is one of the most common and widespread fungal diseases which has a wide incidence area, especially in the southwest and northwest of China, the middle and lower reaches of the Yangtze river, and the southern part of the Huang-huai-hai river basin. To explore the relationship between the epidemic flora of leaf rust and transmission, a total of 709 isolates from Beijing, Hebei, Henan, Shandong, Shanxi, Shaanxi, Anhui, Jiangsu, Hubei, Yunnan, Sichuan, Gansu, Qinghai, Heilongjiang, Inner Mongolia 15 provinces was genotyped using 13 simple sequence repeat (SSR) markers. The*Puccinia triticina* populations from the 15 provinces were obviously divided into three predominant populations including the eastern *Pt* populations consisting of *Pt* samples from eight eastern provinces of Beijing, Hebei, Shandong, Anhui, Henan, Shanxi, Shaanxi and Heilongjiang; the four western *Pt* populations from Gansu, Sichuan, Qinghai and Inner Mongolia provinces; and the bridge*Pt* populations including *Pt* samples from Jiangsu, Hubei and Yunnan provinces which were communicated with the other two populations as a "bridge". And the pathogen source transmission of eastern *Pt* populations was more frequent than western *Pt*populations. The linkage disequilibrium test indicated that the whole*Pt* population was linkage disequilibrium. Beijing, Hebei, Shaanxi, Jiangsu, Henan and Heilongjiang provinces were showed obviously linkage equilibrium phenomena while the five provinces of Qinghai, Hubei, Anhui, Shandong and Inner Mongolia were supported clonal mode of reproductions. In addition, provinces of Shanxi, Yunnan, Gansu, and Sichuan showed weak linkage disequilibrium phenomena. We systematically revealed the genotypic diversities, population differentiation and reproduction of *P. triticina* in 15 wheat producing areas in China.

Key words: *Puccinia triticina*, SSR, Population genetic structure, Pathogen exchange.

Introduction

Wheat is the third largest grain crop in China leading after corn and rice, accounting for 20.7% of Chinese total grain output, which is of great significance to Chinese grain stability (Jin, 1996). Although China attaches great importance to the production and supply of wheat, it will be still facing facts of various production crises (Lu et al., 2017). Leaf rust of wheat caused by *P. triticina* is the most common and widespread fungal disease among the three major wheat rust diseases, which seriously threatens the world wheat safety (Peng, 2013). Wheat leaf rust has a wide incidence area, especially in the southwest and northwest of China, the middle and lower reaches of the Yangtze river, and the southern part of the Huang-huai-hai river basin (Huerta-Espino et al., 2011). In recent years, the occurrence of wheat leaf rust has been increasing. In 2008, 2009, 2012 and 2013, it occurred on a large scale throughout the country, causing serious losses in some areas of Shandong, Henan and Xinjiang. In 2015, it broke out in Huang-huai-hai region, especially in Henan, Anhui and other provinces (Kang et al., 2016; Zhang et al., 2018).

The prevention and control of wheat rust should be based on the strengthening of disease monitoring and integrated measures of planting disease-resistant varieties, supplemented by fungicides and cultivation control (Chen, 2011). Planting disease-resistant varieties is the most effective and environmentally friendly method to control the growth of wheat leaf rust. However, the resistant varieties of wheat will lose their rust resistance due to the virulence variations of rust pathogen (Bolton et al., 2008). In addition, wheat leaf rust was spread by uredospores in the wind, which enabled it to colonize quickly in new areas and migrate between different regions, causing it to establish a new population in new areas (Kolmer and OrdoñEz, 2007).

Although it is simple, intuitive and practical to study the population virulence of *P. triticina* by traditional virulence analysis, the results are easily affected by the differential host, environmental conditions and human

factors, and it is difficult to comprehensively reflect the population genetic structure and genetic relationship of*P. triticina* (Xu, 2012). SSR molecular marker technology effectively reveals diminutive variation among individuals within the same species, using a series of primer pairs (Ashley and Dow, 1994). Duan (Duan et al., 2003) and Szabo (Szabo and Kolmer, 2006) reported SSR primers for genetic polymorphism analysis of *P. triticina* in 2003 and 2007, respectively. In 2007, Ordoñez et al. (Ordoñez and Kolmer, 2007) used SSR markers to analyze the molecular polymorphisms of the world durum *P. triticina* and found that strains from South America, North America and Europe had highly similar SSR genotypes, indicating that they might originate from one single strain. In 2010, Ordoñez et al. (Ordoñez et al., 2010) conducted SSR analysis of *P. triticina* in South America for nearly 20 years and compared its genetic structure with that of North America. They found that SSR genotypes of*P. triticina* in north and South America were highly similar. In 2007, Kolmer and Ordoñez (Kolmer et al., 2007) applied SSR technique to analyze *P. triticina* population in central Asia and the Caucasus and compared with *P. triticina* of common wheat and durum wheat on north American, the results showed that strains of central Asia and the Caucasus had similar genotypes, and clear differences of *P. triticina* populations genetic structure of North America were demonstrated with that of pathogens from these two regions. In 2011, Kolmer and Ordoñez (Kolmer et al., 2011) analyzed the genetic structure of *P. triticina* in the Middle East and compared it with the genetic structure of it in central Asia. The results showed that the middle east and central Asia did not have the same genotype, which proved the lack of gene communication in the two regions at the molecular level. In 2012, Xu Minqing et al. in China studied 120 strains of wheat leaf rust with 25 pairs of SSR primers, and found that the genetic diversity of *P. triticina* was relatively rich, and there was a correlation between genetic polymorphisms and geographical source. Kolmer et al. (Kolmer et al., 2013) studied the European population and divided the European population into 8 groups by using 23 SSR markers. SSR genotypes were correlated with virulence phenotypes and revealed that the European population of leaf rust had higher genetic diversity than other continental populations. In 2015, Kolmer (Kolmer, 2015) analyzed *P. triticina* samples from seven provinces in China and found that SSR genotypes and independent phenotypes did not differentiate as a whole according to the province of origin, and the seven provinces were contiguous, which was likely to form a single epidemiological zone for *P. triticina* .

At present, we do not know the relationship between the epidemic divisions of leaf rust and pathogen source transmission, and the evolutionary relationship between the physiological species of leaf rust is not clear. Thus, identifying these relationships are significant for formulating correct control strategy and rational distribution of wheat resistant varieties. Our main objectives are to determine if distinct regional division between 15 provinces (Beijing, Hebei, Henan, Shandong, Shanxi, Shaanxi, Anhui, Jiangsu, Hubei, Yunnan, Sichuan, Gansu, Qinghai, Heilongjiang, Inner Mongolia) of *P. triticina* are present and to reveal reproduction of *P. triticina* populations. Finally, we aim to provide further insight on the transmission of this important wheat pathogen and disease prevalence in China.

Materials and Methods

Collections of *P. triticina*were from locations in Beijing, Hebei, Shanxi, Yunnan, Jiangsu, Gansu, Qinghai, Sichuan, Hubei, Shaanxi, Anhui, Shandong, Henan, Heilongjiang, Inner Mongolia provinces in 2018 (most of the collections were obtained were sent by scientists from Chinese Cereal Rust and Powdery Mildew Research Group) (Figure 1 & Supplementary Table S1). DNA were extracted by Plant Genomic DNA Kit (TIANGEN Biotech (Beijing), China) according to instructions.

Thirteen SSR primer pairs developed from genomic libraries of *P. triticina* were used to characterize the collections (Duan et al., 2003) (Table1). The Polymerase Chain Reaction (PCR) reaction was carried out in a total volume of 25 μL including 12.5 μL of 2×Unique^{TM} *Taq* Super Master Mix (Novogene, Beijing, China), 1μL of DNA, 9.5 μL of sterile ddH_2O and 1 μL of forward and reverse primer. The conditions were as following: initial denaturation at 94 for 5 min, 35 cycles at 94 for 30s, 56 for 30s (according to the annealing temperature of the primers), 72 for 60s and with a final extension at 72 for 10min.

Electrophoresis were carried out in DYY- 6B electrical swimming instrument after PCR for testing the concentrations of extracted DNA. The amplification products, which were amplified by electrophoresis were

diluted at the ratio of 1:50. Then add 1μL of diluted product to the mixture of 9 μL Hi-Di (Hi-Di Formamide for 3500 Dx/3500xl Dx) and Liz 500 (GeneScan 500 LIZ Size Standard), and mixing ratio of Hi-Di and Liz is 1000:15. SSR analysis was performed with ABI 3500xl Genetic Analyzer (Applied Biosystems, USA). The length of the fragment was read by GeneMarker v2.7.0.

Isolate genotypes were determined by using molecular weights of alleles at the 13 SSR loci. An R package called Poppr was used to analysis of data from admixed, clonal, mixed, and/or sexual populations (Kamvar et al., 2014). Simpson's index (1-D) calculate the diversity of alleles of every locus; Genotypes richness and evenness reflected by expected MLG (eMLG) and E.5 (John A. Ludwig, 1989; Grünwald et al., 2003); Genotypes diversity measured by the Shannon-Wiener index (H) (Shannon, 1948), Stoddart and Taylor's index (G) (Stoddart and Taylor, 1988), and Simpson's index (lambda) (Simpson, E.H., 1972). And we used Corrected Simpson's Index (Corrected lambda) to avoid the influence by sample size.

Analysis of Molecular Variance (AMOVA) attempted to analyze the variations of SSR genotypes in three levels between region, between samples within region and within samples (Excoffier, 1992); Discriminant Analysis of Principal Components (DAPC) was a multivariate, model-free approach to clustering based on prior population information (François et al., 2010). We can analyze the population structure by assessing how well samples can be reassigned into previously defined populations.

A Bayesian approach was also used to group the SSR genotypes with STRUCTURE v2.3.4 (Pritchard et al., 2000). Global analysis was run for SSR genotypes together for K ranging from two to eighteen with ten iterations for each K. Running results get on to structure harvester website, with delta K value to determine the best population. Finally used Distruct software (Rosenberg, 2004) to generate histograms and divided the population structure.

Analysis of gene flow (Nm) in each province was calculated by GENALEX, a user-friendly cross-platform package that runs with in Microsoft Excel (Li, 2008). Gene flow (Nm) was the movement of genes in a population (Husband and Barrett, 1995) and an important factor that affects the degree of genetic variation within and between populations (Slatkin, 1987). It can reveal the interaction of genes between populations.

Results

From the genotype accumulation curve, it had reached the plateau with 12 loci indicted that the 13 loci we used is enough for distinguishing all the observed MLGs (Supplementary Figure S1). All the 13 SSR loci used in this study was polymorphic and the average number of alleles was 5.23. Except that RB26, RB12 had two alleles produced, the other SSR markers would produce 3-13 microsatellite alleles. The values of 1-D and Hexp was similar which ranged from 0.076 to 0.729 while the range of evenness was 0.435-0.997. RB26 locus had low diversity, low expected heterozygosity and was very uneven in allele distribution. This is a sign that this might be a phylogenetically uninformative locus, which we have two alleles and one is occurring at a minor frequency. The diversity and evenness of RB25 and RB27 was low, but the alleles were abundant. RB29 had highest diversity and containing the most alleles. RB12 had the most evenly distributed alleles (0.997) (Table2).

To ensure the accuracy of analysis, 0.25 percent missing data was removed by loci. A total of 428 genotypes were observed in 622 samples of*P. triticina* , indicating that 68.8% of the isolates belonged to unique genotype. From the value of eMLG, population of Inner Mongolia had high eMLG (10.00) while the eMLG value of Beijing (7.65) and Yunnan (7.64) is low. Genotype evenness is a measure of the distribution of genotype abundance, where the genotype evenness of a population with the same genotype abundance is equal to 1, while the genotype evenness of a population dominated by a single genotype is close to 0. Evenness ranged from 0.464 to 1.000 and the total was 0.564, showing that genotypes were fairly evenly distributed. From the value of corrected lambda, the genotypic diversity of the population was from 0.875 to 0.980, and the overall genotypic diversity was 0.998, indicating a high genotypic diversity (Table3).

This clonal spread of the pathogen from 15 provinces was supported partially by randomization test showing significant AMOVA results across within samples (132.12, P =0.001) and between regions also had a few

variations (7.12, P =0.001), which was consistent with previous studies (Kolmer and Ordoñez, 2007; Xu et al., 2013). (Supplementary Table S2). Furthermore, the significant differences between regions make it possible to analyze the population relationship between different provinces.

The relative position of the *P. triticina* populations in DAPC scatter plot (Figure 2) suggest that the populations from different provinces was closely related. It indicated that the first discriminant component separated Inner Mongolia from other regions and the second discriminant component showed a gradient from Shandong to Qinghai (Figure 2). Dividing the figure into four quadrants, with the samples of Beijing, Hebei, Shandong, Anhui, Shanxi, Shaanxi, Henan, Heilongjiang were mainly distributed in the third quadrant while the samples of Qinghai, Sichuan, Gansu were in the first quadrant. Jiangsu, Yunnan, Hubei populations was located in the second and fourth quadrants, connecting the first and third quadrants samples, and Inner Mongolia population was mainly located in the fourth quadrant and part of samples were connected with the samples in Qinghai, Sichuan and Gansu provinces, far away from the samples in Beijing, Hebei, and other cities in the third quadrant. It indicated that the populations of Beijing, Hebei, Shandong, Anhui, Shanxi, Shaanxi, Henan, Heilongjiang eight central and eastern provinces had close genetic exchanges and Qinghai, Sichuan, Gansu had close genetic exchanges, which might be two independent epidemic areas. Jiangsu, Yunnan, Hubei populations might serve as a bridge between two independent epidemic areas. Except for Qinghai, Gansu and Jiangsu, the genetic differences of Inner Mongolia population were significant from that in other provinces.

To make sure a more clearly signal about an optimal number of clusters, population analysis by STRUCTURE was also run to determine the cluster of SSR genotypes in 15 provinces. At K =2, samples of Beijing, Hebei, Shanxi, Shaanxi, Anhui, Henan, Heilongjiang, Yunnan split from Jiangsu, Hubei, Gansu, Qinghai, Sichuan, Inner Mongolia; At K =3, fifth populations divided into three clusters: The first cluster was samples of Beijing, Hebei, Hubei, Shandong, Henan, Heilongjiang; The second cluster was samples of Shanxi, Yunnan, Shaanxi, Anhui; The third cluster was samples of Jiangsu, Gansu, Qinghai, Sichuan, Inner Mongolia; At K =4, sample of Hubei showed differentiation from others, Which were divided into first subpopulation Beijing, Hebei, Shandong, Heilongjiang, Sichuan ; the second subpopulation Shanxi, Shaanxi, Anhui, Henan, Yunnan and the third subpopulation Jiangsu, Gansu, Qinghai, Inner Mongolia (Figure 3). Structure results indicated that the population of 15 provinces showed the structural differences of the eastern and western pathogenic rust fungus.

The Nm value between any two populations is greater than 1, indicating that there is gene exchange between the populations. Among them, populations of Beijing, Hebei, Henan, Anhui, Shanxi, Shaanxi, Shandong, Heilongjiang had closer genetic exchanges, while populations of Jiangsu, Gansu, Qinghai, Sichuan had closer genetic exchanges. Except Jiangsu, Gansu, Qinghai populations, there was little genetic exchange between the Inner Mongolia population and other provinces (Table4).

A genotype is the combination of alleles carried by a given individual at a particular set of loci. Individuals carrying the same set of alleles are considered to have the same multi-locus genotype. The multi-locus genotype indicated except for Qinghai, Sichuan and Inner Mongolia provinces, all the other populations had shared genotypes. Thirty-one genotypes were found in the Yunnan population, sharing genotypes with Shanxi, Shandong, Anhui, Beijing and Shaanxi. There were thirty-six genotypes in Hubei population, among which there were shared genotypes between Shaanxi, Anhui, Shandong, Henan and Hebei five provinces while shared two genotypes in Gansu population. Fifty-one genotypes were found in Jiangsu population which shared genotypes with Shanxi, Shaanxi, Anhui, Shandong, Henan provinces, and a shared genotype with the Gansu population. In addition, gene exchange also existed between Yunnan, Hubei, Jiangsu provinces (Supplementary Table S3).

Discussion

Population Genetic Structure of *P. triticina* Collections

Populations monitored from 15 provinces show pathogen exchange especially in the eastern region. Wheat leaf rust is a typical air-borne disease. Uredospore of *P. triticina* can travel long distances by the wind,

resulting in regional communication (Xu et al., 2002). Whitlock and Mc Cauley believe when $Nm > 4$, the gene exchange between populations is relatively sufficient, which can play the role of homogenization and eliminate the genetic difference of populations (Whitlock and McCauley).Strong gene flow of *P. triticina* was found among eight populations including Beijing, Hebei, Henan, Anhui, Shanxi, Shaanxi, Shandong, Heilongjiang and four populations including Qinghai, Gansu, Sichuan, Inner Mongolia, indicating both eight populations and four populations had a closer pathogen relationship. The result of cluster analysis also proved that there was little difference between Beijing, Hebei and other eight populations and Qinghai, Gansu and other four populations. Because most of the eight samples are adjacent to each other and there is almost no mountain barrier, which provides geographical condition for pathogen communication (Kolmer, 2015). In addition, most of eight populations including Shandong, Anhui, Henan, Hebei, Shaanxi, Shanxi belong to the Huang-huai winter wheat area. Similar natural selection conditions make these populations closely related to each other.

Qinghai, Gansu, Sichuan and Inner Mongolia populations are located in the western region of China, which is geographically far away from the middle and eastern groups and different from these groups. Qinghai and Gansu populations are relatively high in elevation and Sichuan, Inner Mongolia population is located in a basin. For complex and diverse terrain, the pathogen exchange among the four western populations are not as close as that among the eight eastern populations. Furthermore, the population of Inner Mongolia exhibit obvious difference between eight provinces population in terms of *Nm* value and DAPC analysis. From the geographical position, the Inner Mongolia group mainly comes from Bayannur city, which is located in the west of Inner Mongolia and belongs to Hetao plain irrigation area (Li et al., 2000). Spring wheat is mainly planted in this area, with an average temperature of 18-20 from June to July and abundant rainfall. Both humidity and temperature are favorable to the epidemic of leaf rust (Wu,2014). It is adjacent to Qinghai and Gansu provinces, but far away from other provinces. According to the *Nm*value, there were close pathogen exchange between Jiangsu and Gansu, Shanxi; Hubei and Gansu, Anhui; Yunnan and Gansu, Shaanxi populations. In other words, there were shared genotypes between Jiangsu, Hubei populations and eight provinces in the middle and east, and Gansu populations. The eight populations in the middle and east and the four populations in the west probably spread through the three populations of Jiangsu, Hubei and Yunnan. From the geographical position, the samples of Hubei lie between the eight provinces in the mid-east region and the four provinces in the west; Jiangsu samples are close to the eight provinces in the mid-east region, and Yunnan samples lies in the mountains in the southwest. The fungus in Yunnan mainly came from falling seedlings in mountainous areas, which went down mountain in autumn and went up mountain in spring, invaded back and forth (Ran, 1993).

Prediction of Pathogen Transmission Between Different Regions in China

The occurrence of wheat leaf rust is closely related to temperature and humidity, which affects the time of disease onset. Due to the vast territory and changeable climate in China, the onset time of wheat leaf rust is different. The samples of *P. triticina* from 15 provinces were distributed in the northeast spring wheat region, the north spring wheat region, the northwest spring wheat region, the north winter wheat region, the Huang-huai winter wheat region, the middle and lower Yangtze river winter wheat region and the southwest winter wheat region. The Lincang city of Yunnan belongs to the southwest winter wheat region, which has mild climate and suitable hydrothermal conditions so the onset time of *P. triticina* is early. The pathogen in Yunnan mainly came from falling seedlings in mountainous areas, which went down mountain in autumn and went up mountain in spring, invaded back and forth (Ran, 1993). The uredospores may be carried by wind to the northwest spring wheat region and the eastern wheat growing region then spread to the northern spring wheat region in Hetao plain by the northwest spring wheat region. The winter wheat region in the middle and lower reaches of the Yangtze river has a warm and humid climate, with the precipitation of 360mm to 830mm during the wheat growth period. The latitude of the wheat is higher than that in Beijing, Shandong and other regions and the incidence of *P. triticina* is earlier. The uredospores in the winter wheat region in the middle and lower reaches of the Yangtze river may be introduced into the winter wheat region in Huang-huai and the northern wheat region. Meanwhile, the samples of Zhenjiang city in Jiangsu province, located in the winter wheat region of the middle and lower reaches of the Yangtze

river also communicated with the population in the west. Therefore, taking Zhenjiang samples in Jiangsu province as the representative, the samples of Hubei and Xinyang which located in the middle and lower reaches of the Yangtze river winter wheat region spread to the west wheat leaf rust population represented by Gansu population.

In conclusion, the *P. triticina* in 15 provinces communicated with each other but based on the genetic differences and pathogen communication, the *P. triticina* populations from the 15 provinces were obviously divided into eastern and western regions In this study, the samples were collected in multiple areas, involving 15 provinces in China. It reflects that the classification of *P. triticina*populations in China is affected by topography and geographical location to some extent. Due to its highly thermal adaptability, *P. triticina* may have extensive communication in most areas of China, but its infection cycle is formed in small areas. Therefore, the *P. triticina* population in China can be preliminarily divided into eastern, western and "bridge" regions, and on this basis, the genetic structure, pathogen transmission route and disease prevalence can be studied in a small area.

ACKNOWLEDGMENT

This study was supported by the National Natural Science Foundation of China (Project 31671967), National Key Research and Development Program (2018YFD0200500 & 2016YFD0300700), China Agriculture Research System (CARS-3), Agricultural Science and Technology Innovation Program (CAAS-ASTIP) and Epidemic Detection and Control of Crop Diseases and Insect Pests (2130108).

Data Availability Statement:

All the data including information of locus, genotype diversity information, the value of *Nm* , input the Dryad data repository. And all data are obtained through experiments.

Reference

Ashley, M.V., and Dow, B.D. (1994). The use of microsatellite analysis in population biology: background, methods and potential applications.*EXS* 69**,** 185-201. doi: 10.1007/978-3-0348-7527-1_10.

Bolton, M.D., Kolmer, J.A., and Garvin, D.F. (2008). Wheat leaf rust caused by *Puccinia triticina* . *Molecular Plant Pathology*9**,** 563-575. doi:10.1111/j.1364-3703.2008.00487.x.

Chen, W.Q. (2011). The occurrence and control of wheat rust. *China Agriculture Press* .

Duan, X., Enjalbert, J., Vautrin, D., Solignac, M., and Giraud, T. (2003). Isolation of 12 microsatellite loci, using an enrichment protocol, in the phytopathogenic fungus *Puccinia triticina* .*Molecular Ecology Notes* 3**,** 65-67. doi: 10.1046/j.1471-8286.2003.00350.x.

Excoffier, L., Smouse,P.E. ,Quattro,J.M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes : application to human mitochondrial DNA restriction data. *Genetics*131**,** 479-491. doi: 10.1016/1050-3862 (92)90005-P.

Francois, B., Sebastien, D., and Thibaut, J. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* 11**,** 94. doi: 10.1186/1471-2156-11-94.

Grunwald, N.J., Goodwin, S.B., Milgroom, M.G., and Fry, W.E. (2003). Analysis of genotypic diversity data for populations of microorganisms.*Phytopathology* 93**,** 738-746. doi: 10.1094/PHYTO.2003.93.6.738.

Huerta-Espino, J., German, S., Singh, R.P., McCallum, B.D., Park, R.F., Chen, W.Q., Bhardwaj, S.C., and Goyeau, H. (2011). Global status of wheat leaf rust caused by *Puccinia triticina* . *Euphytica*179**,** 143-160. doi: 10.1007/s10681-011-0361-x.

Husband, B.C., and Barrett, S.C.H. (1995). Estimates of gene flow in*Eichhornia paniculata* (*Pontederiaceae*): effects of range substructure. *Heredity* 75**,** 549-560. doi: 10.1038/hdy.1995.174.

Jin, S.B. (1996). Chinese wheat science. *China Agriculture Press.*

John A. Ludwig, A.J.F.R., John Wiley and Sons (1989). Statistical ecology: a primer on methods and computing. *Coenoses* 4**,**38. doi: 10.2307/43461256.

Kamvar, Z.N., Tabima, J.F., and Grunwald, N.J. (2014). Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2**,** 281.doi: 10.7717/peerj.281.

Kang, J., Zhang, L., Zhang, M.Y., Yan, H.F., and Liu, D.Q. (2016). Development of specific molecular markers in*Puccinia triticina* . *Journal of Agricultural University of Hebei* 39**,** 63-67.

Kolmer, J.A. (2015). Collections of *puccinia triticina* in different provinces of China are highly related for virulence and molecular genotype. *Phytopathology* 105**,** 700-706.doi: 10.1094/PHYTO-11-14-0293-R.

Kolmer, J.A., Hanzalova, A., Goyeau, H., Bayles, R., and Morgounov, A. (2013). Genetic differentiation of the wheat leaf rust fungus*Puccinia triticina* in Europe. *Plant Pathology* 62**,**21-31. doi: 10.1111/j.1365-3059.2012.02626.x.

Kolmer, J.A., Ordonez, M.E., Manisterski, J., and Anikster, Y. (2007). Genetic differentiation of *Puccinia triticina* populations in central Asia and the Caucasus. Phytopathology 97, 1141-1149. doi: 10.1094/PHYTO-97-9-1141.

Kolmer, J.A., Ordonez, M.E., Manisterski, J, and Anikster, Y. (2011). Genetic differentiation of *Puccinia triticina* populations in the Middle East and genetic similarity with populations in Central Asia.*Phytopathology* 101**,** 870-877. doi: 10.1094/phyto-10-10-0268.

Li, X. (2008). Introduction to a population genetic software-GENALEX6.*Chinese Wild Plant Resources* 27**,** 62-65. doi: CNKI:SUN:ZYSZ.0.2008-04-018.

Li, Y.Q., Wu, X.H., Cui, G.H., Wang, X.B., and Yu, M.L. (2000). Present situation and problems of wheat production in Inner Mongolia.*Inner Mongolia Agricultural Science And Technology* **,**139-140. doi: 1007-0907 (2000)-0139-02.

Lu, Y.J., Li, X., and Jiang, N. (2017). Analysis on the development and trend of the world wheat industry in 2017. *China Market* **,**63-65. doi: 10.13939/j.cnki.zgsc.2017.26.063

Ordonez, M.E., German, S.E., and Kolmer, J.A. (2010). Genetic differentiation within the *Puccinia triticina* population in South America and comparison with the North American population suggests common ancestry and intercontinental migration. *Phytopathology*100**,** 376-383. doi: 10.1094/PHYTO-100-4-0376.

Ordonez, M.E., and Kolmer, J.A. (2007). Simple sequence repeat diversity of a worldwide collection of *puccinia triticina* from durum wheat.*Phytopathology* 97**,** 574-583. doi: 10.1094/PHYTO-97-5-0574.

Peng, S.Y. (2013). Comprehensive control technology of wheat leaf rust.*Modern Agriculture* **,** 30-31. doi: CNKI:SUN:XDNY.0.2013-02-026.

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*155, 945-959.

Ran, Y. (1993). Review of wheat rust control in yunnan province.*Yunnan Agricultural Science and Technology* **,** 5-9. doi: CNKI:SUN:YNKJ.0.1993-06-000.

Rosenberg, N. A. (2004). Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes* 4, 137-138.doi: 10.1046/j.1471-8286.2003.00566.x

Shannon, C.E. (1948). A mathematical theory of communication. *Bell System Technical Journal* 27. doi: 10.1002/j.1538-7305.1948.tb00917.x.

Simpson, E.H. (1972).Measurement of diversity. *Cardiothoracic & Vascular Anesthesia* 27.doi: 10.1136/thx.27.2.261

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science* 236**,** 787-792. doi: 10.1126/science.3576198.doi: 10.1126/science.3576198.

Stoddart, J.A., and Taylor, J.F. (1988). Genotypic diversity: Estimation and prediction in samples. *Genetics* 118**,** 705-711.

Szabo, L.J., and Kolmer, J.A. (2006). Development of simple sequence repeat markers for the plant pathogenic rust fungus *Puccinia triticina* . *Molecular Ecology Notes* 7**,** 92-94. doi: 10.1111/j.1471-8286.2006.01540.x.

Whitlock, M.C., and Mccauley, D.E. (1999). Indirect measures of gene flow and migration: *Fst* [?]1/ (4*Nm* +1). *Heredity*82**,** 117-125. doi: 10.1038/sj.hdy.6884960.

Wu, X.L. (2014). Analysis on climatic conditions of wheat growth in Inner Mongolia. *Beijing Agriculture* **,** 39-40. doi: CNKI:SUN:BJNY.0.2014-33-029.

Xu, D., Huang, G., Yang, W., and Liu, D. (2002). Analysis of the virulence and molecular polymorphism of Puccinia recondita f.sp tritici.*Journal of Agricultural Biotechnology* 10**,** 41-45.

Xu, M.Q. (2012). *Genetic Diversity of Puccinia triticina by SSR in Some Regions of China* Hebei Agricultural University.

Xu, M.Q., Wang, Q., Meng, Q.F., and Liu, D.Q. (2013). Genetic diversity of *Puccinia triticina* by SSR in some regions of China.*Journal of Agricultural* 21**,** 89-96. doi: 10.3969/j.issn.1674-7968.2013.01.011.

Zhang, M.Y., Meng, Q.F., Zhang, L., Gao, Y., Yan, H.F., and Liu, D.Q. (2018). Analysis of genetic diversity of*Puccinia triticina* isolated from different wheat cultivars.*Journal of Henan Agricultural Sciences* 47**,** 77-81. doi: CNKI:SUN:HNNY.0.2018-09-014.

TABLE 1 Repeat motif, primer sequence, size, amplification conditions (Tm: annealing temperature), number and size in base pair (bp) of alleles, of the 13 microsatellite locus isolated from *Puccinia triticina*

Locus	**Locus**	**Repeat Motif**	**Primer sequences (5'-3')**	**Tm()**	**Florescence**	**Product sizes(bp)**
RB1	$(GT)_5$	$(GT)_5$	TTGTCGTTCTCGAATGATGC TGCCCACAACCCTCCTC	56	FAM	126-134
RB25	$(AT)_4$+$(GT)_7$	$(AT)_4$+$(GT)_7$	ATGTCTGTAGTGGGCAGGGC GCCTCTGCGGGATCGGT	58	ROX	226-228
RB35	$(AC)_9$+$(TA)_5$+$(AC)_5$	$(AC)_9$+$(TA)_5$+$(AC)_5$	AGCTGCGATATCCAGTACACACACATC TGATGGGCTCGCAGTGG	62	FAM	244-248
RB29	$(CA)_{15}$	$(CA)_{15}$	CTCACCAAACACCAAGCACC GAGCCTAGCATCAGCATCC	60	HEX	118-180
RB4	$(GT)_8$	$(GT)_8$	CAGTATTGTGCCGGTTGGATG ACTCAAGAATAATGGGGAACAC	62	ROX	230-244
RB17	$(TGC)_5$+$(TGG)_6$	$(TGC)_5$+$(TGG)_6$	CTTCGGTAGGAGTTCGAGCG CAGCTCCAAATCCTTTGCC	60	FAM	89-92
RB8	$(TGG)_7$	$(TGG)_7$	CGCCGTTCCCACACGTTC TAAAACACTCCACCCACGCC	64	HEX	138-147
RB11	$(CA)_{17}$	$(CA)_{17}$	AGCAGTGAGCAGCAGCGTC ACTACTGTGAGTGTCGGCTTGG	58	ROX	178-208
RB26	$(CT)_{8+6}$	$(CT)_{8+6}$	TCGTCCTGCCTCCTCTGAC AAAGTGCATGATCTGCATGTG	58	FAM	340-346
RB27	$(CA)_{4+3}$	$(CA)_{4+3}$	CTATCGAGTCCGGAACCGAAC CAAGCCAAGACCTGAGCTATC	60	HEX	170

Locus	Locus	Repeat Motif	Primer sequences (5'-3')	Tm()	Florescence	Product sizes(bp)
RB12	$(AG)_{5+3}$	$(AG)_{5+3}$	CCACAAGCAAC TGGTCCATGAAGAAGTCTCTGAAC	60 60	ACATACCACC ROX	288-298
RB10	$(GT)_{7+4+4}$	$(GT)_{7+4+4}$	TAAGATTGGTG TTGTCTTTCATCTCATCCAGCC	58 58	TATGTGGTGG FAM	218
RB28	$(TGG)_5$	$(TGG)_5$	CATCTGGCTGG GAAGCCCGCCGAGCAGC	58 58	TGAGGTCGC HEX	315-318

Locus: Reference Duan et al., 2003

TABLE 2 Allele, 1-D, Hexp and Evenness of the 13 microsatellite locus isolated from *Puccinia triticina*

Locus	Allele	1-D	Hexp	Evenness
RB1	3.000	0.495	0.495	0.959
RB25	3.000	0.076	0.076	0.435
RB35	3.000	0.530	0.530	0.829
RB29	13.000	0.729	0.729	0.708
RB4	6.000	0.560	0.560	0.711
RB17	4.000	0.215	0.215	0.591
RB8	5.000	0.508	0.509	0.856
RB11	6.000	0.651	0.652	0.895
RB26	2.000	0.113	0.113	0.499
RB27	6.000	0.154	0.154	0.448
RB12	2.000	0.498	0.499	0.997
RB10	12.000	0.600	0.601	0.714
RB28	3.000	0.231	0.231	0.505
mean	5.231	0.412	0.413	0.704

Allele: Number of the observed alleles; 1-D: Simpson index; Hexp: Nei's unbiased gene diversity;

Evenness:Evenness of the observed alleles

TABLE 3 Genotype diversity information of different*P. triticina* populations

Province	N	MLG	eMLG	H	G	lambda	Corrected lambda	E.5
Beijing	18	11	7.65	2.29	9.00	0.889	0.909	0.898
Hebei	35	30	9.59	3.38	27.00	0.963	0.964	0.920
Shanxi	20	14	8.32	2.53	11.11	0.910	0.909	0.878
Shaanxi	48	46	9.92	3.81	44.31	0.977	0.974	0.977
Anhui	75	48	9.12	3.64	28.27	0.965	0.974	0.734
Shandong	61	37	8.72	3.33	20.33	0.951	0.955	0.718
Henan	63	50	9.53	3.80	37.09	0.973	0.976	0.830
Heilongjiang	28	27	9.88	3.28	26.13	0.962	0.955	0.980
Jiangsu	59	51	9.77	3.88	45.21	0.978	0.980	0.932
Hubei	42	38	9.67	3.57	31.50	0.968	0.971	0.881
Yunnan	54	31	7.64	2.92	9.11	0.890	0.966	0.464

Province	N	MLG	eMLG	H	G	lambda	Corrected lambda	E.5
Gansu	45	42	9.86	3.71	39.71	0.975	0.971	0.967
Qinghai	29	21	8.83	2.92	15.87	0.937	0.952	0.848
Sichuan	8	8	8.00	2.08	8.00	0.875	0.875	1.000
Inner Mongolia	37	37	10.00	3.61	37.00	0.973	0.973	1.000
Total	622	428	9.83	5.77	177.14	0.994	0.998	0.564

N: Number of individuals observed; MLG: multilocus genotype; eMLG: The number of expected MLG at the smallest sample size [?] 10 based on rarefaction; H: Shannon-Wiener Index of MLG diversity; G: Stoddart and Taylor's Index of MLG diversity; Lambda: Simpson's Index; Corrected Lambda: Corrected Simpson's Index; E.5: Evenness.

TABLE 4 The value of *Nm* between different pair of populations

	Beijing	Hebei	Shanxi	Shaanxi	Anhui	Shandong	Henan	Heilongjiang	Jiangsu	H
Beijing	****	22.804	10.592	11.137	13.288	14.439	16.472	12.661	6.081	5
Hebei		****	8.852	9.777	10.670	19.790	15.516	9.381	6.354	6
Shanxi			****	27.330	17.675	15.738	15.850	10.297	11.384	7
Shaanxi				****	29.722	17.275	22.931	10.971	9.710	1
Anhui					****	17.000	34.712	12.817	8.281	1
Shandong						****	20.344	10.567	9.615	7
Henan							****	26.657	7.339	8
Heilongjiang								****	5.882	5
Jiangsu									****	7
Hubei										*
Yunnan										
Gansu										
Qinghai										
Sichuan										
Inner Mongolia										

The value at the top is *Nm*

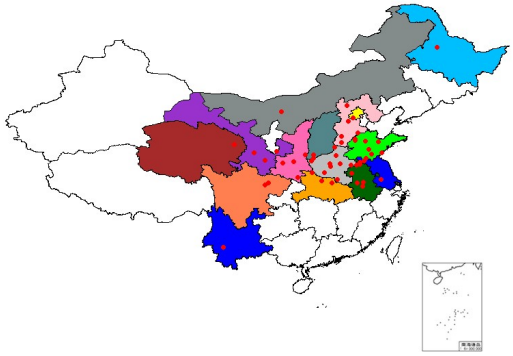


FIGURE 1 The collection site of *P. triticina* samples

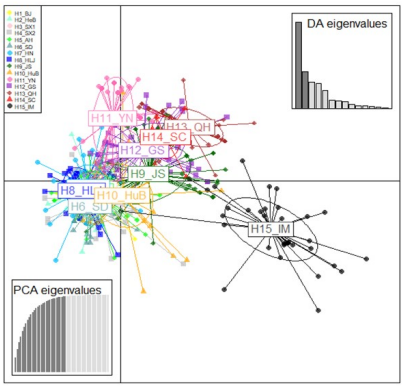


FIGURE 2 Scatterplot from discriminant analysis of principal components (DAPC) of the first two principal components discriminating*P. triticina* populations by regions. Points represent individual observations. Lines and shapes represent population membership

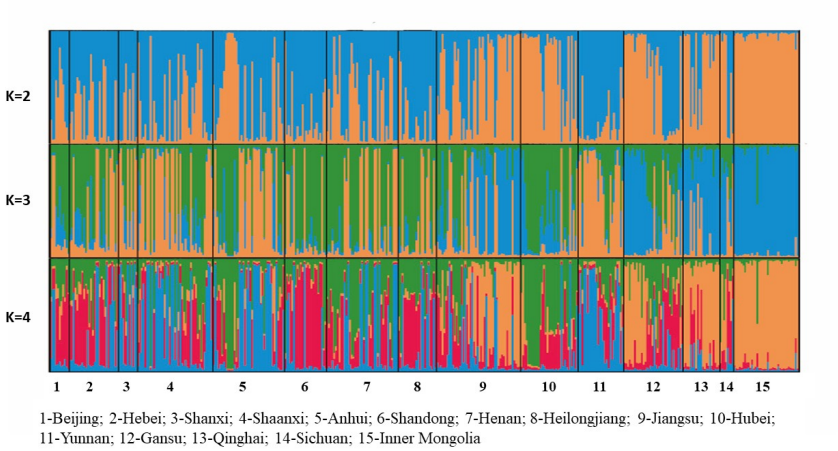
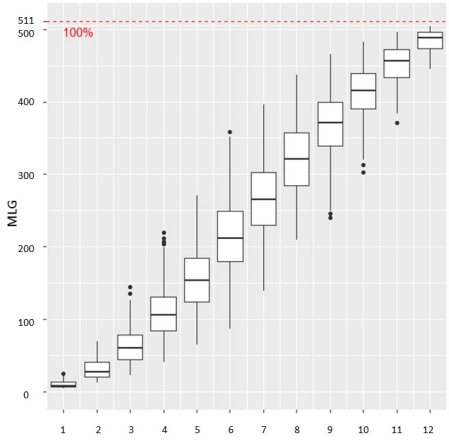


FIGURE 3 Distruct software divided 15 populations which based on the results of Structure for K =2 to K =4. They are partitioned into different colors and populations are divided by black line. Each vertical bar represents an individual, where the proportion of the color bar representing membership coefficient for each subpopulation. A bar with only a single color represents its ancestry to a single population, and a mixture of colors represents admixture from different populations



Supplementary FIGURE S1 Genotype accumulation curve is a tool that allows you to assess how much power you have to discriminate between unique individuals given a random sample of nn loci. It had reached the plateau with 12 loci indicted that the 13 loci we used is enough for distinguishing all the observed MLGs