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GENETIC DIVERSITY AND POPULATION STRUCTURE OF *POPULUS YUNNANENSIS* REVEALED BY SSR MARKERS

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Abstract

Populus yunnanensis, a native poplar in southwest China, is a potential candidate to improve the quality of poplars due to its high growth rate, wide adaptability, and strong disease-resistant ability. However, the genetic structure of *P. yunnanensis* has not been determined. To illustrate the genetic diversity and population structure of *P. yunnanensis*, 6 SSR markers, amplified from 208 specimens of 7 populations, were used in this study. In total, 35 alleles with a mean of 6 were acquired, and the genetic diversity was at a moderate level. Populations from Qujing (QJ) and Dali (DL) showed abundant genetic diversities, whereas the population from Lijiang (LJ) had low genetic diversities due to a relatively recent population bottleneck. Both *F*-statistics and AMOVA results supported that the main genetic variation was attributed to individual differences within populations, and a high frequency gene flow (*Nm* = 5.560) occurred among populations. There was an overall increasing trend of genetic differences within populations from north to south and from east to west. Based on UPGMA, PCoA, and Bayesian analyses, populations from LJ, Liangshan (LS), QJ, and Zhaotong (ZT) were closely related to each other. A high barrier was found between the Kunming (KM) population and the Chuxiong (CX) population, and there was no correlated relationship (R = -0.159, P = 0.175) for the genetic and geographic distances between these two populations. We concluded that human activity was the decisive factor for shaping the present population distribution. Many potential resource types disappear at a fast rate, and the germplasm protection and scientific plan is imperative for breeding *Populu*s and its genetic improvement.

Key words: Genetic diversity, Population structure, Genetic improvement, SSR, *Populus yunnanensis*.

Introduction

Tree species of the genus *Populus*, collectively known as poplars, are some of the world's most forested trees (Mc Carthy *et al*., 2018; John *et al*., 2014; Ellis *et al.,* 2010; Jansson *et al.,* 2007; Eckenwalder, 1996). More than one hundred species are widely distributed across the northern hemisphere, and they play important ecological and biological roles in their native habitats (Yang *et al*., 2010; Taylor, 2002; Wang & Fang, 1984). Based on rich germplasm resources, China has achieved great success in cross-breeding and ploidy breeding of *Populus* since the mid-20th century (Ma, 1994). However, the development and utilization of *Populus* resources have been focused on a few species (e.g., *P. tomentosa*), and a number of species types are almost ignored despite their potential values as genetic resources. This makes the artificial breeding of *Populus* and its genetic improvement fall into a bottleneck. Therefore, it is crucial to fill the knowledge gap and explore new gene resources from the understudied species.

Yunnan poplar (*Populus yunnanensis*) is an important native dioecious poplar in southwest China and mostly lives in pure forests in high-altitudes (> 2,000 m) or as mixed forest or scattered individual in low-altitudes (< 1,900 m) (Ren *et al*., 2018; Li *et al*., 2014; Li *et al*., 2013). *P. yunnanensis* is planted for greening cities and roads because of its high growth, strong adaptability, easy asexual propagation, early maturity, accumulation of high biomass in a short period, cold resistance, leaf rust (*Melampsora larici-populina*) resistance, and leaf spot (*Marssonina brunnea*) resistance (Ren *et al*., 2018; Jiang *et al*., 2013). These features of *P. yunnanensis* make it an important species in forestry production, afforestation and environmental conservation. In the early 18th century, *P. yunnanensis* was introduced to Australia as a representative high mountain tree and to New Zealand as a gene resource of leaf rust resistance and leaf spot resistance (Ma, 1994). These cases show that *P. yunnanensis* is qualified as a potential gene resource.

However, the females of *P. yunnanensis* are currently threatened with extinction, and the males form almost the whole forest. Males could have more capacity to adjust the developmental program to respond to changes in the environment than females, such as water deficiency and high temperature (Melnikova *et al*., 2017; Jiang *et al*., 2013; Li *et al*., 2013; Chen *et al*., 2010). The physiological analysis showed that the inhibition of growth, photosynthesis and ROS protection was stronger in females than that in males, resulting in the verge of extinction of female trees (Melnikova *et al*., 2017; Li *et al*., 2014; Peng *et al*., 2012). Moreover, artificial propagation of *P. yunnanensis* is frequent in history. Because of high rootability, local residents plant *P. yunnanensis* by branch cuttings. When these trees reach growth height, their branches are propagated without juvenile strategies. This process is repeated again and again. Negative characteristics occur in posterior cuttings because of the age effect, and a good example includes the dwarf of tree and black core of timber (He *et al*., 2004). Lacking protection of germplasm and scientific plans of breeding, the potential *P. yunnanensis* resource types are disappearing at a fast rate. Therefore, it is imperative to protect and genetically improve *P. yunnanensis*.

The genetic diversity of a species can reflect its evolutionary history (Shinwari *et al.*, 2018; Jan *et al.*, 2016; Shinwari *et al.*, 2013), adaptation ability and population size (Jan *et al.*, 2018; Jan *et al.*, 2017). Despite the asexual reproductive pattern and selective propagation by humans, the observed genetic differentiation among and within *P. yunnanensis* populations was not as low, as we thought. A study on leaf traits in the seedling duration of *P. yunnanensis* plus tree clones showed that the variations were abundant and were not relative to their geographical habitats, which indicated a rich genetic diversity of *P. yunnanensis* (He *et al*., 2009). In extensive fieldwork, we also found clear differences among *P. yunnanensis* forests.

Simple sequence repeats (SSRs) as codominant markers are sensitive to investigating the genetic diversities and population structures of the species of *Populus* (Gao *et al*., 2018; Bisen *et al*., 2015; Akfirat & Uncuoglu, 2013). SSR polymorphisms are often used for the parentage of clones (Khasa *et al*., 2003), hybrid identification (Schroeder & Fladung, 2010), and population genetics (Shah *et al.*, 2015; Du *et al*., 2012; Turi *et al.*, 2012; Lee *et al*., 2011; Rabbani *et al.*, 2010). In this study, we employed six SSR markers to examine the genetic differentiation and structure of seven populations of native *P. yunnanensis*, which almost covered its ranges. The results provide a scientific basis for protection, development and utilization of the species resources only found in southwest China.

Materials and Methods

Sampling: A total of 208 samples were collected from seven areas throughout the range of *P. yunnanensis* between 2014 and 2017 (Fig. 1). These areas were Liangshan (LS), Lijiang (LJ), Qujing (QJ), Kunming (KM), Dali (DL), Chuxiong (CX), and Zhaotong (ZT). With a minimum of 3,000 m apart, we randomly selected the samples that represented the area and were regarded as one population. The *P. yunnanensis* leaves were rapidly dried and preserved in silica gel.

DNA extraction, PCR amplification and SSR scoring: Total DNA was extracted with the modified CTAB method (Murray & Thompson, 1980; Doyle, 1989). A total of six loci from Van der Schoot *et al*., (2002) and Ghfistoph *et al*., (2005) was amplified for all samples (Table 1). PCR amplification was carried out in a 10 µL reaction containing 1 µL 10 × PCR buffer with 25 mmol/L Mg^{2+}, 0.7 µL dNTPs (2.5 mmol/L), 1.5 µL primer pair (10 µmol/L), 0.25 µL Taq DNA polymerase (2.5 U/µL), 2.5 µL template DNA, and 2.55 µL ddH_2O. The reactions were performed using the following procedure: initial denaturation with 5 min at 94°C, 30 cycles with 30 s at 94°C for denaturation, 30 s at 53 ~ 64°C (depending upon the primer set used) for annealing and 1 min at 72 °C for extension, and final extension at 72°C for 10 min. The SSR fluorescent primer synthesis and capillary electrophoresis were both completed by Sangon Biotech Co., Ltd. (Shanghai, China).

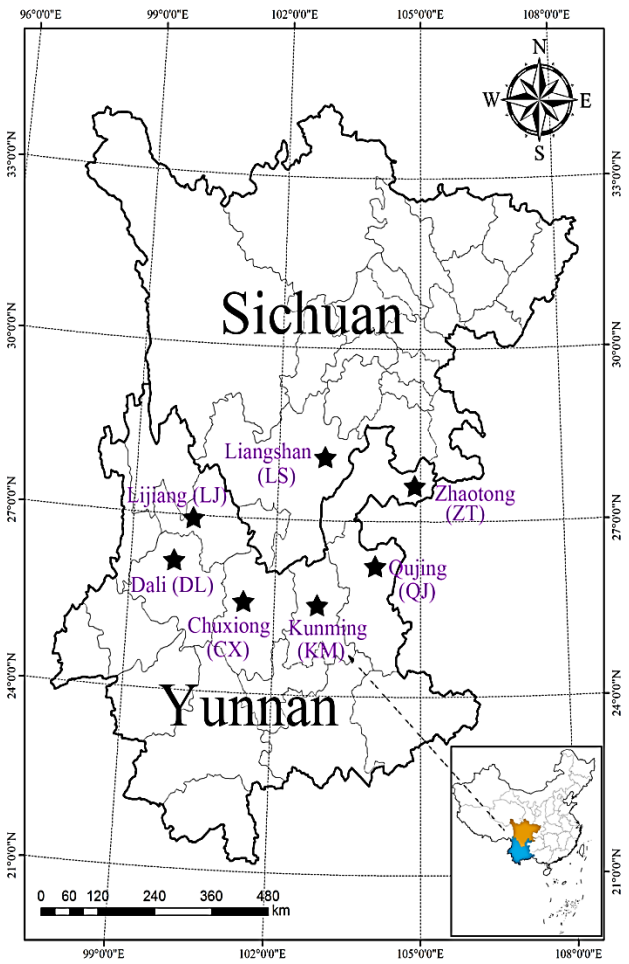

Fig. 1. Sampling localities of the populations of *Populus yunnanensis* used in this study. Populations are presented as black stars. The inset in the lower right corner shows the map of China.

Data analysis: To statistically evaluate levels of genetic diversity, the number of alleles (*Na*), effective number of alleles (*Ne*), Shannon's Information index (*I*), observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were obtained using PopGen 1.32 (Yeh *et al*., 1999). Allelic richness (*Ar*) and polymorphism information content (*PIC*) were calculated using FSTAT 2.9.3.2 (Goudet, 2001) and PICcalc (Nagy, 2012), respectively. These values were averaged for expressing the mean genetic variation.

Bottleneck was tested for each population with replications of 1000 in a two-phase-mutation model (TPM, Di Rienzo *et al*., 1994) and a stepwise mutation model (SMM, Ohta *et al*., 1973). Statistical analysis of heterozygote excess was performed using the Standardized Differences Test and Wilcoxon Test with Bottleneck 1.02 (Piry *et al*., 1999).

F-statistics (*Fit*, total inbreeding coefficient; *Fis*, actual inbreeding; *Fst*, inbreeding-like effects of population genetic structure), or the inbreeding coefficient, were used to describe the distribution of genetic variation of population using PopGen 1.32 (Yeh *et al*., 1999). Similarly, a hierarchical analysis of variance was carried out for partitioning the all genetic variance into components using the AMOVA (Analysis of Molecular Variance) module of Arlequin 3.5 (Excoffier *et al*., 2010).

Gene flow (*Nm*), typically expressed as the mean number of migrants per generation, was obtained using PopGen 1.32 (Yeh *et al*., 1999). The mean posterior distribution of migration for population pairs was used to interpret the proportion of individuals in one population recent migrated from other as their ancestral location using BayesAss 1.3 (Wilson & Rananla, 2003). It was run with parameters settings including the Markov Chain Monte Carlo (MCMC) iterations of 10^7, burn-in of 10^6 and sampling frequency of 2000.

Landscape shape analysis with a distance weight value (a) of 1 was conducted to produce a 3D surface plot showing the spatial distribution of genetic diversity using Alleles In Space 1.0 (Miller, 2005). The Mantel test was performed to estimate the coefficient of association using GenAlEx 6.41 (Peakall *et al*., 2006). Barriers, based on a connection generated by the Delaunay triangulation between geographical coordinates and Nei's genetic distance, were investigated using Barrier 2.2 (Guerard *et al*., 2004). Barriers in triangulation were identified by the Monmonier's maximum distance algorithm, and their strengths depended on the number of loci supporting them.

A 2D map generated from the principal coordinates analysis (PCoA) was drawn using GenAlEx 6.41 (Peakall *et al*., 2006). On the basis of Nei's genetic distance generated from GenAlEx 6.41, an unweighted pair-group method with arithmetic means (UPGMA) was performed to look for a better picture of genetic structure using Mega 5.02 (Tamura *et al*., 2011). Furthermore, the Bayesian clustering algorithm was used to evaluate the proportion of mixed ancestry using STRUCTURE 2.1 (Pritchard *et al*., 2000). In this case, the number of genetic clusters (*K*) was tested from 1 to 8 with 20 independent runs under the admixture model and correlated allele frequencies model through a burn-in-period of 10^4 and MCMC iterations of 10^5. The most likely *K* was obtained according to the maximum of ΔK (Evanno *et al*., 2005), and the bar graph was graphically generated using Distruct 1.1 (Rosenberg, 2004).

Results

Genetic diversity: A total of 35 alleles were obtained for all samples (Table 2). The mean number of six loci was 5.83, ranging from 2 (ORPM 203) ~ 8 (WPMS 7). The values, including *Ne* (2.4558), *I* (1.0610), *Ho* (0.9904), *He* (0.5516) and *PIC* (0.5119), were highest in the locus WPMS 5. The highest *Ar* was obtained in the WPMS 7 locus, whereas it was found to be lowest in the locus ORPM 203.

As shown in Table 3, the mean value of populations was 2.762 for *Na*, ranging from 2.167 (LJ) to 3.833 (QJ); 1.829 for *Ne*, ranging from 1.718 (LJ) to 1.967 (DL); 0.633 for *I*, ranging from 0.537 (LJ) to 0.716 (QJ); 2.668 for *Ar*, ranging from 2.127 (LJ) to 3.574 (QJ); 0.656 for *Ho*, ranging from 0.618 (CX) to 0.677 (LS); and 0.397 for *He*, ranging from 0.364 (LJ) to 0.434 (DL). Among the seven populations, QJ and DL showed abundant genetic diversity, and LJ was poor in genetic diversity.

Table 1. SSR primer information used in this study.

SSR loci	Direction	Sequence (5'-3')	Motif	Annealing (°C)
WPMS 5	F	TTCTTTTTCAACTGCCTAACTT	$(GT)_{27}$	53
	R	TGATCCAATAACAGACAGAACA		
WPMS 7	F	ACTAAGGAGAATTGTTGACTAC	$(GT)_{24}$	54
	R	TATCTGGTTTCCTCTTATGTG		
WPMS 14	F	CAGCCGCAGCCACTGAGAAATC	$(CGT)_{28-3}$	64
	R	GCCTGCTGAGAAGACTGCCTTGAC		
WPMS 18	F	CTTCACATAGGACATAGCAGCATC	$(GTG)_{13}$	60
	R	CACCAGAGTCATCACCAGTTATTG		
ORPM 30	F	ATGTCCACACCCAGATGACA	$(TC)_9$	58
	R	CCGGCTTCATTAAGAGTTGG		
ORPM 203	F	CCACCAGGCATGAGATATGA	$(TA)_4$	55
	R	TCAAACCGAAAGGTCAACAA		

Table 2. Diversity information parameters at six SSR loci of *P. yunnanensis*.

Locus	*Na*	*Ne*	*I*	*Ar*	*Ho*	*He*	*PIC*	*Fis*	*Fit*	*Fst*
WPMS 5	7.00	2.4558	1.0610	4.2020	0.9904	0.5516	0.5119	-0.7956	-0.6511	0.0805
WPMS 7	8.00	1.3414	0.6210	4.5770	0.1587	0.2387	0.2473	0.3405	0.3773	0.0557
WPMS 14	6.00	2.2623	0.9514	3.8310	0.9615	0.5372	0.4638	-0.7804	-0.7158	0.0363
WPMS 18	5.00	2.1672	0.8741	3.2280	0.8990	0.5264	0.4391	-0.7074	-0.6583	0.0288
ORPM 30	7.00	2.0821	0.8091	2.9220	0.9327	0.5104	0.4057	-0.8287	-0.7979	0.0168
ORPM 203	2.00	1.0194	0.0542	1.3890	0.0000	0.0181	0.0189	1.0000	1.0000	0.0237
Mean	5.83	1.8880	0.7285	3.3582	0.6571	0.3971	0.3478	-0.2953	-0.2410	0.0403

Table 3. Diversity information parameters of seven populations.

Pop.	*Na*	*Ne*	*I*	*Ar*	*Ho*	*He*	Standardized differences test (*P* - value)		Wilcoxon test (*P* - value)	
							TPM	SMM	TPM	SMM
LS	2.333	1.750	0.584	2.316	0.677	0.388	0.065	0.171	0.078	0.344
LJ	2.167	1.718	0.537	2.127	0.656	0.364	0.029	0.122	0.047	0.313
QJ	3.833	1.875	0.716	3.574	0.667	0.422	0.127	0.002	0.922	1.000
KM	2.667	1.898	0.677	2.599	0.667	0.420	0.074	0.289	0.078	0.313
DL	2.833	1.967	0.712	2.754	0.667	0.434	0.116	0.420	0.313	0.406
CX	2.667	1.828	0.616	2.667	0.618	0.381	0.247	0.464	0.313	0.500
ZT	2.833	1.765	0.586	2.636	0.641	0.370	0.352	0.235	0.313	0.594
Mean	2.762	1.829	0.633	2.668	0.656	0.397	-	-	-	-

Bottleneck effect test: The results from the Wilcoxon test (Table 3) showed that population LJ provided a significant deviation from the mutation-drift equilibrium under the TPM (P = 0.047), but this was not supported under the SMM (P = 0.313). Similarly, standardized differences test (Table 3) exhibited that the deviation was significant for population LJ under TPM (P = 0.029) and for population QJ under SMM (P = 0.002). Furthermore, the graphical method based on distributions of allele frequency (Fig. 2) showed the abundant proportion of alleles of population LS and LJ at intermediate frequency classes of 0.6, which indicated that the two populations have undergone recent genetic bottlenecks. Therefore, it could be concluded that population LJ was likely to be a genetic bottleneck.

The divergence and gene flow: The mean values of *Fit* and *Fis* of the *P. yunnanensis* species were -0.580 and -0.651 respectively, indicating heterozygote excess. The mean *Fst* value was 0.043, ranging across the loci from 0.0168 (ORPM 30) to 0.0805 (WPMS 5). Only 19% of the total molecular variances were attributed to the divergence among populations by AMOVA (Table 4). Both of these results suggested that the most variation of the species was in the individuals of the populations.

Nm was estimated to be 5.560 by *Fst* analyses, indicating a high frequency migrant occurred among seven populations of *P. yunnanensis*. Furthermore, the total proportions of migrants (Fig. 3) were estimated to range from 4.07 (ZT) to 32.31 (LS). Within population LS, 32.31% of individuals were immigrated from other populations, in which QJ contributed the highest immigrant proportions (24.27%). Within populations LJ and DL, the immigrant proportions were 32.28% and 32.29%, respectively, mostly from population ZT (29.90% and 29.91%). Within populations QJ and CX, the individuals from LS were the main immigrants, and their proportions were 23.32% and 20.58%, respectively. The highest immigrant in population KM was associated with individuals of QJ (14.91%), followed by ZT (10.23%). There was the lowest immigrant proportion at 4.07 in population ZT.

Genetic and Geographical relatedness: Resulting from the 3D surface plot (Fig. 4), differentiation decreased from high genetic surfaces in southwestern plot edges to low genetic surfaces in northeastern areas. The genetic distances within populations DL, CX and KM were all relatively high (peak as shown in Fig. 4). There were notable barriers in population KM and CX (Fig. 4). There was no correlation between genetic and geographic distances identified by the Mantel test (Fig. 5), where the mantel relevance (R) was negative and estimated at -0.159 and was significant (P = 0.175). This suggests that geographic distances did little work as barriers to gene flow.

Clustering: As presented from the PCoA biplot (Fig. 6), the first two eigenvectors (coord. 1 = 50.95% and coord. 2 = 29.25%) explained most of the genetic variation (80.20%). Populations QJ, LJ and LS were designated into a group, and population ZT was related to them. The other three populations (KM, CX and DL) were separated in the first or second coordinates. The UPGMA tree (Fig. 7) supported the genetic relationships among these populations. At the threshold of 0.01 based on genetic distance, populations LS, LJ, QJ, ZT were similar as a group, and the population KM added this group at a threshold of 0.02, followed by the population DL at 0.03.

The values of *L(K)*, representing the log likelihood of the multilocus genotypes, stagnated at K=5, suggesting five clusters. Using the ΔK method, the location of maximum of ΔK further confirmed that five clusters were the best for *P. yunnanensis*, and individuals in each population were therefore assigned into five clusters, as shown in Fig. 8. Except for the genetic cluster 5 (yellow), every population was associated with a mixture of the four clusters, indicating a power genetic similarity among populations. Cluster 1 (red) contains a few individuals who mainly existed in populations LS and QJ. *P. yunnanensis* individuals were almost captured by clusters 2 (green), 3 (blue) and 4 (pink), in which clusters 2 and 3 were predominant in populations LS, LJ, QJ and ZT, and cluster 4 was abundant in populations CX and KM. Cluster 5 was only found in population DL, and a few individuals were assigned to populations QJ, LJ and ZT.

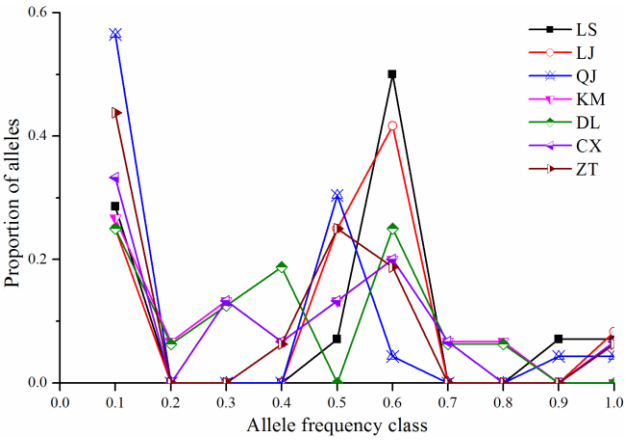

Fig. 2. Allele frequency distributions of SSRs. Identifying from the mode-shift distortion, a high proportion of alleles are found to be at a low frequency in nonbottlenecked populations, whereas it is at an intermediate frequency in bottlenecked populations.

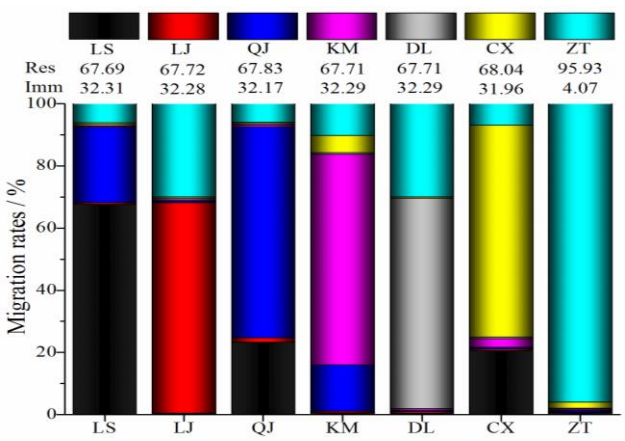

Fig. 3. Migration rates among seven populations. Each stacked bar is expected proportions of individuals in the population that have migrated from populations represented by color: Black = LS; Red = LJ; blue = QJ; Magenta = KM; Light Gray = DL; Yellow = CX; Cyan = ZT. Values in "Res" are proportions of individuals from the population of residents, and the values in "Imm" were the total proportions of individuals immigrated from others.

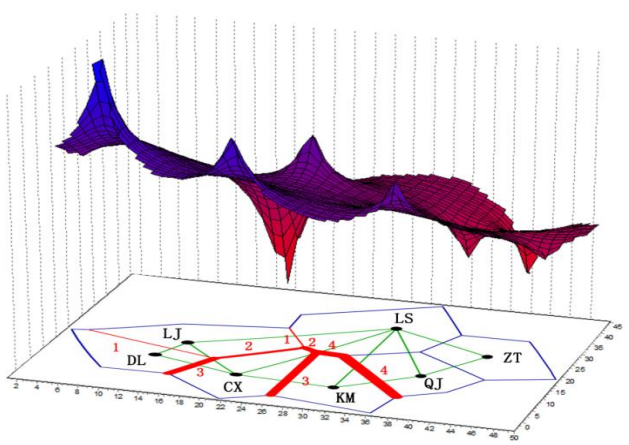

Fig. 4. Results of the genetic landscape shape interpolation analysis and the barrier analysis. In the 3D surface plot, heights show genetic distances, where peaks represent high differentiation and valleys represent low differentiation, and smooth genetic surfaces show areas with low barriers to gene flow. In the barrier plot, the thickness of the line shows the strength of the barriers determined by the number of loci as supported.

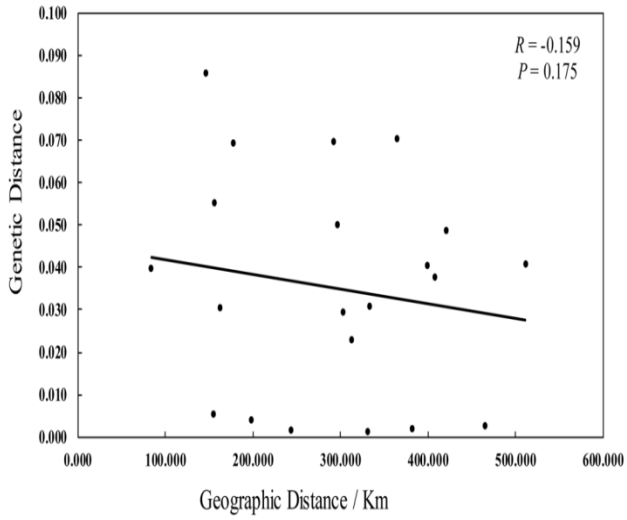

Fig. 5. Relevance between genetic distance and geographic distance.

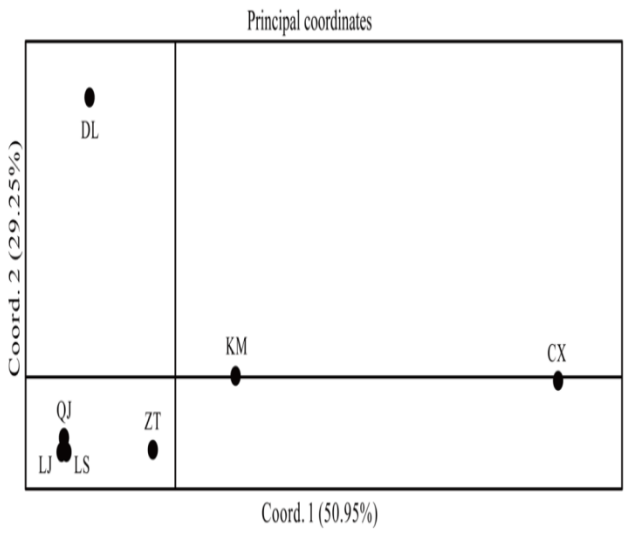

Fig. 6. Principal coordinates analysis of seven populations. The first two eigenvectors explained 80.20% of all variance.

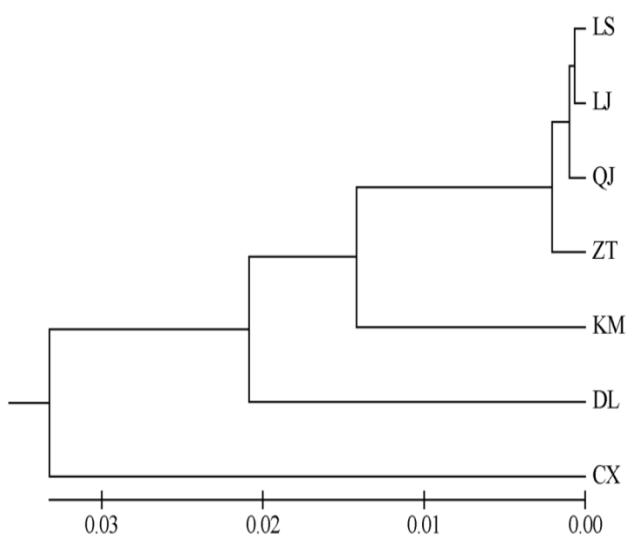

Fig. 7. Dendrogram generated by UPGMA of seven populations in *P. yunnanensis*.

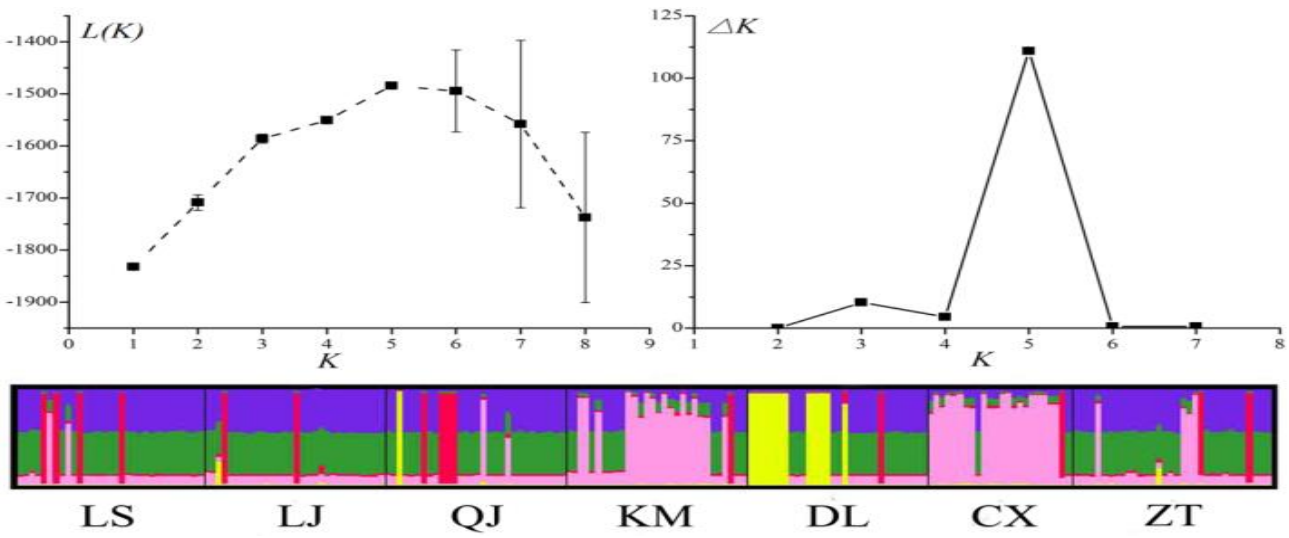

Fig. 8. Bar graph of seven populations. Five clusters are determined for Bayesian analysis according to the identification of *K*. Every thin vertical line is an individual that is classified with colored partitioning: red represents cluster 1; green represents cluster 2; blue represents cluster 3; pink represents cluster 4; and yellow represents cluster 5, and the black vertical lines are delimitation of populations whose names are given below the graph.

Table 4. Analysis of molecular variance for 208 individuals of *P. yunnanensis* from seven populations.

Source	df[a]	SS[b]	MS[c]	Est. var.[d]	% Var.[e]
Among populations	6	41.635	6.939	0.205	19%
Within population	201	171.134	0.851	0.851	81%
Total	207	212.769			

[a] "df" refers to the degrees of freedom; [b] "SS" refers to the sum of squares; [c] "MS" refers to the mean square; [d] "Est. var." refers to the estimated variance; [e] "%Var." refers to the percentage of variance

Discussion

At the species level, all parameters explaining the genetic diversity based on the codominant marker (SSR) were noticeably higher than those based on the dominant markers (AFLP, ISSR, RAPD), as shown in Table 5, which is associated with differences in characters among marker types. To obtain a more accurate result, we compared these values only from the codominant data set (Table 5). The genetic diversity of *P. yunnanensis* (*Ne* = 1.8880, *I* = 0.7285, *Ho* = 0.6571, *He* = 0.3971, section *Tacamahaca*) from this study was higher than the genetic diversity of *P. szechuanica* var. *tibetica* (*Ne* = 1.6796, *I* = 0.5038, *He* = 0.3464, section *Tacamahaca*) according to Bo (2012), *P. maximowiczii* (*Ho* = 0.235, section *Tacamahaca*) according to Rahman and Rajora (2002), *P. balsamifera* (*Ho* = 0.351, section *Tacamahaca*) according to Rahman and Rajora (2002) and *P. trichocarpa* (*Ho* = 0.181, section *Tacamahaca*) according to Rahman and Rajora (2002) but was lower than that of *P. ussuriensis* (*Ne* = 2.5421, *I* = 1.0424, section *Tacamahaca*) according to Lin (2003), *P. deltoides* (*Ne* = 5.37, *He* = 0.75, section *Aigeiros*) according to Li *et al*., (2006), *P. davidiana* (*Ne* = 2.6951, *I* = 1.1001, *H*= 0.5957, *He* = 0.5971, section *Leuce*) according to Bai (2010), and *P. pruinosa* (*Ne* = 3.074, *I* = 185, *H* = 0.541, *He* = 0.560, section *Turaga*) according to Zhang *et al*., (2012). These results suggested that the genetic diversity of *P. yunnanensis* was the middle level.

When we compared the results among *P. yunnanensis* populations, the difference of variations within the population were notable. There was higher genetic diversity in populations QJ (*Na* = 3.833; *Ne* = 1.875; *I* = 0.716; *Ar* = 3.574; *Ho* = 0.667; *He* = 0.422), DL (*Na* = 2.833; *Ne* = 1.967; *I* = 0.712; *Ar* = 2.754; *Ho* = 0.667; *He* = 0.434) and KM (*Na* = 2.667; *Ne* = 1.898; *I* = 0.677; *Ar* = 2.599; *Ho* = 0.667; *He* = 0.420). Similar results have been shown by Yan *et al*., (2016) using SRAP markers. This finding suggested an abundant genetic basis for the development of *P. yunnanensis* in populations QJ, DL and KM. In contrast, the genetic diversity was low in populations LJ (*Na* = 2.167; *Ne* = 1.718; *I* = 0.537; *Ar* = 2.127; *Ho* = 0.656; *He* = 0.364), LS (*Na* = 2.333; *Ne* = 1.750; *I* = 0.584; *He* = 0.388) and ZT (*Ne* = 1.765; *I* = 0.586; *He* = 0.370). In particular, population LJ might have undergone recent genetic bottlenecks (Table 3 and Fig. 2), leading to a sudden reduction of genotypes. Due to a lack of female plants, the frequent selective propagation may be the main cause of the direct bottleneck effect and the low genetic difference of population LJ.

Seven populations showed frequent gene flow (5.560 for *Nm*), and the variation was mainly attributed to diversity within the population (95.7% from *Fst* and 81% from AMOVA). This finding is similar to the result revealed by AFLP (*Gst* = 0.259, Feng, 2006). In the distribution range, the genetic difference within the population showed an increasing trend from north to

south and from east to west (Fig. 4). The barriers were found in populations KM and CX (Fig. 4), and no correlation was found between genetic variation and geographic distance (Fig. 5). In other words, the geographic nature failed to decide the population structure. For instance, there is a large geographic distance between population LJ and population ZT, but they showed a similar clustering position (Figs. 6, 7 and 8). Population KM exhibited a genetic barrier to its neighbors (CX and QJ), but there is no notable nature isolation (e.g., mountain and river) among them. These genetic data provide important information for the genetic structure of native *P. yunnanensis.* We deduced that its current distribution pattern was mainly decided by human activity.

Table 5. The genetic diversity of Populus based on various molecular markers.

Section	Species	Marker	*Ne*	*I*	*H*	*Ho*	*He*	Reference
Leuce	*P. tomentosa*	AFLP	1.479	0.290	0.289	-	-	He, 2005
	P. davidiana	SSR	2.6951	1.1001	0.5957	0.5421	0.5971	Bai, 2010
		SRAP	1.1955	0.2137	0.1309	-		He *et al.*, 2015
	P. tremuloides	SSR	-	-	-	-	0.797	Callahan *et al.*, 2013
	P. grandidentata	SSR	-	-	-	0.24	-	Rahman & Rajora, 2002
Aigeiros	*P. nigra*	SSR	-	-	-	0.308	-	Rahman & Rajora, 2002
		SSR	6.51	-	-	0.793	0.829	Pospiskova & Bartakova, 2004
		SSR	1.3967	0.3348	0.2187	0.3452	0.2200	Zheng, 2014
	P. deltoides	SSR	5.37	-	-	0.39	0.75	Li *et al*., 2006
		SSR	-	-	-	0.225	-	Rahman & Rajora, 2002
Turaga	*P. euphratica*	RAPD	-	-	-	-	0.124	Saito *et al.*, 2002
	P. pruinosa	SSR	3.074	1.185	0.541	0.321	0.560	Zhang *et al*., 2012
Leucoides	*P. lasiocarpa*	SRAP	1.0343	0.0301	0.0201	-	-	Yun *et al*., 2015
Tacamahaca	*P. xiangchengensis*	SRAP	1.1220	0.1208	0.0767	-	-	Yun *et al*., 2015
	P. schneideri	SRAP	1.0997	0.0972	0.0620	-	-	Yun *et al*., 2015
	P. szechuanica	SRAP	1.1412	0.1325	0.0852	-	-	Yun *et al*., 2015
	P. haoana	SRAP	1.1197	0.1178	0.0749	-	-	Yun *et al*., 2015
	P. trinervis	SRAP	1.0708	0.0704	0.0445	-	-	Yun *et al*., 2015
	P. qamdoensis	SRAP	1.2041	0.1991	0.1274	-	-	Yun *et al*., 2015
	P. kangdingensis	SRAP	1.1634	0.1618	0.1030	-	-	Yun *et al*., 2015
	P. koreana	SSCP	2.6571	1.0651	-	-	-	Lin, 2003
	P. cathayana	ISSR	1.556	0.500	0.331	-	-	Lv, 2006
	P. ussuriensis	RAPD	-	0.739	-	-	-	Su *et al.*, 1997
		SSCP	2.5421	1.0424	-	-	-	Lin, 2003
	P. simonii	SRAP	1.1160	0.1058	0.0691	-	-	Yun *et al*., 2015
		SSR	3.877	1.443	0.677	0.511	0.691	Wei, 2010
	P. szechuanica var. *tibetica*	SRAP	1.1402	0.1436	0.0897	-	-	Yun *et al*., 2015
		SSR	1.6796	0.5038	-	0.3074	0.3464	Bo, 2012
	P. trichocarpa	SSR	-	-	-	0.181	-	Rahman & Rajora, 2002
	P. laurifolia	SSR	3.8872	0.9185	0.3924	0.4249	0.3940	Zheng, 2014
	P. balsamifera	SSR	-	-	-	0.351	-	Rahman & Rajora, 2002
	P. maximowiczii	SSR	-	-	-	0.235	-	Rahman & Rajora, 2002
	P. yunnanensis	SRAP	1.1468	0.1402	0.0902	-	-	Yun *et al*., 2015
		SSR	1.8880	0.7285	-	0.6571	0.3971	This study

Conclusion

Populus yunnanensis plays an important role in forestry production and environmental protection, but fundamental research has not kept pace with its utilization. Our study has focused on revealing the genetic diversity of *P. yunnanensis*. Human activity is the decisive factor for losing genotypes and shapes the present population distribution. The collection and protection of *P. yunnanensis* germplasm resources is proposed for breeding of *Populus* and its genetic improvement.

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