

AFLP ANALYSIS OF GENETIC DIVERSITY IN *POPULUS CATHAYANA* REHD ORIGINATING FROM SOUTHEASTERN QINGHAI-TIBETAN PLATEAU OF CHINA

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Abstract

The wide geographical and climatic distribution of *Populus cathayana* Rehd indicates that there is a large amount of genetic diversity available, which can be exploited for conservation, breeding programs and afforestation schemes. In our study, genetic diversity was evaluated in the natural populations of *P. cathayana* originating from southern and eastern areas of the Qinghai-Tibetan Plateau of China by means of AFLP markers. For four primer combinations, a total of 175 bands were obtained of which 173 (98.9%) were polymorphic. Six natural populations of *P. cathayana* possessed different levels of genetic diversity, high level of genetic differentiation existed among the populations ($G_{ST}=0.489$) of *P. cathayana*. Individuals cluster and PCO analysis based on Jaccard's similarity coefficient also showed evident population genetic structure with high level of population genetic differentiation. The long evolutionary process coupled with genetic drift within populations, rather than contemporary gene flow, are the major forces shaping genetic structure of *P. cathayana* populations. Moreover, there was no correspondence between geographical and genetic distances in the populations of *P. cathayana*, seldom gene exchange among the populations and different selection pressures may be the causes. Our findings of different levels of genetic diversity within populations and high level of genetic differentiation among the populations provided promising condition for further breeding and conservation programs.

Introduction

Populus cathayana Rehd is a native species of China and it belongs to *Sect. Tacamahaca* Spach. It mainly occurs in the northern, southwestern and central parts of China and possesses a large geographic range (Wu & Raven, 1994). In the southern and eastern areas of the Qinghai-Tibetan Plateau, many *P. cathayana* populations occur in the mountains and canyon belts between the plateau and plain at altitudes varying between 1,500 – 3,900 m above sea level (Zhao & Gong 1991). Usually, the species has strong resistance against stressful environments, especially to cold and wet conditions, such as those in the southern and eastern areas of the Qinghai-Tibetan Plateau, which are generally harsh for other woody plants (Liu, 1997; Yu *et al.*, 2003; Chen, 2004). The wide geographical and climatic distribution of this species indicates that there is a large amount of genetic diversity available, which can be exploited for conservation, breeding programs and afforestation schemes. However, the species does not benefit from specific breeding and conservation programs, although their genetic resources deserve attention and can be severely affected by human activities.

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In most countries, interest in poplar research and cultivation in recent decades has concentrated primarily on a few species native to Europe or North America, eg., *P. deltoides*, *P. nigra*, *P. trichocarpa* and their hybrids. Many native poplar species of China are notable for their remarkable site adaptation even in harsh conditions and also for their fast and vigorous growth, so it is expected to be suitable for international breeding and cultivation programs, especial in resistance breeding program of poplar in the future (Weisgerber & Han 2001). In order to facilitate breeding programs and conservation priorities for *P. cathayana*, it is clearly advisable to assess genetic variation and investigate genetic structure. As a widespread species, the spatial scale of genetic diversity studies should be increased to better understand the historical, physical and environmental factors that have shaped intraspecific variation.

The amplified fragment length polymorphism (AFLP) technique is regarded as a powerful tool to evaluate genetic diversity with a high level of reproducibility in poplar and other species, and the fact that no previous knowledge of DNA sequences was required for its application, made this technique useful for the characterization of the natural populations of plants (Jones *et al.*, 1997; Arens *et al.*, 1998; Suyama *et al.* 2000; Lamote *et al.*, 2002). In our study, AFLP markers were employed to evaluate the genetic diversity of six populations of *P. cathayana* originating from their natural range in southern and eastern areas of the Qinghai-Tibetan Plateau of China. The aim of this study was to assess the levels and pattern of genetic variation and the level of genetic differentiation, to assess the effect of gene flow and genetic drift on population structure in this species, and to provide valuable information for further breeding or conservation programs.

Materials and Methods

Plant materials: A total of 143 individuals from six natural populations of *P. cathayana* were collected from the southern and eastern parts of the Qinghai-Tibetan Plateau in China (Table 1). The six sampled populations, three populations (SHY, JZ and PW) from the Sichuan province, two populations (QHY and LED) from the Qinghai province and one (TS) from the Gansu province, these populations represented the major areas of natural resources for *P. cathayana* in southwestern China (Fig. 1).

DNA extraction: DNA was extracted using 0.5 g of leaf material from each individual following the procedure as described by Castiglione *et al.*, (1993). Approximate DNA concentrations were estimated on agarose gels using a DNA standard and Ethidium bromide staining (Maniatis *et al.*, 1982). The total DNA was diluted to a concentration of about 20 ng μl^{-1} with the buffer TE (Tris-HCl pH 8.0 and 0.1 mM EDTA pH 8.0).

AFLP analysis: The AFLP reaction protocol was adapted from Vos *et al.*, (1995) with some modification. Restriction digestion of DNA samples was conducted using two restriction enzymes (EcoR I and Mse I). All amplification reactions were performed in a GeneAmp® PCR System 9700 (perkin Elmer Corp., Norwalk, CT, USA). Initially 64 primer combinations with three selective bases were tested for selective amplification. From those primer pairs, the following four primer combinations resulted in more and distinct polymorphic loci were selected for further analysis: E-AGC/M-CAA, E-AGG/M-CTG, E-AAG/M-CAT and E-AAG/M-CAG. Amplified products were electrophoresed on 6% denaturing polyacrylamide gels, and gels were silver stained as described by Bassam *et al.*, (1991).

Table 1. The ecological and geographical parameters of *P. cathayana* populations sampled.

Population	Landform feature	Water system	Longitude (E)	Latitude (N)	Altitude (m)	Annual rainfall (mm)	Annual mean temperature (°C)	Sample size
SHY	Riparian and basin	Dadu river	102°40'	29°25'	1500	750	17.7	29
JZ	Alpine and canyon	Bailong river	103°57'	32°13'	1600	553	12.7	22
PW	Canyon and basin	Peijing river	104°25'	32°01'	1620	566	14.7	18
QHY	Plateau and mountain	Huangshui river	100°23'	37°54'	3100	412	3.3	29
LED	Plateau and mountain	Huangshui river	102°28'	36°31'	3160	335	6.9	28
TS	Canyon and basin	Wei river	105°40'	34°37'	1650	531	11.5	17

Table 2. Genetic variation parameters of *P. cathayana* based on AFLP.

Population	Number of polymorphic loci	Percentage of polymorphic loci	N_a	N_e	h	Private bands
SHY	66	37.7%	1.377	1.203	0.117	6
JZ	106	60.6%	1.606	1.385	0.210	10
PW	95	54.3%	1.543	1.250	0.148	10
QHY	69	39.4%	1.394	1.217	0.122	5
LED	74	42.3%	1.423	1.276	0.152	1
TS	83	47.4%	1.474	1.328	0.181	3
Mean	82.2	47.0%	1.470	1.276	0.155	5.8
Total	175	98.9%	1.989	1.525	0.306	35

N_a , observed number of alleles per locus; N_e , effective number of alleles per locus (Kimura and Crow 1964); h , Nei's (1978) gene diversity.

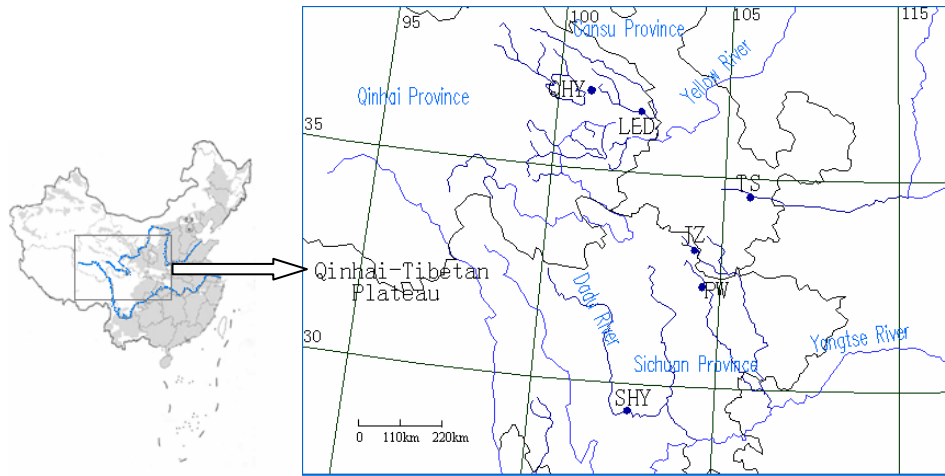


Fig 1. The geographical distribution of *P. cathayana* in China. The locations of the natural populations sampled in this study are shown. Population abbreviations are given in Table 1.

Data analysis: The fragments amplified by AFLP primers were scored as either present (1) or absent (0) on the basis of size in comparison with external standards using the Gel Doc 1000™ image analysis system (Biorad). Only those fragments, which had a molecular weight between 100 bp and 400 bp were visualized with medium or high intensity were considered for data analysis. The following parameters were generated using the population genetic software package POPGENE, program version 1.32 (Yeh *et al.*, 1997): the observed number of alleles (N_a) and the effective number of alleles (N_e), and Nei's gene diversity (h) (Nei, 1973). We counted the number of common alleles (defined as bands with frequency >0.05 common to all populations) and the number of private alleles (defined as bands restricted to a single population).

The genetic structures were investigated as described by Nei's (1978) analysis. The proportion of the total genetic variation found among the populations (G_{ST}) was calculated from $G_{ST} = D_S/H_T$. Gene flow (Nm) was estimated from $Nm = 0.5(1 - G_{ST})/G_{ST}$. Genetic divergence between the populations was investigated using Nei's unbiased genetic distances (D) and genetic identities (I) (Nei, 1978). A Mantel test was used to test the relationship between the matrix of genetic distances and the matrix of geographical distances (Mantel, 1967).

Genetic relationships among individuals were quantified by the Jaccard's coefficient of similarity using NTSYS-pc version 2.02 software (Rohlf, 1998). The matrix of similarity between the pairs of individuals was subject to cluster analysis by the unweighted pair-group method with the arithmetic averages (UPGMA) and a principal coordinate (PCO) analysis according to the extracted Eigen vectors (Rohlf, 1998).

Results

AFLP polymorphism: For six natural populations of *P. cathayana*, four primer combinations (E-AGC/M-CAA, E-AGG/M-CTG, E-AAG/M-CAT and E-AAG/M-CAG) were selected for complete data analysis. A total of 175 bands were produced, with the number of bands per primer combination equaling 39, 35, 56 and 45 bands, respectively.

When taking into account the 175 polymorphic bands across the entire sample, the percentage of polymorphism per population was 98.9%. Of the total of 175 bands scored, 35 bands (20%) were private, presented in single population only, while 16 bands (9.14%) were common to all populations tested.

Genetic variation within population: Genetic variability among the populations is presented in Table 2. In individual populations, the per population percentage of polymorphic loci (P) ranged from 37.3% to 60.6%, with an average of 47.0%. The observed number of alleles (N_a) varied from 1.377 to 1.606, while the effective number of alleles (N_e) varied from 1.203 to 1.385. Nei's gene diversity (h) ranged from 0.117 to 0.210, with an average of 0.155. When across the whole sample, the N_a , N_e and h equaled 1.989, 1.525 and 0.306, respectively.

Six natural populations of *P. cathayana* showed different levels of genetic diversity. A comparison of genetic diversity was performed among six populations of *P. cathayana*. The highest level of genetic diversity was detected in population JZ, while the lowest level was detected in population SHY. The order of genetic diversity based on genetic parameters (h) was found to be JZ > TS > LED > PW > QHY > SHY.

Population genetic structure and differentiation: *P. cathayana* showed high level of genetic differentiation among the populations. The total gene diversity (H_T) and gene diversity among the populations (D_S) were 0.303 and 0.148, respectively. The proportion of genetic variation among the populations (D_S) accounted for 48.9% of the whole genetic diversity, indicating a high degree of genetic differentiation among the populations. The level of gene flow (Nm , the number of migrating individuals among the populations per generation) was estimated to be 0.523. The observed value indicated that gene exchange between the populations was low.

Cluster analysis of individuals: For 143 individuals of all sampled populations, the Jaccard's coefficient of similarity for all possible pair-wise comparisons ranged from 0.537 to 0.994 (data not shown), with an average of 0.688. However, for one population, higher individual genetic similarity was detected and the order of the average value of Jaccard's coefficient of similarity was found to be SHY (0.914) > QHY (0.913) > LED (0.880) > PW (0.860) > TS (0.844) > JZ (0.830). Clustering of individuals in the dendrogram based on the Jaccard's coefficient of similarity matrix for all individuals is shown in Fig. 2. Population genetic structure was evident from this dendrogram with the individuals from the same population clustering together regularly. The results further showed a high level of genetic similarity within populations and a high level of genetic differentiation among the populations.

By providing spatial representation of relative genetic distances among individuals, the PCO analysis was performed to determine the consistency of the differentiation among the populations defined by the cluster analysis (Fig. 3). For all loci, the first three axes explained 51.1% of the variation. In agreement with what was found in the cluster analysis, the individuals from each population formed separated plot and could clearly distinguish from other populations. Population SHY had the highest level of genetic differentiation from other populations, showed the greatest spatial representation of relative genetic distances. Population JZ formed a looser clustering of individuals, with less individuals identity comparing to any other in the PCO analysis.

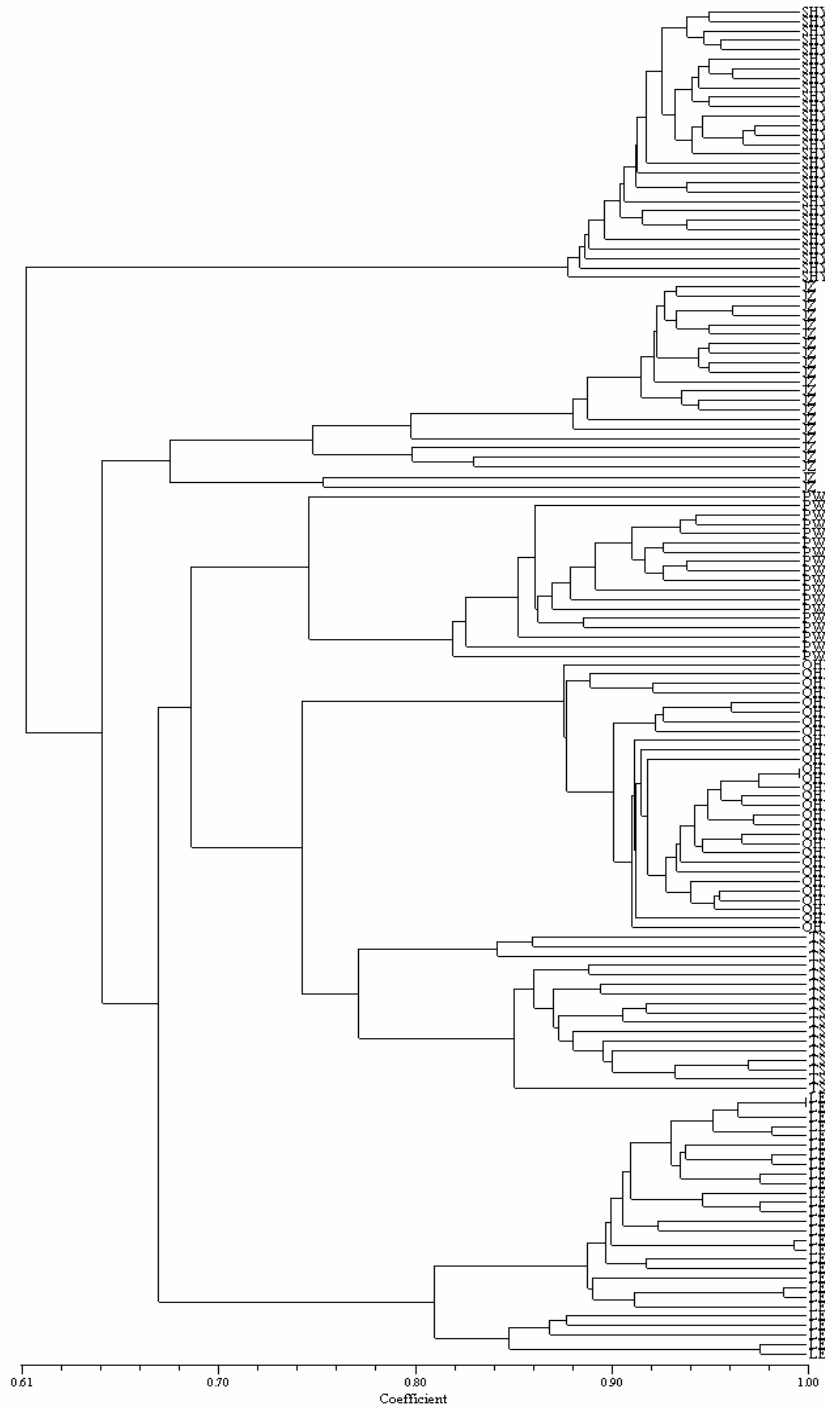


Fig. 2. Dendrogram of 143 individuals resulting from the UPGMA cluster analysis based on Jaccard's similarity coefficients obtained from 175 AFLP fragments.

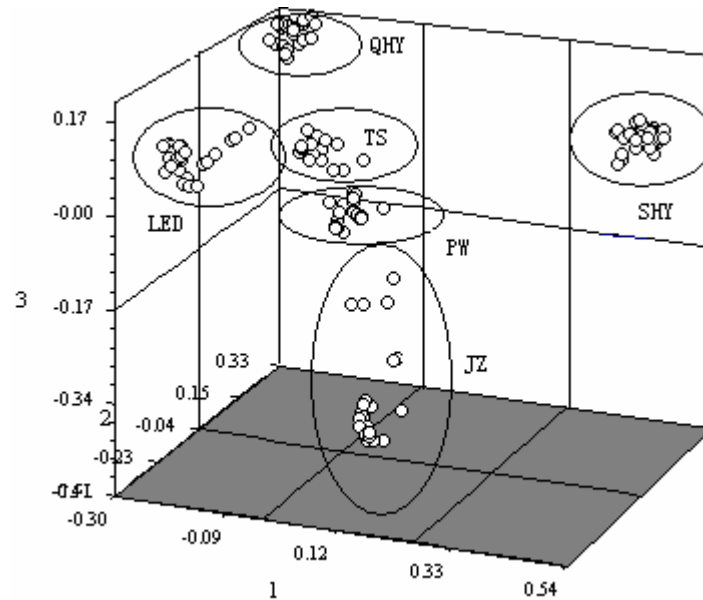


Fig. 3. Principal coordinate (PCO) analysis plot estimates based on Jaccard's similarity coefficients obtained from 175 AFLP fragments. Variance explained: 23.6% (axis 1), 15.0% (axis 2) and 12.5% (axis 3).

Table 3. Genetic distances among the populations of *P. cathayana*, based on Nei's (1978) unbiased measurements.

Population	SHY	JZ	PW	QHY	LED	TS
SHY	—	0.767	0.755	0.702	0.702	0.777
JZ	0.266	—	0.826	0.776	0.795	0.858
PW	0.282	0.191	—	0.811	0.784	0.839
QHY	0.353	0.254	0.209	—	0.774	0.866
LED	0.354	0.230	0.176	0.256	—	0.825
TS	0.252	0.153	0.243	0.144	0.193	—

Nei's genetic identity (*I*) (above diagonal) and Nei's genetic distance (*D*) (below diagonal).

Populations genetic relationships: The genetic relationship between the populations was determined by calculating genetic distances (*D*) and genetic identify (*I*) (Table 3). For population pairs, the greatest genetic distance ($D = 0.354$) was found between the populations SHY and LED and the lowest genetic distance ($D = 0.144$) was found between the populations TS and QHY, with an average equaling 0.237. The estimates of genetic distance (*D*) further confirmed the presence of a high level of genetic differentiation among the populations over great geographic distances. However, Mantel's test with 1000 random Permutations showed that there was no significant correlation between genetic and geographical distances ($r = -0.3866$, $p > 0.05$) among the six populations of *P. cathayana*.

Discussion

AFLP markers were high effective to evaluate genetic diversity since a single PCR assay could reveal many bands at once and which was confirmed again (Maguire *et al.*,

2002; Portis *et al.*, 2005). In our study, a total of 175 bands were produced for four primer pair combinations in all *P. cathayana* populations. The percentage of polymorphic was 98.9%. As a widespread species, the level of genetic diversity contained in *P. cathayana* populations (the mean value of Nei's genetic diversity equaled 0.155) confirmed the assumption that the widespread species maintain more genetic diversity than do restricted species (Karron 1987; Hamrick & Godt 1989; Xue *et al.*, 2005). Compared with previous studies in poplar species, the level of genetic diversity was moderate, which was higher than that reported in *P. nigra* subsp. *betulifolia* in the Upper Severn area of the UK using AFLP markers (Winfield *et al.*, 1998), also higher than that detected in *P. cathayana* within a wide geographical scale in China based on RAPD markers (Li *et al.*, 1997), while slightly lower than that detected in European gene banks of *P. nigra* from nine countries using AFLP markers (Storme *et al.*, 2004).

In our study, *P. cathayana* populations showed significant levels of genetic differentiation among the populations, with 48.9% of the total variation ($G_{ST} = 0.489$) due to differences among the populations. This was also consistent with the result of individual cluster analysis and PCO analysis, which indicated a high level of genetic similarity within population and a high level of genetic differentiation among the populations. The level of genetic differentiation among the populations ($G_{ST} = 0.489$) was considerably higher than that of poplar species investigated using isozymes, RAPD, AFLP and SSR (Agnes & Francois 1996; Li *et al.*, 1997; Imbert & Lefèvre 2003; Storme *et al.*, 2004).

It was reported that high genetic differentiation was attributed to restricted gene flow (Loveless & Hamrick 1984). Analysis of AFLP variation allows an insight into the contemporary genetic connectivity between the populations *via* gene flow (Cavers, 2004). As widespread species, the interaction of gene flow (hundreds of metres) and distribution (thousands of metres), exposure to diverse environmental regimes with differential selection pressures and extinction/colonization sequences creates structure on multiple scales. When the distance is not a limiting factor for the dispersal and gene flow is unlimited, the populations will form a single uniform genetic unit, but when gene flow rate is greatly reduced, population genetic differentiation will increase due to genetic drift (Slatkin, 1977; Hutchison & Templeton, 1999). The populations of *P. cathayana* occur in disjunctive mountain areas with high degree of geographical isolation (at least 100 km between the populations) including plateaus and valleys and complex topography with physical obstacles and variable climate conditions present in the region, which can block gene flow even in a species with generally good dispersal ability. In fact, the level of gene flow was found to be very low (only 0.523), thus genetic drift may be a primary factor for high level of genetic differentiation.

Individuals cluster and PCO analysis based on AFLP information did not show any significant geographical pattern, suggesting no correspondence between geographical and genetic distances in the populations of *P. cathayana*, as also shown by Mantel's test. The presence was accord with the results of other tropical species with widespread distributions and disjunct populations (Rivera-Ocasio *et al.*, 2002). Local currents and stochastic colonization events may be responsible for the geographical and genetic correspondence. However, in our study such large geographical scale, because of seldom gene exchange among the populations and differing selection pressures, genetic distance may not be explained by the geographic distances.

The southeastern part of the Qinghai-Tibetan Plateau is regarded as the natural distribution and variation center of the genus *Populus* in China. During the long

evolutionary process, changes in distributions and adaptations to the harsh conditions have taken place (Yu *et al.*, 2003). In our study, the large geographic range and complex topography, where the natural populations of *P. cathayana* occur, cause a wide range of climatic conditions, including humid monsoon climate (populations TS and PW), humid and cold climate (population JZ), dry and cold plateau climate (populations LED and QHY), dry and hot climate (population SHY). Under such conditions, it is likely that different selective pressures may increase population genetic differentiation by local adaptation. The presence of a number of private AFLP bands found in single population only, perhaps, was the result of several independent evolution events. The long evolutionary process coupled with genetic drift within local populations, rather than contemporary gene flow, are the major forces shaping genetic construction of the studied populations.

The finding of different genetic diversity level within population and high level of genetic differentiation among the populations provided promising condition for further breeding or conservation programs. Three genetic criteria (number of alleles, number of locally common alleles and number of private alleles) have been proposed for defining priorities for ex situ germplasm programs or *In situ* conservation programs (Marshall & Brown 1975; Brown & Briggs 1991; Falk 1991). In our study, the levels of genetic diversity within population detected were not similar among the six populations. This information may assist plant breeders in their decisions of what germplasm to include in their breeding program. The populations JZ and PW contained relatively high level of genetic diversity and had a few private bands, and population SHY, although with the lowest level of genetic diversity, had more private bands than the populations QHY, TS and LED, which suggested these populations have potential for providing further gain from selection within a breeding program and were the priority populations for germplasm collections or *In situ* protection. However, taking into account the complex topography and a wide range of climatic conditions that the natural populations of *P. cathayana* occurred, different populations maybe evolved under different environmental selection pressure and generate special adaptability to local environment. Therefore, for breeders, it is important to make early statements on the adaptation of the populations on their natural distribution area.

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