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Genetic Diversity of *Toona ciliata* Populations based on SSR Markers

WANG Yang, YUE Dan*, LI Xinzhi*

Hubei Ecology Polytechnic College, Wuhan 430200, China

Abstract: In order to provide a theoretical basis for the protection and development of *T. ciliata* germplasm resources, we studied the genetic diversity of *T. ciliata* by using SSR (Simple Sequence Repeat) primers to evaluate the genetic diversity of 192 *T. ciliata* germplasm samples from 24 populations of 5 provinces. DataFormater, Popgene, NTSYS, TFPGA and other software were used for genetic data conversion, genetic parameter estimation, dendrogram construction and genetic variation analysis. The results showed that: 1) a total of 17 alleles (N_a) were detected in seven pairs of primers, with an average of 2.260 for each primer. Among them, the highest numbers of alleles (4) were detected in primers S11 and S422.The mean value of Nei's genetic diversity index (H) was 0.4909, the mean value of Shannon information index (I) was 0.7321, and the mean value of polymorphic information content (PIC) was 0.5182. The mean expected heterozygosity (H_e) and observed heterozygosity (H_o) were 0.1055 and 0.4956, respectively. The Nei's genetic distances of the populations ranged between 0.0002 and 2.6346, and the mean was 0.5477. The average genetic diversity level (H=0.1044) of the 24 populations was lower than that of the species (H=0.4909). 2) The genetic differentiation coefficients (F_{st}) varied from 0.2374 to 0.9148, with an average value of 0.7727. The mean of population gene flow (N_m) was 0.0735, indicating a low level of genetic exchange between populations, and suggesting that the genetic variation mainly came from within populations. 3) With the UPGMA method, the 24 populations were clustered into 3 groups at Nei's genetic identity (0.99): the populations from Guizhou Province and Guangxi Zhuang Autonomous Region were clustered into one group, the populations from Hunan Province were in another group, and the populations from Hubei Province were in the third group. The Mantel test analysis showed a significant correlation between Nei's genetic distance and geographic distance (r=0.6318, P=0.009<0.05). The genetic diversity of the 24 populations of *T. ciliata* was at a low level. Geographic isolation was the main reason for genetic differentiation among *T. ciliata* provenances. In the protection of germplasm resources of *T. ciliata*, emphasis should be placed on breeding genetic resources from the populations with higher genetic diversity (P14, for example). As for the populations with low genetic diversity, an ex-situ protection strategy as well as ecological and timber objectives, should be taken into account to maximize the conservation and utilization of the diversity of *T. ciliata*.

Key words: *Toona ciliata*; SSR marker; natural population; genetic diversity; genetic differentiation

1 Introduction

Toona ciliata Roem. is a tall deciduous or semi-evergreen tree, and a precious timber species belonging to genus *Toona* (Meliaceae). *T. ciliata* was listed as a wild endangered species under secondary national protection (Yu, 1999). The natural distribution area of *T. ciliata* is limited to East Asia, South Asia and Australia. In China, *T. ciliata* is mainly distributed in South China, Central China, East Chi-

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First author: WANG Yang, E-mail: 13296698026@163.com
***Corresponding author:** YUE Dan, E-mail: 190098250@qq.com; LI Xinzhi, E-mail: 794942134@qq.com

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na and Southwest China, and the natural population has sporadic distribution characteristics (Long et al., 2011; Li et al., 2016; Wang et al., 2018). In these areas, it is known as "Chinese mahogany" (Long et al., 2011; Wang et al., 2018) due to its high timber quality, so it has been overexploited and its natural regeneration is slow, leading to the reduction of its natural distribution (Wen et al., 2012; Chen et al., 2014).

So far, studies on *T. ciliata* at home and abroad have mainly focused on plant physiology and biochemistry, ecology, plant introduction and breeding, and related topics (Malairajan et al., 2007; Duan et al., 2015; Wang et al., 2016a; Wang et al., 2016b; Wang et al., 2016c; Huang et al., 2017; Wang et al., 2018). Genetic studies on *T. ciliata* have been reported with respect to phenotypic variation (Cai et al., 2018; Wang et al., 2018; Wang et al., 2019) and molecular markers (SRAP) (Li et al., 2016; Zhan et al., 2016). The SSR (Simple Sequence Repeat) molecular marker is a neutral molecular marker randomly distributed in the genome (Zietkievicz et al., 1994). Due to its advantages of co-dominant inheritance, wide distribution, good stability and repeatability (Powell et al., 1996), SSR technology has become an ideal genetic marker technology. It has been widely used in the genetic studies of some endangered and rare tree species, such as *Eucommia ulmoides* (Miao et al., 2017), *Taxus wallichiana* var. *mairei* (Yi et al., 2013), *Parashorea chinensis* (Zhang and Li, 2011), *Pteroceltis tatarinowii* (Fan et al., 2018) and *Phoebe bournei* (Liu et al., 2019). However, the application of SSR markers in the study of genetic diversity in genus *Toona* is seldom reported. Liu et al. (2013) compared the genetic diversity of central and peripheral populations of *Toona ciliata* var. *pubescens* by using SSR markers. Zhan et al. (2016) established an optimal reaction system of SSR-PCR and screened the highly polymorphic primers fitted for the SSR analysis of *T. ciliata*, which laid an academic foundation for genetic research on natural populations of *T. ciliata*.

Studies of the genetic characteristics of *T. ciliata* should consider the selection of provenances in different geographical distribution areas and the use of different molecular marker technologies in the investigation and evaluation of *T. ciliata* resources. We studied 192 germplasm samples from 24 natural *T. ciliata* populations from five provinces using SSR markers. Through the analyses of genetic diversity, population genetic differentiation, clustering and correlations between geographic distance and genetic distance, we aimed to reveal the genetic diversity level of *T. ciliate,* and the cause of that diversity, to provide a theoretical basis for the protection and development of *T. ciliata* germplasm resources.

2 Materials and methods

2.1 Testing materials

In May 2016, experimental samples were collected based on the investigation of 14 naturally-distributed populations of *T. ciliata* in the provinces of Hubei, Hunan, Jiangxi, Guizhou and Guangxi Zhuang Autonomous Region (Table 1). Eight samples were collected for each population and the distances between sampled plants were all greater than 40 m. Small healthy leaflets on adult plants without pests and diseases were collected for a total of 192 testing samples. All leaflets were rapidly dehydrated with a large amount of color-changing silica gel and then kept at low temperature for subsequent DNA extraction. See Table 1 for specific sampling data for each population.

2.2 Experimental method

2.2.1 DNA extraction

Genomic DNA was extracted from leaflets of *T. ciliata* using the CTAB method. Purity and quality of extracted DNA were measured with 1.0% agarose gel electrophoresis (AGE), and the DNA concentration was measured with a UV spectrophotometer.

2.2.2 SSR-PCR amplification

Based on the published literatures (Liu et al., 2009; Liu

Table 1 Locations and altitudes of the 24 sampled populations of *Toona ciliata*

Population	Location	East longitude	North latitude	Altitude (m)	Population	Location	East longitude	North latitude	Altitude (m)
P1	Xingyi of Guizhou	105°02′08″	24°58′03″	779	P13	Laifeng of Hubei	109°15′57″	29°25′58″	521
P2	Changde of Hunan	111°31′08″	29°18′54″	399	P14	Hefeng of Hubei	110°12′29″	30°10′12″	559
P3	Ceheng of Guizhou	105°52′38″	24°52′16″	972	P15	Enshi of Hubei	109°14′51″	30°01′13″	738
P4	Tianlin of Guangxi	106°39′08″	24°02′12″	311	P16	Xuan'en of Hubei	109°41′59″	30°02′26″	1013
P5	Shaoyang of Hunan	111°22′15″	27°22′30″	540	P17	Lichuan of Hubei	108°33′49″	29°51′22″	521
P6	Jinggangshan of Jiangxi	114°09′37″	26°39′20″	907	P18	Zhushan of Hubei	110°01′59″	31°39′58″	660
P7	Zhenfeng of Guizhou	105°46′17″	25°22′46″	477	P19	Gucheng of Hubei	111°15′49″	32°01′36″	402
P8	Huaihua of Hunan	110°05′14″	27°31′47″	613	P20	Badong of Hubei	110°23′44″	30°36′49″	720
P9	Anlong of Guizhou	105°26′25″	25°06′23″	1377	P21	Chongyang of Hubei	113°46′25″	29°26′37″	338
P10	Youmai of Guizhou	105°59′41″	25°03′19″	695	P22	Tongshan of Hubei	114°38′39″	29°24′18″	567
P11	Xian'an of Hubei	114°19′18″	29°45′42″	356	P23	Huangshi of Hubei	115°04′51″	30°11′26″	356
P12	Xianfeng of Hubei	109°00′07″	29°47′59″	806	P24	Jianshi of Hubei	110°05′59″	30°19′26″	541

et al., 2013; Liu et al., 2016; Zhan et al., 2016), 29 pairs of primers with good polymorphism were selected. The SSR-PCR (polymerase chain reaction) was performed on the Bio-Rad ptc-200 PCR apparatus (Bio-Rad Laboratories, USA). The reaction system was amplified in 19 μl PCR molecular marker solutions, the specific components of which (after being optimized) were: 12.1 μl dd H_2O, 2 μl template DNA, 0.1 μl Taq enzyme, 2.0 μl 10×PCR buffer, 1.8 μl $MgCl_2$ (25 mmol L^{-1}) and 1.0 μl primer mixture (10 μmol).

The PCR thermal cycling was: pre-denaturation at 94 ℃ for 5 min, denaturation at 94 ℃ for 45 s, annealing at 55 ℃ for 45 s, with a total of 30 cycles, and extension at 72 ℃ for 45 s. Then, the sequences amplified by PCR were extended at 72 ℃ for 10 min and maintained at 4 ℃ for 5 min. At the end of PCR, the amplified solutions were stored in the refrigerator at 4 ℃ for future use. A total of seven pairs of primers with stable amplification and good repeatability were selected for SSR analysis using the *T. ciliata* samples. Primer information is shown in Table 2.

Table 2 Primer sequences used in the SSR analysis of *T. ciliata*

Primer	Primer combination sequences	
S5	F: GTGGCGTAACAGACCAAAAC	R: CCAGAGATACTCCATTCCAG
S11	F: AGTAATAGCCTGTAGAGCAG	R: GAAGAAGGGTGAGCGAGA
S22	F: GAAACCAGCAGGCAGAGC	R: ACCGCATTAGTACCAGTAG
T02	F: TAGGAAAGGCAAGGTGGG	R: GGGTGGTCGATGAGGGTT
T05	F: AGTAATAGCCTGTAGAGCAG	R: AGAGTGGGGTGGTCGATGAG
T07	F: ATGGATGAGTGTGCGATAGG	R: TGTGATGTAGGAGTCTGAAC
S422	F: ATGGATGAGTGTGCGATAGG	R: TGTGATGTAGGAGTCTGAAC

2.3 Data analysis

The amplified banding patterns were recorded as 0 or 1. In the positions with the same mobility rate, each position with a band was denoted as 1, while positions without a band were denoted as 0. DataFormater software (Fan et al., 2016) was used to transform the data to meet the input requirements of the different analysis software programs. POPGENE 1.32 was employed to obtain the required genetic parameters, including observed number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), Shannon information index (I), polymorphism information content (PIC), population inbreeding coefficient (F_{is}), genetic differentiation coefficient (F_{st}), the number of migrants per generation (N_m) and Nei's genetic distance (Nei et al., 1983). NTSYS-pc 2.10s was used to draw the UPGMA-based dendrogram. TFPGA software was used to conduct the Mantel test for geographical distance and Nei's genetic distance (Mantel, 1967). SPSS 22 and Excel 2013 were applied for data processing.

3 Results and analyses

3.1 Polymorphism of SSR loci

From 29 pairs of SSR primers, seven pairs of markers were obtained which had stable amplification, effective polymorphic information content and uniform genome-wide distribution (Table 2). A total of 17 alleles (N_a) was detected, yielding a mean value of 2.7143 alleles for each marker, with the variation ranging from 2 to 4, as can be seen in Table 3. The number of detected effective alleles (N_e) was 15.8214, for an average of 2.2602 alleles for each marker. The percentage of polymorphic bands (PPB) ranged from 49.92%–93.70%, and the mean was 78.82%, indicating that there was little difference between N_a and N_e, and that the detected loci were evenly distributed in each population. The average polymorphism information content (PIC) of each marker was 0.5182 and the arrangement of loci by PIC values was: S11 (0.7473) > T07 (0.5789) > S5 (0.5340) > T05 (0.5211) > S422 (0.5122) > S22 (0.4429) >T02 (0.2909). Shannon indexes (I) varied from 0.1447 to 1.2094, with an average of 0.7321. The observed heterozygosity (H_o) spanned 0.0000 to 0.5965, with an average value of 0.1055. The expected heterozygosity (H_e) ranged from 0.4266 to 0.6749, with an average of 0.4956. Nei's genetic diversity

Table 3 Summary of genetic variation statistics of the seven Loci

Locus	N_a	N_e	PPB (%)	PIC	H_o	H_e	H	I
S5	2	2.1460	89.30	0.5340	0.0000	0.4444	0.4401	0.6603
S11	4	3.9574	75.20	0.7473	0.0870	0.6749	0.6675	1.2094
S22	2	1.7950	86.63	0.4429	0.0000	0.4266	0.4228	0.6205
T02	2	1.4102	93.53	0.2909	0.5965	0.4695	0.4654	0.1447
T07	3	2.3750	63.50	0.5789	0.0192	0.4796	0.4750	0.8395
T05	2	2.0880	93.70	0.5211	0.0000	0.4708	0.4664	0.6757
S422	4	2.0498	49.92	0.5122	0.0357	0.5037	0.4992	0.9745
Mean	2.7143	2.2602	78.82	0.5182	0.1055	0.4956	0.4909	0.7321

Note: N_a: the number of alleles; N_e: the number of effective alleles; PPB: the percentage of polymorphic bands; PIC: the polymorphic information content; H_o: the observed heterozygosity; H_e: the expected heterozygosity; H: Nei's genetic diversity index; I: Shannon diversity index.

index (*H*) values were between 0.4228 and 0.6675, with a mean of 0.4909. These results showed that the polymorphism of the seven pairs of SSR primers in the populations of *T. ciliata* was lower but effective, so these primers could be applied to provide a good analysis of the genetic diversity of *T. ciliata*.

3.2 Genetic diversity of the population

The analyses of the genetic diversity parameters of the 24 populations showed that the number of alleles (N_a) varied between 1.0000 and 2.4286, with an average of 1.2629. The number of effective alleles (N_e) ranged from 1.0000 to 2.2286, with an average of 1.2081. The polymorphic information content (PIC) was 0–100.00%, and the mean was 19.05%. The observed heterozygosity (H_o) was between 0.0000 and 0.2857, with an average of 0.1136. The expected heterozygosity (H_e) spanned 0.0000 to 0.6190, with a mean of 0.1493, indicating that the diversity level of all populations was lower. Nei's genetic diversity index (*H*) was between 0.0000 and 0.5159, with an average of 0.1044. Only P16 had a higher genetic diversity than that of the species level (H = 0.4909) while the other populations with H > 0.1000 were ranked in value as: P16 > P6 > P13 > P15 > P10 > P22 > P8. Shannon information indexes (*I*) were in the range of 0.0000–0.8015, with a mean of 0.1546, indicating a low level of genetic diversity (Table 4).

Table 4 Genetic diversity parameters of 24 *T. ciliata* populations

Population	N_a	N_e	PIC	H_o	H_e	H	I
P1	1.1429	1.1429	14.29%	0.1429	0.1429	0.0714	0.0990
P2	1.1429	1.1429	14.29%	0.1429	0.1429	0.0714	0.0990
P3	1.1429	1.1429	14.29%	0.1429	0.1429	0.0714	0.0990
P4	1.1429	1.1429	14.29%	0.1429	0.1429	0.0714	0.0990
P5	1.1429	1.1429	14.29%	0.1429	0.1429	0.0714	0.0990
P6	2.2857	1.8138	100.00%	0.0714	0.4481	0.4107	0.6533
P7	1.1429	1.1429	14.29%	0.1429	0.0952	0.0714	0.0990
P8	1.2857	1.1829	28.57%	0.0357	0.1310	0.1027	0.1528
P9	1.1667	1.1000	14.29%	0.0833	0.0714	0.0625	0.0937
P10	1.2857	1.2101	28.57%	0.0857	0.1302	0.1171	0.1705
P11	1.1429	1.1429	14.29%	0.1429	0.1429	0.0714	0.0990
P12	1.1429	1.1213	14.29%	0.1020	0.0706	0.0656	0.0931
P13	1.4286	1.2527	42.86%	0.2381	0.1810	0.1508	0.2278
P14	1.0000	1.0000	0.00%	0.0000	0.0000	0.0000	0.0000
P15	1.2857	1.2857	28.57%	0.2857	0.2857	0.1429	0.1980
P16	2.4286	2.2286	0.00%	0.1429	0.6190	0.5159	0.8015
P17	1.0000	1.0000	0.00%	0.0000	0.0000	0.0000	0.0000
P18	1.1429	1.1143	14.29%	0.0952	0.0762	0.0635	0.0909
P19	1.1429	1.1429	14.29%	0.1429	0.1429	0.0714	0.0990
P20	1.1429	1.1429	14.29%	0.1429	0.1429	0.0714	0.0990
P21	1.0000	1.0000	0.00%	0.0000	0.0000	0.0000	0.0000
P22	1.2857	1.1708	28.57%	0.0857	0.1175	0.1057	0.1588
P23	1.1429	1.0857	14.29%	0.0714	0.0714	0.0536	0.0803
P24	1.1429	1.1429	14.29%	0.1429	0.1429	0.0714	0.0990
Mean	1.2629	1.2081	19.05%	0.1136	0.1493	0.1044	0.1546

Note: N_a: the number of alleles; N_e: the number of effective alleles; PIC: the polymorphic information content; H_o: the observed heterozygosity; H_e: the expected heterozygosity; *H*: Nei's genetic diversity index; *I*: Shannon diversity index.

3.3 Population genetic differentiation

The coefficient of inbreeding (F_{is}) reveals the deletion or excess of heterozygous genotypes in the total group of samples (Table 5). Among the seven loci, there were excess hybrid genes in five of them, and deleted hybrid genes in two loci (S11 and T02); overall, the mean heterozygosity of the populations was higher, indicating an inbreeding phenomenon of *T. ciliata*. This inbreeding phenomenon might be related to the characteristics of a small population or a high degree of geographic or environmental isolation (Wang et al., 2016a).

The genetic differentiation index (F_{st}) is an important in-

dicator of inter-population genetic differentiation. The mean value of F_{st} was 0.7727, indicating a high degree of genetic differentiation among the populations. The F_{st} of T02 (0.2374) was the lowest, but even it reached a high level of genetic differentiation, while the F_{st} of S5 (0.9148) and S22 (0.9148) were both at the highest value. Gene flow (if N_m > 1) can play a homogenizing role, that is, it can effectively inhibit the differentiation between populations. But when N_m < 1, genetic differentiation between populations definitely occurs (Wright, 1951). The mean N_m of the 24 *T. ciliata* populations was 0.0735, showing a low level of genetic exchanges between the populations, which inevitably resulted in the higher F_{st}.

Table 5 Coefficients of genetic differentiation and gene flow between *T. ciliata* populations

Locus	F_{is}	F_{st}	N_m
S5	1.0000	0.9148	0.0233
S11	–0.1232	0.8288	0.0516
S22	1.0000	0.9148	0.0233
T02	–0.7084	0.2374	0.8029
T07	0.7857	0.8233	0.0537
T05	1.0000	0.8921	0.0302
S422	0.6548	0.7914	0.0659
Mean	–0.0096	0.7727	0.0735

Note: F_{is}: population inbreeding coefficient; F_{st}: genetic differentiation coefficient; N_m: number of migrants per generation.

3.4 Genetic relationship and cluster analysis of the populations

Table 6 shows that the Nei's genetic distances of the 24 *T. ciliata* populations were between 0.000 and 2.635, with an average of 0.548. The genetic distance between P9 and P18 was the longest, while the genetic distance between P1 and P3 was the shortest. The geographical distances among the 24 populations ranged from 20.202 to 1154.471 km. The geographical distance between P1 and P23 was the longest, while that between P14 and P24 was the shortest, with an average of 491.180 km. By using the UPGMA method, the genetic consistency among the populations of *T. ciliata* was clustered (Fig. 1). The populations of Guizhou Province and Guangxi Zhuang Autonomous Region were grouped together; while the populations of Hunan Province were clustered in another group; and the populations of Hubei were in a third group. The results showed that the 24 populations of *T. ciliata* were clearly clustered according to geographical distances.

The Mantel test (Mantel, 1967) was performed on the normalized logarithmic geographic distances between the different populations and Nei's genetic distances (Fig. 2). The results revealed an extremely significant correlation between Nei's genetic distance and geographic distance for the group of 24 *T. ciliata* populations (r=0.631, P=0.009 <0.05).

Table 6 Geographic distance and Nei's measures of genetic distance between the different populations

Population	P1	P2	P3	P 4	P5	P 6	P7	P8	P9	P10	P11	P12
P1	0.000	0.184	0.000	0.169	0.164	1.427	0.023	0.191	0.048	0.020	1.250	1.495
P2	813.899	0.000	0.502	0.284	0.224	0.000	0.253	0.136	0.321	0.355	0.151	0.397
P3	94.239	745.562	0.000	0.134	0.238	1.499	0.087	0.624	0.010	0.079	1.559	0.914
P4	200.399	764.182	121.327	0.000	0.261	0.379	0.357	0.191	0.321	0.251	0.679	0.759
P5	694.831	216.008	615.055	600.545	0.000	0.128	1.861	0.076	0.321	0.251	0.151	0.397
P6	942.630	394.233	854.178	809.441	288.084	0.000	0.594	0.220	1.261	1.101	0.100	0.317
P7	94.373	717.911	58.421	174.249	600.937	851.510	0.000	1.852	1.826	0.001	1.482	1.338
P8	588.999	244.576	513.711	518.222	128.105	415.348	491.837	0.000	0.273	0.250	0.150	1.033
P9	51.623	764.833	51.287	170.344	643.819	890.660	46.619	536.283	0.000	0.011	1.826	1.624
P10	105.825	719.245	28.112	130.579	595.541	937.695	43.327	491.987	56.546	0.000	1.378	1.329
P11	1102.194	295.379	1031.610	1033.003	438.119	409.258	1006.608	518.472	1051.834	1009.778	0.000	0.092
P12	667.923	249.735	627.351	679.819	354.799	614.673	584.291	273.074	628.524	603.785	518.927	0.000
P13	658.233	215.922	610.919	655.850	306.996	569.457	570.941	227.165	615.754	587.346	496.102	47.796
P14	784.345	155.414	734.238	772.719	331.558	547.032	685.329	297.541	740.855	710.735	390.338	131.978
P15	702.516	233.378	660.920	711.379	351.351	608.949	618.035	288.427	662.272	637.127	491.349	34.232
P16	731.596	193.604	686.204	730.671	337.601	576.007	645.371	280.881	690.102	662.422	447.588	32.267
P17	648.599	292.523	612.780	672.126	388.540	653.713	567.327	297.783	610.703	662.329	559.069	43.109
P18	896.381	298.413	859.041	911.302	494.726	686.210	814.512	460.657	858.245	835.246	435.728	231.839
P19	997.076	302.490	952.453	995.339	516.427	659.615	911.266	511.782	955.825	928.686	348.442	328.029
P20	824.102	180.008	777.377	817.424	371.063	572.418	737.151	343.091	782.254	753.221	379.222	161.860
P21	1004.299	222.903	932.128	926.337	329.956	305.026	910.554	418.748	955.163	910.704	117.445	467.081

(*Continued*)

Population	P1	P2	P3	P 4	P5	P 6	P7	P8	P9	P10	P11	P12
P22	1078.720	303.619	1003.264	992.667	391.714	308.371	984.091	492.168	1028.344	982.642	108.409	548.314
P23	1154.471	357.847	1082.811	1077.884	477.909	401.988	1060.605	569.301	1105.364	1061.561	73.747	588.123
P24	789.9930	174.5340	742.3150	783.321	351.456	409.226	702.080	314.612	747.541	718.582	399.826	129.477
Population	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24
P1	1.321	1.320	1.411	1.550	1.398	1.254	1.283	1.279	1.264	1.260	1.339	1.269
P2	0.353	0.147	0.376	0.514	0.315	0.547	0.540	0.184	0.081	0.087	0.103	0.208
P3	1.506	1.234	1.006	0.503	1.075	1.176	0.903	0.645	0.549	0.504	0.538	0.527
P4	0.671	0.484	0.515	0.565	0.372	0.461	0.460	0.349	0.266	0.345	0.342	0.582
P5	1.321	0.670	0.716	0.565	0.996	0.893	0.789	0.338	0.166	0.139	0.136	0.423
P6	0.266	0.330	0.262	0.317	0.354	0.368	0.379	0.272	0.114	0.125	0.120	0.305
P7	0.172	1.322	1.322	0.249	1.349	1.338	1.337	1.337	1.349	2.305	1.340	1.337
P8	0.668	0.725	0.080	0.564	0.514	0.526	0.553	0.191	0.135	0.063	0.128	0.289
P9	2.477	1.784	2.050	1.641	1.636	2.635	2.624	1.672	1.636	1.670	1.839	0.981
P10	1.299	0.935	1.314	1.719	1.866	1.329	1.328	1.328	1.866	2.285	1.331	1.328
P11	0.100	0.103	0.096	0.092	0.093	0.097	0.123	0.138	0.156	0.087	0.093	0.092
P12	0.090	0.089	0.090	0.090	0.090	0.090	0.097	0.093	0.097	0.091	0.095	0.100
P13	0.000	0.097	0.097	0.090	0.091	0.092	0.089	0.102	0.107	0.111	0.103	0.091
P14	126.121	0.000	0.092	0.091	0.094	0.088	0.089	0.236	0.094	0.107	0.114	0.091
P15	62.808	43.537	0.000	0.091	0.094	0.092	0.093	0.236	0.094	0.093	0.100	0.090
P16	75.212	59.243	43.576	0.000	0.091	0.095	0.099	0.233	0.099	0.116	0.118	0.091
P17	84.077	111.518	68.878	111.545	0.000	0.096	0.090	0.232	0.100	0.115	0.095	0.094
P18	257.393	185.494	199.733	185.395	247.608	0.000	0.094	0.206	0.156	0.097	0.097	0.091
P19	341.271	266.155	293.889	266.143	352.536	121.514	0.000	0.154	0.024	0.018	0.029	0.009
P20	166.455	46.169	128.776	92.379	195.463	123.627	177.363	0.000	0.018	0.017	0.028	0.008
P21	437.295	352.559	446.745	404.053	510.539	441.607	381.901	355.899	0.000	0.100	0.090	0.094
P22	518.364	430.732	526.568	483.493	590.942	508.314	436.324	430.933	82.506	0.000	0.099	0.090
P23	564.382	461.774	562.455	518.883	629.691	508.818	418.347	452.369	152.414	101.020	0.000	0.096
P24	131.412	20.202	97.204	58.386	165.387	146.899	211.513	34.708	368.508	443.241	475.439	0.000

Note: Nei's genetic distance (above diagonal) and geographic distance (below diagonal) of the 24 *T. ciliata* populations.

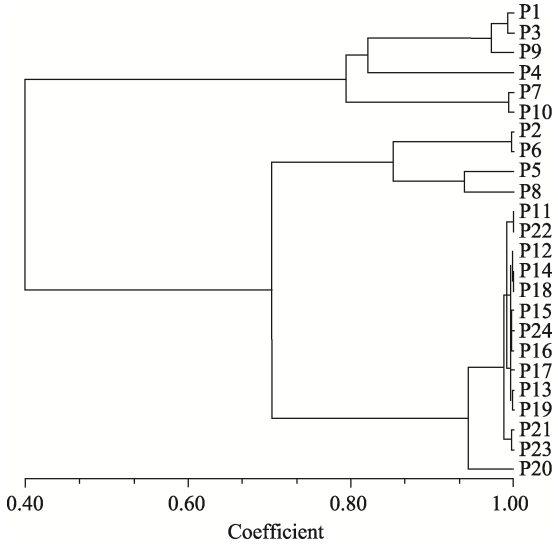

Fig. 1 UPGMA dendrogram based on Nei's genetic distance

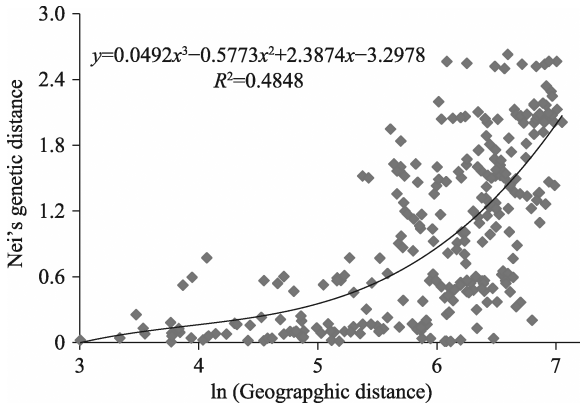

Fig. 2 Spatial genetic correlation of the 24 *T. ciliata* populations

4 Discussion

4.1 Genetic diversity of *T. ciliata*

At the species level, Nei's genetic diversity index (H =

0.4909) was consistent with (but slightly lower than) Shannon diversity index, indicating that the genetic diversity of populations was at a lower level. The level and distribution pattern of genetic diversity of a given plant species are the results of geographical distribution, breeding system, human interferences and many other factors (Wu et al., 2019), among which natural environmental differences can cause isolation between populations. Widely distributed in China, and adapted to complex and diverse environments, *T. ciliata* has derived rich genetic diversity. In this study, the latitudes of sampled *T. ciliata* populations spanned 24°02'12"–32°01'36"N, and the difference between the flowering phases of the northernmost and southernmost distribution areas is greater than 30 d. Therefore, *T. ciliata* trees from the northern populations and the southern populations could not pollinate each other, resulting in reproductive isolation. Limited pollen and seed diffusion might contribute to a lower level of effective gene flow, and can easily cause a high proportion of self-pollination in a species (Liu et al., 2009). *T. ciliata* is mainly distributed in mountainous areas (at altitudes of 300–2260 m), and its sporadic distribution and small population together with overcutting, all add to the declines of its habitats and natural resources (Long et al., 2011), which contribute to the high degree of habitat isolation and obstruct the gene flow, resulting in the low genetic diversity of the *T. ciliata* populations. Except for P16, the genetic diversity levels of the other 23 populations were lower than that of the species level (H = 0.4909), which was higher than that found by Li et al. (2016) for the whole distribution area of *T. ciliata* in China (H = 0.3770). However, the average genetic diversity level of the populations in this study (H = 0.1044) was lower than that found by Li et al. (2016) (H = 0.1805), which might be related to the differences in sampling sites.

T. ciliata is a highly heliophilous species. So, if the plants in the forest cannot reach the canopy, then their competitiveness is insufficient, and the small-and medium-sized plants under the canopy often die (Wang et al., 2019). Therefore, the natural habitats of *T. ciliata* are along streams, rivers or narrow forest margins with optimal luminous conditions. When the suitable habitats shrink, the number of trees declines sharply, and smaller populations become more typical (Wang et al., 2016a). Meanwhile, species with diffusive incompetence are more susceptible to the influence of edge locations, compared with species which have long-distance diffusion competence (Lesica and Allendorf, 1995; Peakal and Smouse, 2012). A strategy of scattered survival would make it difficult for *T. ciliata* trees to maintain extensive gene exchanges, even within the populations, which may lead to low genetic diversity at the population level.

4.2 Genetic differentiation of populations

The high degree of genetic differentiation indicates that the homologous probability of two gametes being randomly selected from any non-cohabitation populations is low, and, therefore, the homogeneity of genetic composition of the population is also low. The genetic differentiation coefficient (F_{st}=0.7727) was higher than that found for *T. ciliata* var. *pubescens* (a *T. ciliata* variety) in central (0.1520) and peripheral populations (0.3045) (Liu et al., 2009), showing that at the species level, the genetic variation (77.27%) within *T. ciliata* populations was higher than the genetic variation between groups (22.73%), and that the genetic differentiation within populations was the main factor causing the genetic variation of *T. ciliata*. This result was consistent with Li's finding that 79.26% of the genetic differentiation existed between populations through AMOVA analysis (Li et al., 2016).

Genetic differentiation is influenced by gene flow, natural selection and mutations (Schaal et al., 1998). Gene flow (N_m) is the flow of genes between populations and an important factor affecting the genetic differentiation of populations. A greater level of gene flow between populations causes more homogeneous populations (Slatkin, 1981). However, as long as the gene flow is in pleiotropy, it can prevent the genetic differentiation caused by genetic drift between populations when the inter-population migration per generation is $N_m \geqslant 1$ (Hamrick et al., 1995). In this study, we found that gene exchange between populations was low (N_m = 0.0735 < 1), and thus increasing the genetic differentiation between populations. Since gene flow mainly comes from seed flow or pollen flow, geographical isolation caused by mountains or rivers could block it (Nagel et al., 2015; Yi et al., 2018).

Nei's genetic distance was significantly related to the geographical distance of the *T. ciliata* populations. First of all, there were six populations in one group, including P4 of Guangxi Zhuang Autonomous Region as well as P1, P3 P7, P9, and P10 from Guizhou Province. In addition, P2, P5 and P8 from Hunan Province were grouped together, while all 14 populations in Hubei were grouped together. Such a clustering reflected the differences in the geographical distribution areas of *T. ciliata* (both in the north and in the south), and, as a result, reproduction between the populations was almost completely isolated and the gene flow was greatly blocked.

4.3 Protection and utilization of germplasm resources of *T. ciliata*

As an important source of genetic diversity, wildlife may possess valuable genetic resources which can serve as the basis for resource utilization. Therefore, systematic research and scientific protection of wildlife should be emphasized (Wu et al., 2019). For protecting germplasm resources of *T. ciliata*, it is necessary to select and breed superior populations according to the higher genetic diversity of the species resources and their genetically-differentiated characteristics. P16, for example, has the highest genetic diversity (H = 0.5159), which is higher than that of the species level (H =

0.4909). As we observed in this investigation, the natural resources of *T. ciliata* in Xuan'en region were very rich. This might give rise to a higher probability of gene exchanges within the populations, which might help to effectively reduce the genetic differentiation.

5 Conclusions

We can generally conclude that the larger distribution area of *T. ciliata* results in the lower genetic diversity of the species, but the higher genetic diversity at the population level as a whole. The differences in the geographical distribution areas of *T. ciliata* can add to reproductive isolation. Furthermore, the geographical and environmental characteristics within smaller areas in each group coupled with the resource pressure from human activities have led to the unique clustering pattern. For example, terrain blockage, human interference, and frequent rainfall in the flowering period could bring the reduction of gene exchanges within the populations, resulting in lower genetic diversity within populations. Meanwhile, natural selection and genetic mutations may increase the genetic differentiation.

The key element of germplasm breeding of *T. ciliata* lies in the selection of families and plants with high genetic diversities within the different populations (Yang et al., 2017; Wu et al., 2019). The populations with the lowest genetic diversities, P14, P17 and P21, for example, might harbor higher potentials, so pursuing on-site protection together with ex-situ protection strategies is recommended (Yi et al., 2018). The selection of parents in crossbreeding, genetic relationships between individuals separated by geographical distances and the parent populations (or individuals) should all be taken into account (Yang et al., 2017), as well as the ecological and timber objectives, so as to maximize the preservation and utilization of the genetic diversity of *T. ciliata*.

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红椿居群遗传多样性的 SSR 分析

汪 洋，岳 丹，李新枝

湖北生态工程职业技术学院，武汉 430200

摘 要：利用 SSR (简单重复序列) 标记研究红椿分布区天然居群的遗传多样性，为红椿种质资源的保护和开发提供理论依据。本文筛选 7 对 SSR 引物，分析了湖北等 5 省（区）24 个居群的 192 份红椿种质的遗传多样性，利用 DataFormater、Popgene、NTSYS、TFPGA 等软件进行遗传数据转换、遗传参数估算、树状聚类图和遗传变异分析。结果显示：(1) 7 对引物共检测到 17 个等位基因(N_a)，平均每个引物为 2.2602 个，其中引物 S11 和 S422 检测到的等位基因数最多，均为 4 个。Nei's 遗传多样性指数均值(H)为 0.4909，Shannon 信息指数均值(I)为 0.7321，多态信息含量(PIC)平均值为 0.5182；平均期望杂合度(H_e)和观测杂合度(H_o)分别为 0.1055 和 0.4956。居群 Nei's 遗传距离介于 0.000–2.635 之间，均值为 0.548；居群遗传多样性平均水平(H=0.1044)低于物种水平(H=0.4909)。(2)居群遗传分化系数(F_{st})介于 0.2374–0.9148，平均为 0.7727；居群基因流(N_m)均值为 0.0735，表示居群间遗传交流水平低，遗传变异主要来自居群内。(3)在遗传一致度为 0.99 时，24 个居群 UPGMA 聚类为 3 大群组：贵州和广西居群聚为一组，湖南聚类为 1 组；湖北聚类为 1 组。Mantel 检验结果显示居群间的遗传距离与地理距离间呈现显著相关性(r=0.6318，P=0.009<0.05)。红椿居群遗传多样性处于偏低水平，地理隔离是红椿种源间产生遗传分化的重要的原因。进行红椿种质资源保护时，应侧重选育群体遗传多样性最高的居群（如 P14）；遗传多样性低的居群，应就地保护与迁地保护相结合，综合生态和用材目标，最大化保存和利用好红椿的多样性。

关键词：红椿；SSR 标记；天然居群；遗传多样性；遗传分化