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RESEARCH ARTICLE

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Genetic diversity of *Betula luminifera* populations at different elevations in Wuyi Mountain and its association with ecological factors

Abstract The random amplified polymorphic DNA (RAPD) technique was used to evaluate the genetic diversity and population structure of 91 genets from four wild populations of *Betula luminifera* at different elevations in the National Nature Reserve of the Wuyi Mountain, Fujian Province, China. Eighteen random primers (from 139 primers) produced a total of 199 scorable amplified fragments, of which 174 (87.44%) were polymorphic across all individuals. The genetic diversities of *B. luminifera* at the population level and species level were $PPL = 60.05\%$, $h = 0.2242$, $I = 0.3181$ and $PPL = 87.44\%$, $h = 0.3442$, $I = 0.4899$, respectively. The value of differentiation ($G_{st} = 0.3486$) and analysis of molecular variance (AMOVA) indicated that there was a relatively high genetic differentiation among populations, and about one-third of the genetic variation occurred among populations. Pearson correlation analysis further revealed that the genetic diversity within populations had significant or very significant correlation with the elevation, climatic factors (annual average temperature and annual precipitation) and soil nutrient factors (total nitrogen, C/N ratio and organic matter). Mantel tests show that there was a significant correlation between the genetic distances among populations and the distance of elevation, and the divergence of soil nutrient factors. The results of the present study suggested that the relatively high genetic differentiation among populations of *B. luminifera* at different elevations might be caused by ecological factors and gene flow.

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1 Introduction

Betula luminifera, a fast-growing deciduous tree of family Betulaceae, is a typical species of genus *Betula* in the subtropical zone of central China and an important broad-leaved plant endemic to China (Zheng et al., 1985; Wu and Wang, 1996). *B. luminifera* has many merits, such as fast growth, good wood quality, strong adaptive capacity, large amount of leaf-fall, and so on. Therefore, it is not only a superior material in the industries of furniture, decoration and pulpwood making, but also plays an important role in improving soil and environment. In recent years, *B. luminifera* has been widely cultivated in southern China, especially in the barren mountains which need ecological recovery. Previous studies on *B. luminifera* focused mostly on its growth characteristics (Dong et al., 2000), seedlings and afforestation (Xie and Li, 2000), community characteristics (Li, 2000) and comprehensive utilization (Zhou et al., 2003). Few molecular ecology studies have been reported yet. The objectives of this study were to use random amplified polymorphic DNA (RAPD) technique to detect the genetic diversity of wild *B. luminifera* populations at different elevations in the National Nature Reserve of the Wuyi Mountain, Fujian, China, to discuss the variation of genetic diversity, and then to disclose ecological factors related to genetic diversity and factors which play an important role in the genetic differentiation of *B. luminifera*. We trust that the study could provide some molecular information to understand the genetic background and further help to make some effective strategies for the germplasm resources conservation, genetic improvement and sustainable utilization of the species.

2 Materials and methods

2.1 Plant materials

The samples were collected from four wild populations of *B. luminifera* at different elevations in the National Nature Reserve of Wuyi Mountain (27°51′42″N, 117°47′18″E), Fujian, China. The ecological factors of different samples are shown in Table 1. Among which, the soil total nitrogen and C/N ratio were tested by the NC-80 Analysis Instrument (made in Japan), soil organic matters was measured by volumetric method, elevations were detected by GPS, and the meteorological data were provided by the meteorological observation station of the Nature Reserve. The distance among individuals sampled in the same subpopulation was kept more than 30 m to avoid deviation produced by sampling within the same family. The fresh leaves for DNA extraction from 20–29 trees in each wild population were collected and stored with improved saturated NaCl-CTAB solution (Li et al., 2006).

Table 1 The environmental data for the four wild populations of *B. luminifera*

ecological factors	population			
	population 1	population 2	population 3	population 4
elevation/m	580	750	980	1250
annual average temperature/°C	17–19	13–18	13–16	11–13
annual precipitation/mm	1700	2000	2100	2200
pH	4.4	4.3	4.3	4.5
content of total nitrogen/(g·kg^{-1})	2.4	3.7	4.0	5.6
C/N ratio	7.12	8.29	9.78	12.98
content of organic matters/(g·kg^{-1})	47.1	77.8	98.6	112.7
soil	red soil	yellow red soil	yellow red soil	red soil
number of plants	29	22	20	20

2.2 RAPD analysis

Genomic DNA was extracted with reference to the method of Zeng et al. (2002) with a minor modification for *B. luminifera* from the leaves which were stored in the improved saturated NaCl-CTAB solution (Xie et al., 2006). PCR amplifications were performed on a DNA thermal cycler (Applied Biosystems 2720 Thermal Cycler, USA). The amplifications were carried out in a volume of 20 μL containing 2 μL 10×PCR buffer, 2.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTP, 0.4 μmol/L 10-base RAPD primer, 30 ng of DNA template, 1 U *Taq* DNA polymerase (produced by TaKaRa of China). PCR conditions were as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles consisting of denaturation at 94°C for 30 s, annealing at 40°C for 1 min and extension at 72°C for 2 min, with a final extension at 72°C for 5 min, and then storing at 4°C. In order to obtain reliable and reproducible data, the primers (produced by Shanghai Sangon of China) which produced clear and reproducible fragments needs to be selected. Amplification products were analyzed by electrophoresis on 1.5% agarose gels. DNA bands were visualized by staining with ethidium bromide, and observed under UV light and photography.

2.3 Data analysis

2.3.1 RAPD data analysis

RAPD bands were scored as binary presence (1) or absence (0) characters to assemble the matrix of the RAPD phenotypes. Then, the indices of genetic diversity, such as the percentage of polymorphic loci (*PPL*), observed number of alleles (N_a), number of effective alleles (N_e), Nei's gene diversity (h), Shannon information index (I), the coefficient for gene divergence (G_{st}) and gene flow (N_m), were calculated using POPGENE 32 software (Yeh et al., 1999) on the basis of gene frequencies. At the same time, the genetic structure within and among populations were detected using the software AMOVA-PREP1.01 (Miller, 1997) and WINAMOVA (Excoffier, 1995).

2.3.2 Correlation analysis

The Pearson correlation between the genetic index within population and ecological factors was analyzed using the SPSS 11.0 software. Meanwhile, the Mantel test was applied to estimate the associations between genetic distance and elevation distance among populations, and between the matrix of Nei's unbiased genetic distances and matrixes of divergence of ecological factors at different elevations of *B. luminifera*.

3 Results

3.1 Genetic diversity of *B. luminifera* populations

Eighteen ideal primers (Table 2) which produced clear and reproducible fragments were selected from 139 random primers for further study. The 18 primers generated a total of 199 RAPD bands (loci), 11.1 bands per primer on average. The number of amplification products per primer varied from 8 to 14, and these primers produced fragments ranging from 200 to 3000 bp in size.

The genetic diversity of *B. luminifera* (such as *PPL*, total genetic diversity (I_T) and total gene diversity (h_T), 87.74%,

0.4899 and 0.3442, respectively) was in accordance with that of *B. alnoides* which has similar life histories (Zeng et al., 2003) (Table 3), but was higher than that of the coniferous or broad-leaved trees (Ge, 1988), which indicated that there was a relatively high genetic diversity in *B. luminifera* at different elevations in the National Nature Reserve of Wuyi Mountain. Biological characteristics and life habits of *B. luminifera* might contribute to its high genetic diversity. As a long-lived, hermaphroditic individual and outcrossing tree, *B. luminifera* can maintain its genetic diversity over a very long time. Moreover, *B. luminifera* itself maybe has high genetic diversity because the populations had been protected effectively.

The genetic diversity among populations of *B. luminifera* from different elevations had obvious differences, and the genetic parameters (*PPL*, *I*, *h*) at mean population level were $PPL = 60.05\%$, $I = 0.3181$, $h = 0.2242$, $N_a = 1.6339$, $N_e = 1.3893$ (Table 3), respectively. According to the genetic diversity parameters, we found that the genetic variation of *B. luminifera* increased with the drop of elevations, i.e., the genetic variation level of the population at the elevation of 580 m (population 1) was the highest, and it became low at the elevation of 750 m (population 2), 980 m (population 3) and 1250 m (population 4).

Table 2 Primers used for RAPD analysis

primers	sequence (5′–3′)	primers	sequence (5′–3′)
S22	TGCCGAGCTG	S152	TTATCGCCCC
S24	AATCGGGCTG	S154	TGCGGCTGAG
S40	GTTGCGATCC	S1401	CCGTCGGTAG
S45	TGAGCGGACA	S1402	GGAAACCCCT
S105	AGTCGTCCCC	S1405	CCCGAAGCGA
S107	CTGCATCGTG	S1406	GTGGCTTGGA
S122	GAGGATCCCT	S1411	GTGCGCAATG
S147	AGATGCAGCC	S1420	CTTCTCGGAC
S151	GAGTCTCAGG	S1430	AGCAGCGAGG

3.2 Genetic structure among populations

The analysis by AMOVA implied that only 32.74% of genetic variation occurred among populations and most of the variation (67.21%) occurred within population (Table 4), which was in accordance with the G_{st} (34.86%) based on the Nei's gene diversity index and the genetic diversity among populations (35.07%) based on the Shannon information index. Based on the above results, we concluded that about 1/3 of the genetic variations existed among populations, and most of genetic variation resided within population, which was similar to some endangered species of China, such as *Liriodendron chinense* (Li et al., 2002) and *Cathaya argyrophylla* (Wang et al., 1997), but dissimilar to the ubiquitous species. Table 4 also shows that N_m based on G_{st} was 0.9343, which indicated that the estimate of gene flow among populations was lower.

Table 5 shows the genetic distance and genetic identity among populations. The variation of the genetic distance ranged from 0.0836 (between population 1 and population 2) to 0.1748 (between population 1 and population 4). The mean distance was 0.1284. The analysis of the genetic identity among populations of *B. luminifera* indicated that the largest genetic identity occurred between population 1 and population 2 (0.8897) and the least between population 1 and population 4 (0.7120). The above results shows that there was higher genetic difference among populations of *B. luminifera* at different elevations, which became obvious with the increasing distance of elevation.

Table 3 Genetic diversity parameters of four natural populations of *B. luminifera* at different elevations

population	sample size	polymorphic loci	percentage population level *PPL*/%	observed number of alleles N_a	number of effective alleles N_e	Shannon's index of diversity *I*	Nei's gene diversity *h*
population 1	29	130	65.33	1.6721	1.4206	0.3484	0.2412
population 2	22	126	63.32	1.6557	1.4013	0.3341	0.2301
population 3	20	115	57.79	1.6252	1.3758	0.3092	0.2189
population 4	20	107	53.77	1.5826	1.3594	0.2806	0.2067
mean		119.5	60.05	1.6339	1.3893	0.3181	0.2242
total	91	174	87.44	1.8622	1.5269	0.4899	0.3442

Table 4 Genetic differentiation for populations of *B. luminifera* at different elevations

variable	POPGENE							AMOVA	
	h_S	h_T	N_m	G_{st}	I_S	I_T	$(I_T-I_S)/I_T$	among populations	within population
species level	0.2242	0.3442	0.9343	0.3486	0.3181	0.4899	0.3507	0.3274 ($p < 0.001$)	0.6726 ($p < 0.001$)
SD	0.1483	0.1571			0.2651	0.2015			

h_S: gene diversity within population; h_T: total gene diversity; N_m: estimate of gene flow; G_{st}: coefficient of gene differentiation; I_S: genetic diversity within population; I_T: total genetic diversity.

Table 5 Genetic identity (above diagonal) and genetic distance (below diagonal) among populations of *B. luminifera*

population	population 1	population 2	population 3	population 4
population 1		0.8897	0.7656	0.7120
population 2	0.0836		0.7415	0.7825
population 3	0.1329	0.1091		0.8397
population 4	0.1748	0.1503	0.1195	

3.3 Correlation between genetic structure and ecological factors

The correlation analysis (Table 6) indicated that the five diversity indices of different subpopulations had significantly ($p < 0.05$) or very significantly ($p < 0.01$) negative correlation with elevation distance and soil C/N ratio. Some genetic diversity indices had also significant correlation with climate factors (annual average temperature and annual precipitation), the content of soil total nitrogen and organic matter. Among them, all the diversity indices had a positive correlation with annual average temperature, but had a negative correlation with the other ecological factors. There was no significant correlation between the genetic diversity parameters and pH of soil. The above correlations implied that the genetic diversity of *B. luminifera* might be the result from the joint effects of one or several ecological factors, i.e., the ecological factors play an important role in influencing the RAPD polymorphism of *B. luminifera*, which are in accordance with the results of the study on *Stipa grandis* (Zhao et al., 2004) and *Triticum dicoccoides* (Fahima et al., 1999).

Table 7 shows the relationships between the divergence ecological factors of different elevations and genetic distances of *B. luminifera* populations. From Table 7, we knew that there were significant correlation between Nei's unbiased genetic distances and elevation-distance among populations, which indicated that elevation had influenced on the genetic differentiation of *B. luminifera* among populations, which is similar to the results of Liu et al. (2003) and Li and Peng (2001). Furthermore, the genetic distance among populations had obvious correlation with the soil factors, such as C/N ratio, the content of total nitrogen, and organic matter, which indicated that the ecological factors might significantly affect the differentiation in population. The above results indicated that the genetic differentiation of *B. luminifera* among populations was actually influenced by the joint effects of many ecological factors.

4 Discussion

The results determined by RAPD markers show that there was a relative high genetic diversity of wild *B. luminifera* populations at different elevations in the National Nature Reserve of Wuyi Mountain. At the species level, the genetic diversity indices *PPL*, *I* and *h* were 87.44%, 0.4899 and 0.3442, respectively. However, according to field investigation, we found that it was difficult for seedlings of *B. luminifera* to survive in the natural forest and most of *B. luminifera* trees were mature ones. From these, we presumed that the high genetic diversity of *B. luminifera* in Wuyi Mountain might be only a temporary phenomenon. Consequently, if we cannot take effective and timely measures of breeding and protection, the genetic diversity of *B. luminifera* will decline in the long run. At the population level, the genetic variation level of *B. luminifera* varied regularly with elevations. That is, the genetic variation of the population at the elevation of 580 m (population 1), in which soil, moisture and light environments were well was the highest, and it became low at the elevation of 750 m (population 2), 980 m (population 3) and 1250 m (population 4), which shows that elevation had a close relation to genetic variation of *B. luminifera*, and the genetic variation level increased with the decrease of elevations. In this study, the change of genetic diversity *B. luminifera* may be a consequence of adaptation to the microtopography. And the effects of geographical distances and elevations on the genetic variation of *B. luminifera*

Table 6 Pearson correlation analyses for the relationships between genetic diversity parameters within populations of *B. luminifera* and ecological factors

ecological factors	genetic diversity parameters				
	PPL	N_a	N_e	Shannon's index of diversity (*I*)	Nei's gene diversity (*h*)
elevation	-0.993^{**}	-0.996^{**}	-0.989^{*}	-0.999^{**}	-0.997^{**}
pH	−0.482	−0.570	−0.365	−0.522	−0.425
annual average temperature	0.921	0.939	0.952^{*}	0.945	0.970^{*}
annual precipitation	−0.899	−0.886	-0.953^{*}	−0.906	-0.951^{*}
total nitrogen	−0.938	-0.965^{*}	-0.950^{*}	-0.964^{*}	-0.975^{*}
C/N ratio	-0.974^{*}	-0.996^{*}	-0.955^{*}	-0.990^{*}	-0.976^{*}
organic matter	-0.952^{*}	−0.935	-0.987^{*}	-0.952^{*}	-0.982^{*}

*, **: significant at 0.05 and 0.01 levels, respectively.

Table 7 Mantel test between matrix of Nei's unbiased genetic distances and matrixes of divergence of ecological factors of different elevations distances at *B. luminifera* populations

divergence of ecological factors of different elevation	genetic distances among different *B. luminifera* populations	
variable	correlation coefficient (r)	level of significance (p)
elevation	0.612	0.044*
pH	0.681	0.176
annual average temperature	0.240	0.344
annual precipitation	0.836	0.108
total nitrogen	0.727	0.042*
C/N ratio	0.873	0.049*
organic matter	0.725	0.037*

*, significant at 0.05 level.

might increase if we enlarge the study area. Of course, further experiments are needed to support this hypothesis.

Pearson correlation analysis further revealed that the change rule of genetic diversity had a close relation to the ecological factors of *B. luminifera* at different elevations. The genetic diversity within population was significantly or very significantly related to elevation, climate factors (annual average temperature and annual precipitation) and soil nutrient factors (total nitrogen, C/N ratio and organic matter), which suggested that the elevation, soil nutrient and climatic factors might play an important role in maintaining the genetic diversity of *B. luminifera*. In our study, the genetic differentiation *B. luminifera* among populations was also significantly correlated to the elevation and climate factors. Therefore, the divergence of elevation and microenvironments influenced not only the genetic diversity of *B. luminifera*, but also the genetic differentiation.

Genetic structure analysis shows that the genetic differentiation percentage of *B. luminifera* at different elevations in Wuyi Mountain was relatively high. Only about 1/3 of genetic variations occurred among populations and most of the variations occurred within populations. The genetic structure among populations of a species was affected by many factors, such as gene mutation, geographic distance, gene flow, genetic drift, natural selection and its biological characteristics (Schaal et al., 1998; Volis et al., 2001). Among them, gene mutation was not often considered as the factor that induced genetic differentiation among populations. To avoid the effect of community edges and gaps, the samples were randomly collected in the *B. luminifera* community, and there were no obvious difference on the geographic distances among the four samples. So, geographic distance was also not the reason that caused the genetic differentiation of *B. luminifera* among populations. Secondly, *B. luminifera* is a heliophilous tree species. Most of them are situated in upper canopy layers of natural community and their seedlings had difficulty surviving in the natural forest, which lead to the fact that most *B. luminifera* were mature trees. Furthermore, the *B. luminifera* natural forests have been seriously destroyed and disappeared from many sites as a result of over utilization and invasion of other species. Therefore, the biological characteristics of *B. luminifera* might be a potential reason that affected the genetic difference of this species. Meanwhile, Volis et al. (2001) pointed out that if the genetic differentiation was caused by genetic drift among populations, then there would be no correlation between genetic differentiation and ecological factors. However, in our study, the genetic variation of *B. luminifera* among populations had significant correlation with ecological factors, which meant that the genetic differentiation of *B. luminifera* could not be affected by genetic drift, but by the natural selection pressure from the microenvironment at different elevations. As a result, except for the biological characteristics, natural selection and the lack of effective gene flow might also affect the genetic difference of *B. luminifera* among populations. Further studies are needed to reveal whether there are some other factors which cause the genetic variation of *B. luminifera*.

Although *B. luminifera* had not been listed as a top conservation plant in China, it is an important economic tree species endemic to China. Therefore, the conservation and further reasonable utilization of the germplasm resources of this species are an urgent task for us. Our results demonstrated that the divergence of elevation and microenvironments had an obvious effect on the genetic diversity and genetic structure of *B. luminifera* in Wuyi Mountain. Consequently, major attention should be paid to the scientific conservation for wild populations of *B. luminifera* at different elevations when strategies for breeding and germplasm conservation will be implemented in the future. Meanwhile, introduction, cultivation and genetic improvement should be carried out for the wild germplasm resources of different elevations, and the new varieties which can adapt to all kinds of ecological environments should be cultivated.

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