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RESEARCH ARTICLE

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Genetic diversity of *Quercus glandulifera* var. *brevipetiolata* populations in three forest communities with different succession stages

Abstract In order to understand the relationship between population succession and its genetic behavior, random amplified polymorphic DNA (RAPD) technique was used to analyze the genetic diversity of *Quercu glandulifera* var. *brevipetiolata* populations in three forest communities with different succession stages (coniferous forest, coniferous and broad-leaved mixed forest, evergreen broad-leaved forest). The results showed that 145 repetitive loci were produced in 60 individuals of *Q. glandulifera* using 11 primers, among which 120 loci were polymorphic, and the total percentage of polymorphic loci was 82.76% with an average of 64.14%. Estimated by the Shannon information index, the total genetic diversity of the three populations was 0.4747, with an average of 0.3642, while it was 0.3234, with an average of 0.2484, judged from the Nei index. Judged from percentage of polymorphic loci, Shannon inform at ion index and Nei index, the genetic diversity followed a decreasing order: coniferous forest > broad-leaved mixed forest > evergreen broad-leaved forest. Analysis of molecular variance (AMOVA) showed that 69.73% of the genetic variance existed within populations and 30.27% of the genetic variance existed among populations. The coefficient of gene differentiation (G_{ST}) was 0.2319 and the gene flow (N_m) was 1.6539. The mean of genetic identity among populations of *Q. glandulifera* was 0.8501 and the mean of genetic distance was 0.1626. The genetic identity between the *Q. glandulifera* population in the coniferous forest and that in the coniferous and broad-leaved mixed forest was the highest. UPGMA cluster analysis based on Nei's genetic distance showed that the population in the coniferous forest gathered with that in the coniferous and broad-leaved mixed forest firstly, then with that in the evergreen broad-leaved forest. The genetic structure of *Q. glandulifera* was not only characteristic of the biological characteristics of this species, but was also influenced by the microenvironment in different communities.

Keywords *Quercus glandulifera* var. *brevipetiolata*, random amplified polymorphic DNA RAPD, genetic diversity, succession

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1 Introduction

Population genetic diversity accumulates in the long-term process of evolution. The developmental history of one species in succession stages is different. When the pioneer species reaches one field, its habitat is new, but light, wetness, competition and feeding plants changes with succession. The offspring would meet other environmental pressures and different genotypes would remain in different succession stages, which results in genetic differences (Cao et al., 2005). Studies on the changes in genetic diversity in different communities and effects of different habitats on genetic diversity are important for the conservation of dominant species and the conservation of degraded ecological systems.

Quercus glandulifera var. *brevipetiolata* is a deciduous tree species belonging to the family Fagaceae. The tree has fine wood quality and strong resistance and is suitable for use as agricultural and architectural material. *Q. glandulifera* is widely distributed in mountains with elevations above 800 m in the Yangtze River Basin. It is one of the main component tree species of deciduous broad-leaved

forests (Editorial Committee of *Zhejiang Flora*, 1992) in mountains of upstream watersheds. The protection of such forests is important for water and soil conservation and water source protection (Editorial Committee of *Zhejiang Forest*, 1993). So far, there have been few reports about *Q. glandulifera* in such aspects as its adaptation to and evolutionary potential in changing environments. In order to understand the adaptability of *Q. glandulifera* to environments, it is necessary to study the genetic diversity of *Q. glandulifera*, which would help narrow the gap in the understanding of genetic diversity of dominant species in these forests and direct forest management, vegetation conservation, habitat reconstruction, utilization and sustainable development of resources.

Among methods used in genetic diversity research, random amplified polymorphic DNA (RAPD) has been widely used in recent years to characterize genetic diversity and genetic structures of woody plants due to its simplicity, rapidness, being economical, using less DNA dosage, and good polymorphism (Massawe et al., 2003; Wang et al., 2004; Wang et al., 2006). Recent studies have shown that RAPD, like AFLP, could be used as reliable molecular markers (Kjølner et al., 2004). In the present study, genetic diversity of *Q. glandulifera* populations in forest communities with different succession stages in the Tiantai Mountain of Zhejiang Province was analyzed using RAPD technology. Our objective is to understand the effect of succession on genetic diversity of *Q. glandulifera.*

2 Materials and methods

2.1 Materials

Tiantai Mountain lies in the central subtropical zone (29°09′–29°28′N, 120°50′–121°24′E) and the vegetation is composed mainly of evergreen broad-leaved forests (Jin, 1998). In May 2002, in different stages of community succession, three types of communities, i.e., coniferous forest, coniferous and broad-leaved mixed forest, and an evergreen broad-leaved forest in the Shiliang Scenery Region in Tiantai Mountain were selected for investigation. The coniferous forest is the *Pinus massoniana* forest, which is characterized by a simple community structure, supreme dominance of *P. massoniana*, low canopy density, sufficient understory light, and dense understory plants. Heliophilous tree species such as *Schima superba* and *L. harlandii* occur. Dominant species in the coniferous and broad-leaved mixed forest communities are *P. massoniana* and some broad-leaved trees. Heliophilous tree species in the coniferous forest developed and became the dominant species in the tree layer, and then the coniferous forest evolved toward a mixed one. The evergreen broad-leaved forest is the subtropical zonal vegetation. During the development of the mixed forest, the canopy density of the community decreased and the mesophytic species, such as *Castanopsis eyrei*, emerged and took the place of the heliophilous species to form an evergreen broad-leaved forest with mesophytic species as the main component. These three types of communities all existed in Tiantai Mountain, representing three different succession stages of forest communities. Because of the short distances among them, their environmental factors are identical.

The sampling site in the coniferous forest is 800 m away from that of the coniferous and broad-leaved mixed forests, and that in the broad-leaved mixed forest is 600 m away from that of the evergreen broad-leaved forest, and that in the evergreen broad-leaved forest is 1300 m away from that of the coniferous forest. We used D1, D2, D3 to represent *Q. glandulifera* populations in, respectively, the coniferous forest, the coniferous and broad-leaved mixed forest, and the evergreen broad-leaved forest. Fresh tender leaves were collected randomly from 20 adult trees, over 30 m apart from each other. The leaves were kept at 4°C and were taken back to the laboratory. Leaves were washed and stored at −70°C for future DNA extraction.

2.2 Methods

2.2.1 DNA extraction and quantification

DNA was extracted following the optimized sodium dodecyl sulfate (SDS) method (Li et al., 2002) and electrophoresis was then run on a 0.8% agarose gel and photographed on a GIS-2008 gel imaging and analysis system (Shanghai Tanon Science & Technology Co., Ltd.). The DNA concentration was determined and stored at –20°C for RAPD amplification.

2.2.2 Primer selection

Random primers were purchased from Sangon Inc., Shanghai, China. Eleven primers with stable and reproducible bands were used for the PCR amplification of all samples. The primer sequences are listed in Table 1.

Table 1 Sequences of 11 random primers used in the RAPD analysis

primers	sequences (5′–3′)
S370	GTGCAACGTG
S335	CAGGGCTTTC
S112	ACGCGCATGT
S333	GACTAAGCCC
S327	CCAGGAGGAC
S306	ACGCCAGAGG
S69	CTCACCGTCC
S94	GGATGAGACC
S309	GGTCTGGTTG
S81	CTACGGAGGA
S380	GTGTCGCGAG

2.2.3 PCR amplification and products identification

After optimization, the reaction system was determined as follows: 1×Taq polymerase buffer (10 mmol/L Tris-HCl, pH 9.0, 50 mmol/L KCl, 0.1% Triton X-100), 0.15 mmol/L each of dATP, dCTP, dGTP or dTTP, 0.75U Taq DNA polymerase (Huamei Inc., Shanghai, China), 10 ng template DNA, 3.6 pmol primer (Sangon Inc., Shanghai, China), 1 μg/μL bovine serum albumin (BSA) in a total of 10 μL reaction volume. The determined optimal PCR cycle program included an initial 5 min denaturation at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 40°C and 1.5 min at 72°C, and 5 min final extension at 72°C. The amplification reaction was performed in a P×2 Thermal Cycler (Thermo Hybaid, Inc.). The PCR product was electrophoresed in a 1.4% agarose gel (stained with 0.5 μg/mL ethidium bromide). The electrophoresis buffer was 0.5×TBE. Images were photographed with a GIS gel imaging and analysis system (Shanghai Tanon Science & Technology Co., Ltd.).

2.2.4 Data analysis

λDNA/*Eco*R I + *Hind* III molecular standard markers were referenced to estimate the molecular weight. A data matrix of RAPD profiles was obtained based on the locations of the reaction products. The presence of the fragment was represented with "1" and the absence with "0". The matrix was used as the input for POPGENE32 software (Yeh and Boyle, 1997). The percentage of polymorphic loci (*P*), Shannon's information index (*I*), Nei's gene diversity (*h*), genetic differentiation coefficient (G_{ST}), genetic identity and genetic distance between populations were estimated. Gene flow (N_m) was estimated according to the G_{ST} value. According to the genetic distance between populations, unweighed pair group method arithmetic average (UPGMA) cluster analysis was performed to demonstrate relationships among populations. At the same time, analysis of molecular variance (AMOVA) software (1.55 edition) was used to analyze the distribution of genetic variation within and among populations (Excoffier et al., 1992; Zhang and Ge, 2002).

3 Results

3.1 Genetic diversity of *Q. glandulifera* populations

Eleven random primers were used for RAPD analysis of a total of 60 individual DNA samples from the three *Q. glandulifera* populations. A total of 145 repetitive loci were produced with an average of 13.2 bands per primer, among which 120 loci were polymorphic and the total percentage of polymorphic loci was 82.76% with an average of 64.14%. The percentage of polymorphic loci of the D1 population was the highest, that of the D2 population took the second place and that of the D3 population was the lowest. Estimated from *P, I* and *h*, the genetic diversity of *Q. glandulifera* in the three succession stages, ranked in decreasing order, was coniferous forest > coniferous and broad-leaved mixed forest > evergreen broad-leaved forest. This indicated that the genetic diversity of *Q. glandulifera* changed regularly in the three succession stages. Light is adequate in the coniferous forest, which benefits the growth of *Q. glandulifera*, and the genetic diversity of *Q. glandulifera* is highest in the coniferous forest. So it is necessary to create adequate light through moderate thinning in the management of the *Q. glandulifera* forests.

Table 2 Genetic diversity of the three populations of *Q. glandulifera*

populations	D1	D2	D3	average	total
number of samples	20	20	20	20	60
total number of loci	145	145	145	145	145
number of polymorphic loci	97	94	88	93	120
percentage of polymorphic loci/%	66.90	64.83	60.69	64.14	82.76
Shannon information index	0.3872	0.3596	0.3459	0.3642	0.4747
Nei index	0.2650	0.2436	0.2365	0.2484	0.3234

3.2 Genetic differentiation among *Q. glandulifera* populations

The total genetic diversity of *Q. glandulifera* estimated by *I* was 0.4747, among which 76.72% of genetic variance existed within populations and 23.28% among populations. The total genetic diversity of *Q. glandulifera* estimated by *h* was 0.3234, and the coefficient of gene differentiation (G_{ST}) was 0.2319. The gene flow (N_m) estimated from G_{ST} was 1.6539 (Table 3). Analysis of molecular variance (AMOVA) showed that 30.27% of genetic variance was found among populations and 69.73% of genetic variance resided within populations (Table 4), and the genetic variances within and among populations were extremely significant (Φ_{st}= 0.3027, $p < 0.001$), which is consistent with those estimated by *I* and *h*.

3.3 Genetic distance and genetic identity among the three *Q. glandulifera* populations

According to the Nei method (Nei, 1972), genetic similarity and genetic distance were calculated among the three populations of *Q. glandulifera* (Table 5). The genetic similarity among the three populations ranged from 0.8310 to 0.8758, with an average of 0.8501. The genetic similarity between the D2 population and the D3 population was the highest, while that between the D1 population and the D3 population was the lowest. The genetic distance

Table 3 Partitioning of the genetic diversity within and among the three populations of *Q. glandulifera*

Shannon information index		Nei index	
genetic diversity within population, H_{pop}	0.3642	gene diversity within population, H_s	0.2484
total genetic diversity, H_{sp}	0.4747	total gene diversity, H_t	0.3234
ratio of genetic diversity within population, H_{pop}/H_{sp}	0.7672	ratio of gene diversity within population, H_s/H_t	0.7681
ratio of genetic diversity among populations, $(H_{sp}-H_{pop})/H_{sp}$	0.2328	the coefficient of gene differentiation, G_{ST}	0.2319
		gene flow, N_m	1.6539

Table 4 Analysis of molecular variance (AMOVA) within and among populations of *Q. glandulifera*

source of variation	degree of freedom	sum of squared deviations	mean squared deviations	variance component	percentage of total variance/%	*p*-value
among populations	2	282.2333	141.1170	6.3269	30.27	< 0.001
within populations	57	830.9500	14.5780	14.5871	69.73	< 0.001

The *p*-value was estimated after 1000 random permutations.

among populations ranged from 0.1326 to 0.1851, with an average of 0.1626. The unweighed pair group method with arithmetic mean (UPGMA) cluster analysis based on the genetic distances among the three populations (Fig. 1) showed that the D2 and D3 populations gathered together firstly, and then clustered with the D1 population.

Table 5 Genetic identity and genetic distance among the three populations of *Q. glandulifera*

population	D1	D2	D3
D1	–	0.8435	0.8310
D2	0.1702	–	0.8758
D3	0.1851	0.1326	–

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

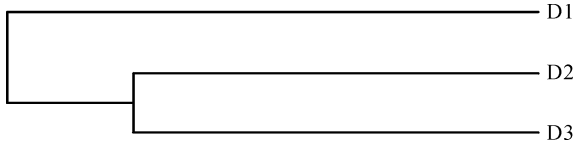

Fig. 1 Cluster figure produced by POPGENE32 soft for the three populations of *Q. glandulifera*

4 Discussion

Genetic diversity of *Q. glandulifera* in the three succession stages, coniferous forest, coniferous and broad-leaved mixed forest, and evergreen broad-leaved forest, was analyzed using a PCR-RAPD technique, and three genetic diversity indices involving *P, I* and *h* were used to describe the genetic conditions of *Q. glandulifera*. It is thought that the genetic diversity at species level is rich when *P* is 50% or so (Liu et al., 2003; Sun et al., 2004; Zhao et al., 2006). In this study, the genetic diversity of *Q. glandulifera* was high, as *P* was 82.76% (more than 50%). It was higher than that of other plants in the same family, such as *Castanea mollissima* ($P = 52.7\%$), *C. sequinii* ($P = 53.7\%$), and *C. henryi* ($P = 44.9\%$) (Shen et al., 2004).

AMOVA showed that, in the total genetic diversity of *Q. glandulifera*, 30.27% of the genetic variance was found among populations and 69.73% within populations. The results estimated using Shannon's information index also showed that genetic variance existed both between and among populations, but mainly within populations. It was mentioned previously that most of the genetic variance of self-pollinated species exists among populations and that of cross-pollinated species within populations (Hamrick and Godt, 1996; Mary et al., 2004). The gene flow of *Q. glandulifera* was 1.6539, which was more than 1, indicating that the gene flow was not inhibited and the genetic differentiation among populations might be due to microenvironmental heterogeneity. The adaptability of different genotypes in different microenvironments differs, and individuals with the same genotype cluster together in a suitable microenvironment to present genetic differentiation. The results in this study showed that, estimated from *P, I* and *h*, the genetic diversity of *Q. glandulifera* in the three succession stages followed a decreasing trend: coniferous forest > coniferous and broad-leaved mixed forest > evergreen broad-leaved forest. This might be the result of the adaptation of *Q. glandulifera* to different microenvironments. In the early stage of succession, canopy density of community is low and the understory light is sufficient. As a heliphilous tree species, *Q. glandulifera* could be well adapted to such a habitat and show high genetic diversity. As the succession develops, however, canopy density increases gradually and the suitable habitat is narrowed, which could lead part of *Q. glandulifera* to be ruled out from the community and parts of the gene to be lost, showing decreased genetic diversity. At the late stage of succession, i.e., evergreen broad-leaved forest, canopy density further increases and the number of residual individuals is reduced further, which results in the lowest genetic diversity.

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