GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genetic variation in and spatial structure of natural populations of *Dipterocarpus alatus* (Dipterocarpaceae) determined using single sequence repeat markers

N.M. Tam[1,2], V.D. Duy[1], N.M. Duc[2], V.D. Giap[3] and B.T.T. Xuan[2]

[1]Department of Experimental Taxonomy & Genetic Diversity, Vietnam National Museum of Nature,
Vietnam Academy of Science and Technology (VAST),
Cau Giay, Hanoi, Vietnam
[2]Department of Molecular Systematics and Conservation Genetics,
Institute of Ecology and Biological Resources (VAST),
Cau Giay, Hanoi, Vietnam
[3]Department of Experimental Biology,
Institute of Natural Products Chemistry (VAST),
Cau Giay, Hanoi, Vietnam

Corresponding author: N.M. Tam
E-mail: ngtam@hn.vnn.vn

Genet. Mol. Res. 13 (3): 5378-5386 (2014)
Received May 29, 2013
Accepted September 18, 2013
Published July 24, 2014
DOI http://dx.doi.org/10.4238/2014.July.24.17

ABSTRACT. *Dipterocarpus alatus* (Dipterocarpaceae) is widely distributed in lowland forests in central and southern Vietnam, Cambodia, Laos, Myanmar, Philippines, Thailand, and India. Due to over-exploitation and habitat destruction, the species is now threatened. The genetic variation within and among populations of *D. alatus* was investigated on the basis of 9 microsatellite (single sequence repeat, SSR) loci. In all, 268 sampled trees from 10 populations in central and southern Vietnam were analyzed in this study. The SSR data showed

a high genetic variability within populations with an average of H_O = 0.209 and H_E = 0.239. Genetic differentiation among populations was high (F_{ST} = 0.266), indicating limited gene flow (N_m = 0.69). Analysis of molecular variance showed that most genetic variation was within populations (74.96%). This study highlights the importance of conserving the genetic resources of *D. alatus* species.

Key words: *Dipterocarpus alatus*; Genetic variation; Species conservation; SSR markers

INTRODUCTION

Dipterocarpus alatus, a species of the Dipterocarpaceae family, is believed to be restricted to central and southern Vietnam, Cambodia, Laos, Myanmar, Philippines, Thailand, and India (Nghia, 2005). This species is bisexual and insect-pollinated. Flowers are large, actinomorphic and scented. Fruiting appears almost every year, with fruit maturation occurring between March and April. The fruit consists of a single-seeded nut with a wing-like calyx. Seeds are dispersed by wind. *D. alatus* is an important timber tree and plays a dominant role in the ecology and economics of lowland rain forests in Vietnam. The wood of *D. alatus* is used for construction purposes such as plywoods, illumination, and waterproofing baskets and boats. Resin is used in paint, varnish, and lacquer. The species is widely distributed in evergreen and dry deciduous forests on the ancient alluvial rocks, granite, and basalt rocks that have low relief and a gentle slope, where water levels rise and fall rapidly during both dry and rainy seasons. The species prefers humidity of 75-85%; precipitation, 1500-2200 mm; mean annual temperature, 25°-27°C; and a dry season lasting 4-6 months.

Due to the exploitation of *D. alatus* for its valuable timber and resin by local people and forestry enterprises, its habitats are heavily affected by deforestation, forest fragmentation, and unsustainable management such as selective logging. Logging results in intense fragmented habitats and low-density populations. This threatens the long-term survival of the *D. alatus* genetic resource.

Conservation and management of a species requires information on the ecological and genetic diversity within and among populations. In order to obtain such information, especially a better understanding of genetic processes, powerful biological techniques are required. Organellar genomes (chloroplast and mitochondrial) are uniparentally inherited. Chloroplast is inherited via pollen, whereas mitochondria are inherited via seeds. Therefore, organellar DNA markers have been used for genetic differentiation between populations. Microsatellite markers (single sequence repeat, SSRs) are useful to analyze the effective pollen flow and seed dispersal within populations. Thus, these markers (high polymorphics) have been used for studies on gene flow, genetic structure, and mating systems (Ujino et al., 1998; Iwata et al., 2000; Takeuchi et al., 2004; Pandey and Geburek, 2009).

The evolutionary potential of a species depends on its genetic variation. Understanding the amount of genetic diversity provides information for the development of conservation strategies and sustainable utilization of a species. The objective of this study was to use SSRs as genetic markers to investigate the level of genetic variability within and between populations of *D. alatus*, and to provide guidelines for the conservation, management, and restoration of this species to the Protection Forestry Department, Vietnam.

MATERIAL AND METHODS

Plant materials

This research was carried out in 10 sites, 4 in Dong Nai Province, and 1 each in Binh Phuoc, Tay Ninh, Ba Ria-Vung Tau, Phu Yen and Dak Lak, and Con Dao Islands (Figure 1; Table 1).

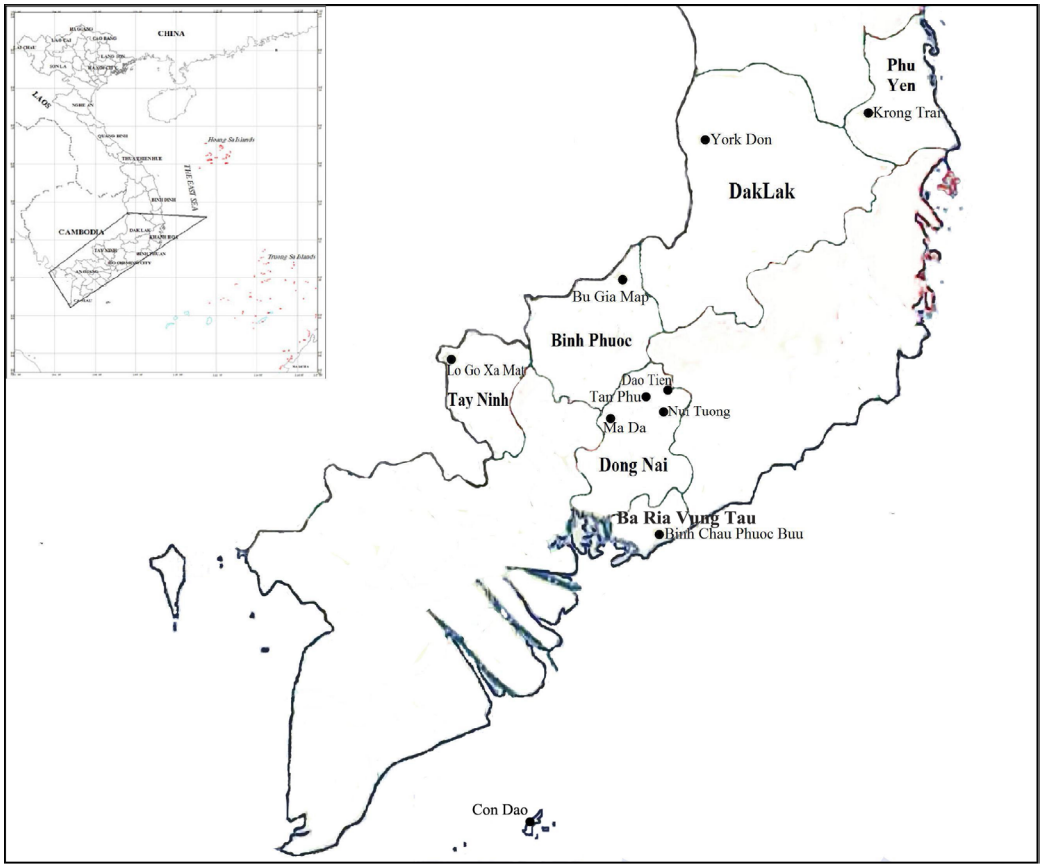

Figure 1. Study sites of *Dipterocarpus alatus*.

The original vegetation at all of these sites has been greatly affected by human activities, including exploitation for commerce, firewood collection, and construction. Some areas of the native vegetations at Lo Go Xa Mat (Tay Ninh), Bu Gia Map (Binh Phuoc), Binh Chau Phuoc Buu (Ba Ria-Vung Tau), and Con Dao Islands have been destroyed because of agricultural expansion. However, three strata also characterize these vegetation structures. These lead to the alteration of the spatial distribution and age class structure of trees in these sites.

Table 1. Collection locations of *Dipterocarpus alatus* sample analysis.

Population	Sample size	Collection locality	Altitude	Latitude	Longitude
Ma Da	32	Vinh Cuu, Dong Nai	129 m	11°12'N	107°09'E
Dao Tien	23	Cat Tien, Dong Nai	120 m	11°44'N	107°27'E
Tan Phu	31	Dinh Quan, Dong Nai	80 m	11°05'N	107°24'E
Nui Tuong	27	Cat Tien, Dong Nai	130 m	11°25'N	107°17'E
Bu Gia Map	33	Bu Gia Map, Binh Phuoc	467 m	12°13'N	107°10'E
Con Dao	29	Con Dao Islands	315 m	8°40'N	106°39'E
Binh Chau Phuoc Buu	19	Xuyen Moc, Ba Ria-Vung Tau	100 m	10°28'N	107°35'E
Lo Go Xa Mat	20	Tan Bien, Tay Ninh	10 m	11°21'N	106°02'E
York Don	30	Buon Don, Dak Lak	230 m	12°49'N	107°34'E
Krong Trai	24	Son Hoa, Phu Yen	158 m	13°03'N	108°51'E

In this study, the inner barks from 19 to 33 mature trees (>25 cm dbh) were randomly sampled for the 10 populations, representing the natural range of *D. alatus* in Vietnam. The samples were placed into plastic bags with silica gel in the field; transferred to Laboratory of Molecular Biology, Vietnam National Museum of Nature; and stored at -30°C until DNA extraction. The samples were identified on the basis of previous taxonomic treatments of collected specimens from these populations.

DNA extraction

Total DNA was extracted from the samples by using the modified CTAB method proposed by Doyle and Doyle (1987). Liquid nitrogen was added to about 100 mg of each sample, which was then ground by hand. Total DNA amount was determined using fluorimetry and then diluted to a concentration of 10 ng/µL.

DNA amplification for SSRs

Polymerase chain reaction (PCR) was performed using 25 µL PCR buffer (Qiagen), 2.5 mM $MgCl_2$, 2 mM of each dNTP, 0.5 pM of each primer, 0.5 U Taq DNA polymerase (Qiagen) and 50 ng template DNA. In all, 9 SSR primer pairs were used in this study (Table 2).

Table 2. SSR loci, primer sequences, marker sizes, optimum annealing temperatures.

Locus	Repeat motif	Primer sequences	PCR product length	Annealing temperature (°C)	GenBank accession No.
Dipt01	$(AG)_{15}$	5'-CTTCCCTAAATTCCCCAATGTT-3' 5'-TAATGGTGTGTGTACCAGGCAT-3'	193	55	Isagi et al., 2002
Dipt03	$(GA)_{24}$	5'-ACAATGAAACTTGACCACCCAT-3' 5'-CAAAAGGACATACCAGCCTAGC-3'	226	56	Isagi et al., 2002
Dipt04	$(AG)_{15}$	5'-TAGGGCATATTGCTTTCTCATC-3' 5'-CTTATTGCAGTCATCAAGGGAA-3'	214	55	Isagi et al., 2002
Dipt05	$(GA)_{25}$	5'-TCTCAAAATCTGCAAAGACAGC-3' 5'-CCATAGTCATCACCTCTAATGGTC-3'	293	55	Isagi et al., 2002
Dipt06	$(TA)_8$	5'-TGGCAAACAAGCTACTGTTCAT-3' 5'-CATGGGTTTAGCAACCTACACA-3'	258	55	Isagi et al., 2002
Dipt07	$(AC)_9$	5'-CAGGAGGGGAATATGGAAAA-3' 5'-AAGTCGTCATCTTTGGATTGC-3'	120	54	Isagi et al., 2002
Dipt08	$(GA)_6$	5'-ATGCTTACCACCAATGTGAATG-3' 5'-CTCGCAGCAGAACAACTTTCTA-3'	170	55	Terauchi, 1994
Shc07	$(CT)_8CA(CT)_5$ CACCC$(CTCA)_3$ $CT(CA)_{10}$	5'-ATGTCCATGTTTGAGTG-3' 5'-CATGGACATAAGTGGAG-3'	169	54	Ujino et al., 1998
Shc11	$(CT)_4TT$ $(CT)_5$	5'-ATCTGTTCTTCTACAAGCC-3' 5'-TTAGAACTTGAGTCAGATC-3'	166	54	Ujino et al., 1998

The reaction mixture was subjected to amplification in the GeneAmp PCR System 9700 (Applied Biosystems) as follows: an initial denaturing step at 94°C for 2 min, 40 cycles of 1 min at 94°C, 1 min annealing for each primer pair at 54°-56°C, 1 min extension at 72°C, and a final extension for 10 min at 72°C; the samples were then stored at 4°C. The amplification products were separated using a Sequi-Gen GT DNA electrophoresis system. Allele sizes were determined against the internal size standard and on QIAxcel Advanced System.

DNA analysis

Genetic parameters were calculated using the Arlequin 3.1 program (Excoffier et al., 2005), including the number of alleles (N_A) per locus, observed (H_O) and expected (H_E) heterozygosities, the coefficient of excesses of homozygotes or heterozygotes compared with panmictic expectations within populations (F_{IS}), and the genetic differentiation (F_{ST}) between populations. Exact tests of deviation from the Hardy-Weinberg equilibrium for all loci and among populations were performed at the significance level (P) = 0.05. F_{ST} values were also used to estimate the levels of gene flow between populations. Significance testing for variance components in analysis of molecular variance (AMOVA) was implemented on the basis of 1000 permutations. Genetic distances and identities, and UPGMA cluster analysis of genetic distances were generated to determine the genetic association among populations by using TFPGA (Miller, 1997).

RESULTS

Genetic variation

The 9 SSR markers produced a total of 38 clear bands ranging in size from 100 to 295 bp, across all 268 trees of the 10 *D. alatus* populations. All 9 SSR loci were polymorphic in *D. alatus* species. The number of polymorphic loci varied among populations. The proportion of polymorphic loci was high (100%) in all populations, except 3 populations (77.8% for Con Dao and Binh Chau Phuoc Buu, and 88.9% for Krong Trai). At the species level, 4 alleles were revealed at 3 loci (Dipt03, Dipt04, and Dipt06); 3 at 4 loci (Dipt01, Dipt05, Dipt08, and Shc07); and 2 at Dipt07 and Shc11. The values of genetic diversity included N_A = 2.2, H_O = 0.209, and H_E = 0.239 (Table 2). The most common alleles (allelic frequencies, >0.8) were revealed at 6 polymorphic loci for 3 populations (Dao Tien, Nui Tuong, and Tan Phu); 7 for 5 populations (Bu Gia Map, Ma Da, York Don, Lo Go Xa Mat, and Krong Trai); and 8 and 9 for Binh Chau Phuoc Buu and Con Dao, respectively. At the population level, 4 populations in Dong Nai had higher levels of genetic diversity than the remaining populations (Table 3).

In all the studied populations, except for 3 populations; Ma Da, Tan Phu, and Lo Go Xa Mat, there were positive fixation index values (F_{IS} > 0.1), indicating an excess of homozygotes and inbreeding. Two populations (Bu Gia Map and Con Dao) had positive values (F_{IS} > 0.2) and suggested a remarkable decrease in hetezygogotes within these populations.

Genetic structure

The results of AMOVA revealed that 74.96% of the total variation was due to the dif-

ference within populations. This was higher than the proportion among populations (Table 4). The variation between populations was significant in *D. alatus* (25.04%; P < 0.001).

Table 3. Genetic variation within populations at nine microsatellite loci.

Population	N	N_A	H_O	H_E	F_{IS}
Ma Da	32	2.3	0.281	0.283	0.005
Dao Tien	23	2.5	0.252	0.298	0.157*
Tan Phu	31	2.4	0.279	0.281	0.004
Nui Tuong	27	2.6	0.248	0.291	0.149*
Bu Gia Map	33	2.0	0.181	0.237	0.238**
Lo Go-Xa Mat	29	1.8	0.158	0.166	0.053
Binh Chau Phuoc Buu	19	1.7	0.150	0.171	0.126
Con Dao	20	2.1	0.161	0.205	0.218*
York Don	30	2.3	0.174	0.213	0.187*
Krong Trai	24	1.9	0.213	0.254	0.164*
Mean	26.8	2.2	0.209	0.239	0.124

N = population size; N_A = mean number of alleles per locus; H_O and H_E = mean observed and expected heterozygosities, respectively; F_{IS} = Wright's inbreeding coefficient with *P < 0.05, **P < 0.01.

Table 4. Analysis of molecular variance in *Dipterocarpus alatus* from ten populations.

Source of variation	d.f.	Sum of squares	Variance component	Total variation (%)	P value
Among populations	9	188.660	0.361	25.04	<0.001
Within populations	542	586.345	1.082	74.96	
Total	551	775.05	1.443		

The population pairwise differentiations (generated from AMOVA) indicated that most of the populations were significantly differentiated (P < 0.05). F_{ST} values ranged from 0.053 to 0.492, except for population pairs in Dong Nai Province, which had F_{ST} values from 0.024 to 0.027 (P > 0.05; see Table 5). These low differentiation values suggest gene exchanges between the 4 populations together in the same province. The results of AMOVA showed F_{ST} value of 0.266 and gene flow N_m of 0.69 in the *D. alatus* species.

Table 5. Population pairwise F_{ST} and significant values.

	BC	BG	MD	DT	CD	NT	TP	YD	LG	KT
BC		+	+	+	+	+	+	+	+	+
BG	0.338		+	+	+	+	+	+	+	+
MD	0.460	0.229		-	+	-	-	+	+	+
DT	0.440	0.215	0.026		+	-	-	+	+	+
CD	0.216	0.405	0.489	0.472		+	+	+	+	+
NT	0.439	0.225	0.024	0.027	0.470		-	+	+	+
TP	0.464	0.230	0.025	0.024	0.492	0.025		+	+	+
YD	0.304	0.111	0.292	0.272	0.327	0.277	0.296		+	+
LG	0.327	0.120	0.301	0.278	0.351	0.283	0.305	0.190		+
KT	0.415	0.053	0.263	0.246	0.433	0.257	0.265	0.156	0.167	

BC = Binh Chau Phuoc Buu; BG = Bu Gia Map; MD = Ma Da; DT = Dao Tien; CD = Con Dao; NT = Nui Tuong; TP = Tan Phu; YD = York Don, LG = Lo Go Xa Mat; KT = Krong Trai. Significance level = 0.05.

Genetic distances and cluster analysis

Table 6 shows the pairwise Nei's (1972) genetic distances and genetic identities be-

tween populations. The largest genetic distance (0.298) was found between populations of Ma Da and Con Dao, and the smallest (0.012) between populations of Nui Tuong and Dao Tien. Similar to the results of genetic distances, the largest identity (0.987) was also recorded between populations of Nui Tuong and Dao Tien and the smallest (0.742) between populations of Con Dao and Ma Da. The mean values of genetic distance between the studied populations ranged from 0.012 to 0.298, with an average of 0.125. The mean value of genetic identity between the populations ranged from 0.742 to 0.987, with an average of 0.819. The obtained value of genetic distance indicated low level of differentiation within populations in Dong Nai Province.

Table 6. Nei's (1972) genetic distance (below diagonal) and genetic identity (above diagonal) for *Dipterocarpus alatus*.

	BC	BG	MD	DT	CD	NT	TP	YD	LG	KT
BC		0.839	0.762	0.780	0.898	0.780	0.769	0.904	0.897	0.841
BG	0.175		0.893	0.897	0.843	0.892	0.891	0.959	0.955	0.957
MD	0.271	0.113		0.982	0.742	0.982	0.986	0.864	0.853	0.871
DT	0.248	0.110	0.015		0.761	0.987	0.985	0.872	0.862	0.878
CD	0.111	0.171	0.298	0.273		0.761	0.748	0.899	0.892	0.845
NT	0.248	0.114	0.015	0.012	0.273		0.986	0.870	0.860	0.872
TP	0.263	0.115	0.013	0.014	0.290	0.013		0.860	0.849	0.870
YD	0.101	0.042	0.146	0.136	0.106	0.139	0.151		0.896	0.944
LG	0.108	0.046	0.159	0.148	0.114	0.151	0.164	0.107		0.938
KT	0.173	0.047	0.138	0.131	0.169	0.137	0.140	0.058	0.064	

BC = Binh Chau Phuoc Buu; BG = Bu Gia Map; MD = Ma Da; DT = Dao Tien; CD = Con Dao; NT = Nui Tuong; TP = Tan Phu; YD = York Don; LG = Lo Go Xa Mat; KT = Krong Trai.

An UPGMA dendrogram (Figure 2) revealed genetic relationships among all the populations investigated on the basis of the Nei's (1972) matrix of genetic distances among populations (Table 6). A total of 10 populations were divided into 3 major groups: the first one corresponding to 4 populations in Dong Nai Province and showing a close relationship to each other with the lowest genetic distances (0.012-0.015), the second one corresponding to 4 populations of York Don (Dak Lak Province), Lo Go Xa Mat (Tay Ninh), Bu Gia Map (Binh Phuoc), and Krong Trai (Phu Yen). The 2 remaining populations of Binh Chau Phuoc Buu and Con Dao were clustered together, with high genetic distance (0.111). According to Mantel tests, the correlation between genetic distance and geographic distance was significant ($R = 0.501$; $P < 0.05$).

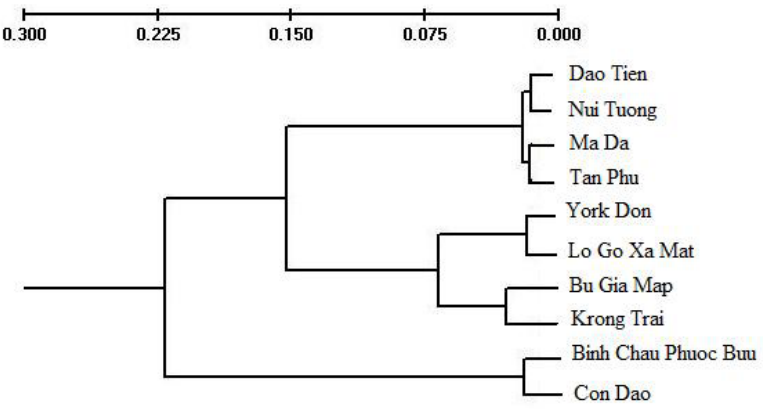

Figure 2. UPGMA dendrogram based on Nei's 1972 genetic distance among the ten populations.

DISCUSSION

Outcrossing species with a great potential for genetic movement will maintain the high levels of genetic diversity within populations and species (Hamrick, 1983; Hamrick and Godt, 1989). Information on the high values of genetic diversity in dipterocarp species such as *Shorea lumutensis* (Lee et al., 2004; Boshier, 2011), *S. leprosula* (Lee et al., 2000; Rimbawanto and Isoda, 2001; Keiya et al., 2001) and population levels was reported previously. Our results showed that *D. alatus* had high levels of genetic diversity within populations, mean H_O = 0.209 (0.150-0.281) and H_E = 0.239 (0.166-0.298). This reflects species ecology and history. The species is regionally distributed, has a long-life, has high fecundity, predominantly outcrosses, pollinated by insects (Appanah and Chan, 1981; Chan, 1981), and late successional. Seeds are dispersed by wind and water (Ashton, 1982). However, some studied populations of *D. alatus* showed lower levels of genetic diversity (e.g., Phu Quoc, Binh Chau Phuoc Buu, Lo Go Xa Mat, York Don, and Bu Gia Map) than the remaining populations (Krong Trai and 4 populations in Dong Nai Province). This might be due to smaller sample sizes, resulting, in part, from human activities (e.g., logging). Small populations might result inbreeding, which can reduce genetic diversity.

AMOVA revealed that most genetic diversity was found within the *D. alatus* populations. The genetic diversity partitioned among populations was significant ($P < 0.001$). The overall degree of population differentiation was higher in *D. alatus* (F_{ST} = 0.266) compared with some other dipterocarp species studied, such as *Dryobalanops aromatica* (G_{ST} = 0.067; Lim et al., 2001), *Shorea leprosula* (G_{ST} = 0.117; Lee et al., 2000) and *Shorea lumutensis* (G_{ST} = 0.048; Lee et al., 2004). Our research showed that 73.4% of the observed genetic variability was contained within populations, and 26.6% was due to the difference among populations. These results are in contradiction with the expectation of low variability among populations of long-lived and outcrossing species (Hamrick and Godt, 1989). The gene flow via limited pollen and seed dispersal plays an important role for these results. Dipterocarp species are insect-pollinated (Appanah and Chan, 1981; Dayanandan et al., 1990). Moreover, dipterocarp species growing in swamps and river banks have fruits with short sepals, and their seeds are dispersed by water. Only populations in Nui Tuong, Dao Tien, Tan Phu, and Ma Da, which were located close to the Dong Nai River showed low and none significant genetic differentiation (F_{ST} = 0.012-0.018, $P > 0.05$). This suggests a high degree of gene flow between these populations and indicates that differentiation in these populations is not due to restricted gene flow. High degrees of differentiation between populations belonging to other provinces, indicates low gene flow ($N_m < 1$). A barrier to gene flow is reflected by significant differentiation (25.04%; $P < 0.001$) in *D. alatus* species.

In conclusion, *D. alatus* maintained the relatively high levels of genetic variability within populations and a high level of genetic population differentiation. From conservation of these species, effective management strategies should be considered for both *in situ* and *ex situ* activities. For example, logging activities should be controlled. Less gene flow resulting from over-exploitation might reduce population viability through, for example, increased inbreeding, genetic differentiation, and genetic erosion by genetic drift.

ACKNOWLEDGMENTS

Research supported by NAFOSTED (#106.15-2011.74).

REFERENCES

Appanah S and Chan HT (1981). Thrips: the pollinator of some dipterocarps. *Malaysian For.* 44: 234-252.

Ashton PS (1982). Dipterocarpaceae. In: Flora Malesiana Series 1 (Van Steenis CGGJ, ed.). Martinus Nijhoff Publishers, The Hague, 9: 237-552.

Boshier D (2011). *Shorea lumutensis*: Genetic Variation and Conservation. A Case Study and Teacher's Notes. In: Forest Genetic Resources Training Guide (Boshier D, Bozzano M, Loo J and Rudebjer P, eds.). Bioversity International, Rome, 13.

Chan HT (1981). Reproductive biology of some Malaysia dipterocarp III. Breeding systems. *Malaysia For.* 44: 28-36.

Dayanandan S, Attygalla DNC, Abeygunasekara AWWL, Gunatilleke IAUN, et al. (1990). Phenology and Floral Morphology in Relation to Pollination of Some Sri Lankan Dipterocarps. In: Reproductive Ecology of Tropical Forest Plants (Bawa KS and Hadley M, eds.). UNESCO, Paris and Parthenon Publishing Group, Carnforth, 103-133.

Doyle JJ and Doyle JL (1987). A rapid DNA isolation procedure for small qualities of fresh leaf material. *Phytochemical Bull.* 19: 11-15.

Excoffier L, Laval G and Schneider S (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* 1: 47-50.

Hamrick JL (1983). The Distribution of Genetic Variation Within and Among Natural Plant Populations. In: Genetics and Conservation (Schonewald-Cox CA, Chambers SM, MacBryde B and Thomas L, eds.). The Benjamin/Cumming Publishing Company, Inc., Menlo Park, 335-348.

Hamrick JL and Godt MJW (1989). Allozyme Diversity in Plant Species. In: Plant Population Genetics, Breeding and Genetic Resources (Brown ADH, Cler MT, Kahler AL and Weir BS, eds.). Sinauer, Sunderland, 43-63.

Isagi V, Kenta T and Nakashizuka T (2002). Microsatellite loci for a tropical emergent tree, *Dipterocarpus tempehes* V. S1 (Dipterocarpaceae). *Mol. Ecol. Notes* 2: 12-13.

Iwata H, Konuma A and Tsumura Y (2000). Development of microsatellite markers in the tropical tree *Neobalannocarpus heimii* (Dipterocarpaceae). *Primer Notes* 1684-1685.

Lee SL, Wickneswari R, Mahani MC and Zakri AH (2000). Genetic diversity of a tropical species, *Shorea leprosula* Miq. (Dipterocarpaceae) in Malaysia: implications for conservation of genetic resources and tree improvement. *Biotropica* 32: 213-224.

Lee SL, Ng KKS, Lee CT, Norwati M, et al. (2004). *Shorea lumutensis*, an endangered dipterocarp with high levels of genetic diversity but low population differentiation. *Conf. For. Forest Prod. Res.* 166-173.

Lim LS, Wickneswari R, Lee SL and Latiff A (2001). Genetic Structure of Natural Populations of *Dryobalanops aromatic* Gaertn. F. (Dipterocarpaceae) in Peninsular Malaysia Using Microsatellite DNA Markers. In: *In-Situ* and *Ex-Situ* Conservation of Commercial Tropical Trees (Thielges BA, Sastrapradja SD and Rimbawanto A, eds.). Gadjah Mada University, Yogyekarta, 309-324.

Keiya I, Irsyal Y, Anto R and Istiana P (2001). Estimation of Genetic Variation of *Drybalanops oblongifolia* Dyer. (Dipterocarpaceae) Planted in Peninsular Malaysia. In: *In-Situ* and *Ex-Situ* Conservation of Commercial Tropical Trees (Thielges BA, Sastrapradja SD and Rimbawanto A, eds.), Gadjah Mada University, Yogyekarta, 377-384.

Miller MP (1997). Tools for Population Genetic Analysis (TFPGA) 1.3: A Windows Program for the Analysis of Allozyme and Molecular Population Genetic Data. Distributed by the author.

Nei M (1972). Genetic distance between populations. *Am. J. Bot.* 72: 1590-1597.

Nghia NH (2005). Dipterocarps of Vietnam. Agricultural Publishing House, Hanoi.

Pandey M and Geburek T (2009). Successful cross-amplification of *Shorea* microsatellites reveals genetic variation in the tropical tree, *Shorea robusta* Gaertn. *Hereditas* 146: 29-32.

Rimbawanto A and Isoda AK (2001). Genetic Structure of *Shorea leprosula* in a Single Population Revealed by Microsatellite Markers. In: *In-Situ* and *Ex-Situ* Conservation of Commercial Tropical Trees (Thielges BA, Sastrapradja SD and Rimbawanto A, eds.). Gadjah Mada University, Yogyekarta, 331-338.

Takeuchi Y, Ichikawa S, Konuma A, Tomaru N, et al. (2004). Comparison of the fine-scale genetic structure of three dipterocarp species. *Heredity* 92: 323-328.

Terauchi R (1994). A polymorphic microsatellite marker from the tropical tree *Dryobalanops lanceolata* (Dipterocarpaceae). *Jpn. J. Genet.* 69: 567-576.

Ujino T, Kawahara T, Tsumura Y, Nagamitsu T, et al. (1998). Development and polymorphism of simple sequence repeat DNA markers for *Shorea curtisii* and other Dipterocarpaceae species. *Heredity* 81: 422-428.