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Research article

Genetic diversity and relationships of *Dipterocarpus alatus* Roxb. (Dipterocarpaceae) on island in freshwater lake and nearby mainland

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Abstract

Importance of the work: *Dipterocarpus alatus* is an economically valuable wood and has been designated as a threatened species.

Objectives: To study the genetic diversity and relationships of *D. alatus* between Don Sawan Island and the nearby mainland for use in developing conservation strategies.

Materials & Methods: In total, 87 accessions of *D. alatus* were analyzed using microsatellite and sequence-related amplified polymorphism (SRAP) markers to investigate the genetic diversity within and among populations of *D. alatus* from Don Sawan Island and six populations from neighboring mainland areas in Sakon Nakhon province, Thailand.

Results: Genetic diversity analysis revealed that the lowest observed heterozygosity (H_o) of *D. alatus* was 0.163 for the Don Sawan Island population compared with populations on the nearby mainland. All tested populations of *D. alatus* had positive values of the inbreeding coefficient, which is a major indication of a decrease in H_o. Thus, the *D. alatus* population on Don Sawan Island is at risk and was assessed as vulnerable due to the loss of conventional genetic diversity. Cluster analysis of *D. alatus* relationships showed coefficients of similarity in the range -0.103 to 0.715. All accessions were clustered into four major groups corresponding to geographical distance.

Main finding: The results should be useful for *D. alatus* conservation in risk areas of extinction and where the species is incapable of increasing genetic diversity solely using natural means. Planting additional trees from outside sources could improve the genetic diversity of *D. alatus* in vulnerable areas.

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Introduction

The Nong Han wetland is a famous freshwater lake in northeastern Thailand; it is the largest in Thailand, with a total area of 123 km^2. This wetland has been described by the Office of Environmental Policy and Planning (2002) as a natural lagoon of international importance, being fed by many steams that flow through to the Mekong River. There are more than 20 islands in this wetland, of which Don Sawan Island is the largest (area approximately 0.24 km^2), being located in the central region of the Nong Han in Mueang district, Sakon Nakhon province. Located in the middle of the Nong Han Lake, it is accessible only by boat. Its land cover varies seasonally. It is surrounded by water all year round, so that many varieties of trees thrive, including large perennial trees for which the area is notable, such as *Dipterocarpus alatus*, *Combretum quadrangulare*, *Streblus asper*, *Shorea siamesis*, *Ficus carica* and *Samanea saman*. In addition, herbaceous plants and mixed deciduous trees with a variety of vines also grow on this island, which is regarded as a native forest area in Sakon Nakhon province (Civil Society Development Institute, 2019). This area is not disturbed by outside activity because it is a conservation area as part of a plant genetic conservation project under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG) in Sakon Nakhon province, Thailand. Additionally, the *D. alatus* trees on Don Sawan Island are an isolated population and older than others on the nearby mainland. One population may be the ancestor of the other or they might share a common ancestor. Since Don Sawan Island is an old island in the Nong Han wetland and is also a fragmented area from mainland, it is an interesting area to study genetic diversity and the relationship of its population of *D. alatus* trees with surrounding populations on the nearby mainland.

The Dipterocarpaceae family contains 17 genera and more than 500 species worldwide (Ashton, 1982). Most of members in this family originate from and are widely distributed in Asia. *Dipterocarpus alatus* Roxb. is often regarded in Southeast Asia as the king of trees because of its large size and although it is originally native to Borneo, it can be found in Bangladesh, Myanmar, Thailand, Laos, Cambodia, Vietnam and Malaysia. In Thailand, *D. alatus* or 'Yang-na' as it is known locally, is found at altitudes in the range 0–350 m above mean sea level (Pooma et al., 2017). *D. alatus* is economically valuable as a versatile wood having both direct and indirect benefits, such as a source of wild food, oleoresin, recreational area enhancement and as a construction material and plywood that is exported to many countries; in addition, oils from *D. alatus* can be applied as boat tar, dammar house varnish base or in herbal mixtures (Dyrmose et al., 2017; Pooma et al., 2017). Yongram et al. (2019) reported that the extracts from its leaves, barks and twigs can inhibit the U937 cancer cell line because of their high antioxidant capacity and total phenolic contents. The distribution and numbers of *D. alatus* at present have been decreasing so noticeably that it is now designated as a threatened species (Tam et al., 2014), partly due to historical carelessness in resource utilization by the forest industries, as well as encroachment of forest areas for agricultural and residential settlement. Consequently, while the distribution of large populations was once widespread, *D. alatus* is now scattered in many small populations that in turn are at risk of loss of genetic diversity and imminent extinction.

The sustainable conservation and management of a valuable resource such as *D. alatus* requires information on its ecology and genetic diversity both within and among its populations. An effective microsatellite or single sequence repeats (SSR) marker has been reported to provide high genetic variation at the population level (Ellegren, 2004) and has been selected to study changes in genetic diversity at the population level, both internally and between populations (Tam et al., 2014; Duc et al., 2016; Wang et al., 2020). In addition, sequence-related amplified polymorphism (SRAP) is a molecular marker that is widely used in the study of plant genetic diversity and is capable of synthesizing DNA from open reading frames (ORF) that is highly reproducible and provides DNA with high polymorphic bands that do not require knowledge of the base sequence information of the organism being studied, as well as being able to test multiple DNA types at once and is easy, fast and relatively low cost (Li and Quiros, 2001). A SRAP marker has been used in forestry research to study genetic diversity and relationships (Liao et al., 2016; Wang et al., 2019; Zagorcheva et al., 2020). Both SSR and SRAP markers were used to support the aim of the current research to study the diversity and genetic relationships of *Dipterocarpus alatus* on Don Sawan Island and the nearby mainland. The results of this study could be used in the evaluation of the genetic resources

and conservation options of *D. alatus* in the future because currently all the mainland stands around Nong-Han wetland are under pressure from urbanization.

Materials and Methods

Study sites and sampling

The plant survey of *D. alatus* was undertaken on Don Sawan Island and six surrounding mainland areas in Sakon Nakhon province within 20 km of the island (Fig. 1 and Table 1): Ban Phan (BP), Ban Muang Lai (ML), Ban Noi Jomsri (NJ), Ban Nong Lad (NL), Phutonphithak temple (PT) and Ban Tao Ngoy (TN). The habitat of *D. alatus* is at altitudes in the range 130–540 m above sea level. *D. alatus* leaf samples without any disease and insect damage from trees which had grown naturally in these seven populations were collected and stored in bags containing silica gel for later DNA extraction.

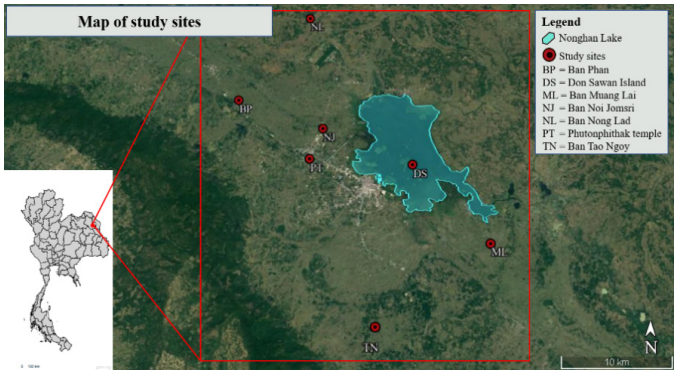

Fig. 1 Geographical location of natural populations of *Dipterocarpus alatus* around Nong Han Lake

DNA extraction

Each genomic DNA sample was extracted from silica gel-dried leaves using a Dneasy® Plant Mini Kit (Qiagen; Valencia, CA, USA), following the manufacturer's protocols. DNA quality was determined using 1% agarose gel electrophoresis and DNA volumes were measured using a DS-11 Spectrophotometer (DeNovix Inc.; Wilmington, DE, USA). The DNA was kept at -20°C for further use.

SSR amplification and genotyping

Eight polymorphic SSR primers were selected following Isagi et al. (2002) and Tam et al. (2014) as shown in Table 2. The amount of DNA was amplified using a polymerase chain reaction (PCR) technique with the GeneAmp® PCR System 9700 (Applied Biosystems Inc.; Foster City, CA, USA). Each sample (20 µL) consisted of a DNA template concentration in the range 30–50 ng, forward and reverse-primers at concentrations of 5 pM and Dream*Taq* Green PCR Master Mix (2x) from Thermo Fisher Scientific (Foster city, CA, USA). The PCR reaction conditions were: 1) pre-denaturation at 94°C for 5 min for 1 cycle; 2) 40 cycles of denaturation at 94°C for 45 sec, an annealing temperature for 45 sec according to Table 3 and then extension at 72°C for 45 sec; 3) extension at 72°C for 5 min; and 4) preserved reactions until removal at 4°C. At the end of the PCR process, the product was separated by size using capillary gel electrophoresis with the QIA*xcel Advanced System* and QIA*xcel* DNA kits (Qiagen; Hilden, Germany) and the PCR fragments were determined using the QIA*xcel* ScreenGel software version 1.6 (Qiagen; Hilden, Germany).

Table 1 Collection areas of *Dipterocarpus alatus* accessions

Locality	Population code	Sample size	Altitude (m)	Latitude (°N)	Longitude (°E)
Ban Phan	BP	18	155-179	17.40361	104.01833
Don Sawan Island	DS	16	410-540	17.18167	104.19000
Ban Muang Lai	ML	14	158-179	17.11111	10425778
Ban Noi Jomsri	NJ	12	150-173	17.21536	104.10589
Ban Nong Lad	NL	6	131-173	17.36208	104.10641
Phutonphithak temple	PT	4	160-198	17.18613	104.09398
Ban Tao Ngoy	TN	17	176-184	17.04083	104.15417

Table 2 Single sequence repeat loci, primer sequences, polymerase chain reaction (PCR) product sizes and annealing temperatures

Locus	Repeat motif	Primer sequence	PCR product length (bp)	Annealing temperature (°C)
Dipt01	(AG)15	5'-CTTCCCTAAATTCCCCAATGTT-3'	193	55
		5'-TAATGGTGTGTGTACCAGGCAT-3'		
Dipt03	(GA)24	5'-ACAATGAAACTTGACCACCCAT-3'	226	56
		5'-CAAAAGGACATACCAGCCTAGC-3'		
Dipt04	(AG)15	5'-TAGGGCATATTGCTTTCTCATC-3'	214	55
		5'-CTTATTGCAGTCATCAAGGGAA-3'		
Dipt05	(GA)25	5'-TCTCAAAATCTGCAAAGACAGC-3'	293	55
		5'-CCATAGTCATCACCTCTAATGGTC-3'		
Dipt06	(TA)8	5'-TGGCAAACAAGCTACTGTTCAT-3'	258	55
		5'-CATGGGTTTAGCAACCTACACA-3'		
Dipt07	(AC)9	5'-CAGGAGGGGAATATGGAAAA-3'	120	54
		5'-AAGTCGTCATCTTTGGATTGC-3'		
Dipt08	(GA)6	5'-ATGCTTACCACCAATGTGAATG-3'	170	55
		5'-CTCGCAGCAGAACAACTTTCTA-3'		
Shc07	(CT)8CA	5'-ATGTCCATGTTTGAGTG-3'	169	54
	(CT)5(CTCA)3(CA)10	5'-CATGGACATAAGTGGAG-3'		

Creation of DNA footprints using sequence-related amplified polymorphism markers

The primer combinations were screened to create DNA footprints using the SRAP markers modified from Comlekcioglu et al. (2010), as shown in Table 3; then, the appropriate primer combinations were selected for further study. In total, DNA footprints were created for 87 DNA samples of *D. alatus* using the SRAP markers. Each reaction (20 µL) consisted of a concentration of *D. alatus* (30–50 ng), forward primer (5 pM/µL), reverse primer (5 pM/µL), 10× PCR buffer (2 µL), dNTP (0.2 mM/2 µL), magnesium chloride (25 mM/1.2 µL) and Taq polymerase (0.2 µL). The strands of DNA were amplified on the GeneAmp® PCR System 9700 (Applied Biosystems Inc.; Foster City, CA, USA) using the following steps: initial denaturation at 94°C for 5 min; followed by 40 cycles with denaturation at 94°C for 1 min, annealing at 47°C for 1 min and extension at 72°C for 2 min; and a final extension of 5 min at 72°C. The PCR products were examined using 2.5% agarose gel electrophoresis with Safe Red (3 µL) at 100 V for 150 min; the images were recorded using the gel documentation equipment.

Data analysis

Microsatellite marker data were analyzed for the average number of alleles per locus ($N_{A,}$), the effective number of alleles (N_e), observed heterozygosity (H_o) and the expected heterozygosity (H_e) using the software program, POPGENE 32 version 1.31 (Yeh and Yang, 1999). Allelic richness (A_R), the F coefficient and an inbreeding coefficient within the population (F_{IS}) and inter-population (F_{ST}) were determined using the FSTAT 2.9.3.2 software (Goudet, 2001). Hardy-Weinberg equilibrium testing was performed using the Genepop 3.4 software (Raymond and Rousset, 1995).

Table 3 Forward and reverse primers for DNA footprints using sequence-related amplified polymorphism markers

Forward primers (5'-3')	Reverse primers (5'-3')
Me1: TGAGTCCAAACCGGATA	Em2: GACTGCGTACGAATTTGC
Me2: TGAGTCCAAACCGGAGC	Em4: GACTGCGTACGAATTTGA
Me5: TGAGTCCAAACCGGAAG	Em14: GACTGCGTACGAATTCTT
Me6: TGAGTCCAAACCGGACA	Em15: GACTGCGTACGAATTGAT
Me10: TGAGTCCAAACCGGAAA	Em16: GACTGCGATCGAATTGTC

The DNA footprints obtained previously using the SRAP and microsatellite data were analyzed based on the following rules. If the DNA bands appeared in the same position, substitute with the value 1; otherwise, substitute with the value 0. The resulting values were analyzed for population structure using the STRUCTURE version 2.3.4 software (Pritchard et al., 2000; Falush et al., 2003) based on a Bayesian clustering approach. Setting the admixture model with correlated allele frequencies, the number of groups in the dataset (K = 1-7) were performed with a burn-in period of 1,000 iterations, followed by 20,000 Markov chain Monte Carlo repetitions. In order to determine the optimal value of K, the number of groups that provided the best fit to the dataset (ΔK) was determined, as described by Evanno et al. (2005) using the Structure Harvester (Earl and vonHoldt, 2012). The correlation coefficient and neighbor joining (NJ) clustering were analyzed using the **PAST** (*PA*leontological *ST*atistics) version 2.17c software (Hammer et al., 2001).

Results

Genetic diversity of Dipterocarpus alatus

The results from the microsatellite marker examination of the 87 Yang-na (*D. alatus*) samples in northeast Thailand showed that 7 from 8 markers could increase the DNA fragments (Dipt01, Dipt03, Dipt04, Dipt05, Dipt06, Dipt07 and Dipt08). No null allele was present at any of the 7 positions. In total, 127 alleles were identified from all samples. The population with highest number of alleles (14) was in the primer Dipt04 from Don Sawan Island, while the population with the lowest number of alleles (2) was the primer Dipt05 from Ban Nong Lad (Table 4).

The genetic variation values of the *D. alatus* populations from the different locations are shown in Table 5. The number of alleles per locus (N_A) values of several populations varied from 4.86 in Ban Nong Lad to 9.00 on Don Sawan Island, with an average of 6.82. The average value of the effective number of alleles (N_e) was between 3.27 (NL) and 6.01 (ML) with an overall average of 4.58. The allelic richness (A_R) values ranged from 4.51 (NL) to 6.19 (ML) with an average of 5.31. The observed heterozygosity (H_o) of *D. alatus* populations was between 0.163 (DS) and 0.355 (TN), with an average of 0.237. The expected heterozygosity (H_e) values were between 0.637 (NL) and 0.800 (ML) with an average of 0.733. There were positive values for the inbreeding coefficient (F_{IS}) in all populations of *D. alatus*. The population from Ban Tao Ngoy had the minimum average value of F_{IS} (F_{IS} = 0.559, p = 0.001), while the population from Don Sawan Island had the maximum average value of F_{IS} (F_{IS} = 0.806, p = 0.001).

Genetic variation of *D. alatus* was assessed using the fixation index (F_{ST}), with values between 0.028 (PT and TN) and 0.151 (NJ and NL), as shown in Table 6. The populations of *D. alatus* at the Phutonphithak temple and at Ban Tao Ngoy had the greatest genetic similarity, while the populations at Ban Noi Jomsri and Ban Nong Lad had the greatest genetic distances.

Table 4 Number of alleles of single sequence repeat markers in *Dipterocarpus alatus* populations

Locus	Population (number of alleles)							Total	Polymorphism (%)
	BP (18)	DS (23)	ML (18)	NJ (14)	NL 10)	PT (10)	TN (17)		
Dipt01	8	11	10	5	5	7	6	18	66.67
Dipt03	8	10	9	9	6	5	8	19	63.16
Dipt04	8	14	10	5	4	5	8	22	45.45
Dipt05	5	9	7	3	2	3	4	17	52.94
Dipt06	7	3	3	6	5	4	5	14	50.00
Dipt07	5	7	7	6	5	5	7	13	61.54
Dipt08	9	9	10	11	7	7	12	24	66.67

See Table 1 for definitions of the population abbreviations (BP–TN)

Table 5 Genetic variability within *Dipterocarpus alatus* populations at seven single sequence repeat loci

Population	N	N_A	N_e	A_R	H_o	H_e	F_{IS}
Ban Phan	18	7.14	4.37	5.30	0.170	0.727	0.782
Don Sawan Island	16	9.00	5.58	5.92	0.163	0.797	0.806
Ban Muang Lai	14	8.00	6.01	6.19	0.195	0.800	0.774
Ban Noi Jomsri	12	6.43	4.45	5.20	0.335	0.718	0.563
Ban Nong Lad	6	4.86	3.27	4.51	0.209	0.637	0.709
Phutonphithak temple	4	5.14	3.64	4.71	0.230	0.690	0.702
Ban Tao Ngoy	17	7.14	4.71	5.31	0.355	0.763	0.559
Mean	-	6.82	4.58	5.31	0.237	0.733	0.699
SD	-	1.48	0.98	0.60	0.078	0.637	0.709

N = number of samples, N_A= number of alleles per locus, N_e = effective number of alleles per locus, A_R = allelic richness, H_o = observed heterozygosity, H_e = expected heterozygosity; F_{IS} = inbreeding coefficient

Table 6 Fixation index of *Dipterocarpus alatus* populations

Population	DS	BP	ML	NJ	NL	PT	TN
DS	-	0.110	0.082	0.078	0.124	0.089	0.047
BP		-	0.077	0.098	0.135	0.059	0.081
ML			-	0.092	0.087	0.071	0.050
NJ				-	0.151	0.055	0.030
NL					-	0.126	0.101
PT						-	0.028
TN							-

See Table 1 for definitions of the population abbreviations (BP–TN)

Genetic relationships

The genetic relationships of the 87 *D. alatus* accessions were tested using the SRAP markers for the nine pairs of primers (ME1Em2, Me1Em4, Me2Em14, Me2Em15, Me2Em16, Me5Em15, Me6Em15, Me10Em2 and Me10Em4). In total, there were 219 SRAP bands, in the range 100–3,000 bp with 118 polymorphic bands (53.88%). The number of bands per primer ranged from 12 (Me6Em15) to 44 (Me2Em14). The Ban Tao Ngoy population had the highest number of bands (95), while the Ban Nong Lad population had the lowest (29), as shown in Table 7.

Table 7 Sequence-related amplified polymorphism (SRAP) primers with number of amplified products

SRAP primer	Size range (bp)	Total bands/ primer	Monomorphic bands	Polymorphic bands	Polymorphism (%)	Number of bands in each population						
						BP	DS	ML	NJ	NL	PT	TN
Me1Em2	180–1,000	29	17	12	41.38	6	2	3	11	5	5	11
Me1Em4	250–1,000	24	14	10	41.67	4	3	5	5	3	3	13
Me2Em14	100–2,000	44	18	26	59.09	21	2	13	11	4	6	24
Me2Em15	150–3,000	25	3	22	88.00	10	6	15	10	6	6	16
Me2Em16	130–1,400	29	16	13	44.83	15	7	16	3	1	3	11
Me5Em15	100–2,000	15	6	9	60.00	5	6	3	4	2	2	6
Me6Em15	150–1,400	12	3	9	75.00	2	6	2	2	2	2	3
Me10Em2	100–1,300	27	14	13	48.15	11	9	15	7	3	6	5
Me10Em4	120–1,500	14	10	4	28.57	1	4	1	2	3	1	6
Total	100–3,000	219	101	118	53.88	75	45	73	55	29	34	95

See Table 1 for definitions of the population abbreviations (BP–TN)

The genetic relationships of *D. alatus* populations were analyzed using the combined data of SRAP and SSR based on the NJ method; the coefficients of similarity were in the range -0.103 (NL009 versus PT005) to 0.715 (DS012 versus DS013). All but two accessions of *D. alatus* could be classified into one of four major groups (Fig. 2). Group I was composed of *D. alatus* populations from Ban Muang Lai with 13 accessions, Don Sawan Island with 14 accessions and 2 accessions of Ban Nong Lad. Group II could be divided into two subgroups: Subgroup 1 consisted of populations from Ban Nong Lad (4 accessions) and 1 accession of Phutonphithak temple; Subgroup 2 contained 15 accessions of Ban Tao Ngoy and 2 accessions of Don Sawan Island. Group III comprised of Ban Phan (17 accessions) and Ban Noi Jomsri (1 accession). Group IV consisted of populations from Ban Noi Jomsri (11 accessions), 1 each of Ban Tao Ngoy and Ban Pan, and 3 accessions of Phutonphithak temple. However, 1 accession in each of Ban Tao Ngoy (TN003) and Ban Muang Lai (ML006) could not be clustered into any group.

Population structure

The population structure analysis, using SRAP and the SSR dataset, indicated that the best possible dataset (ΔK) for the 87 *D. alatus* accessions was 6 (Fig. 3) with the value of -4,608.9 for the average ln likelihood, a value of 50,569.1 for the ln likelihood variance and a value of -29,893.4 for the Ln Prob estimator. The bar plot revealed that the seven populations were divided into six clusters: 1) populations from Don Sawan Island, Ban Muang Lai and one of Tao Ngoy were grouped together with red bars; 2) most of the Tao Ngoy population (green bars); 3) some of Ban Phan, Ban Noi Jomsri and the Phutonphithak temple were clustered into the same group with dark blue bars; 4) yellow group contained some of the populations from Ban Noi Jomsri and Don Sawan Island; 5) most of the Ban Phan population (pink bars); and 6) light blue group consisted of each one accession from Don Sawan Island, Ban Muang Lai and Phutonphithak temple.

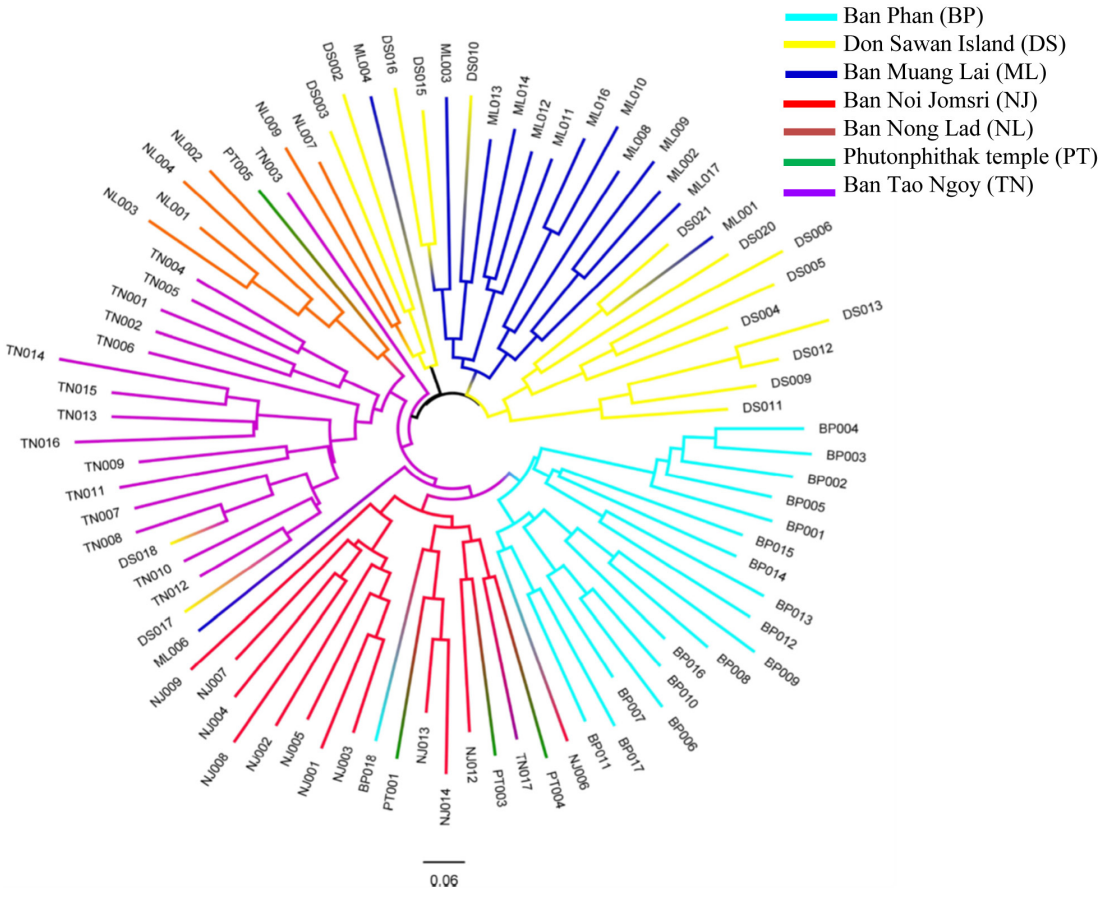

Fig. 2 Dendrogram based on combined data among 87 dipterocarp accessions using neighborhood joining approach

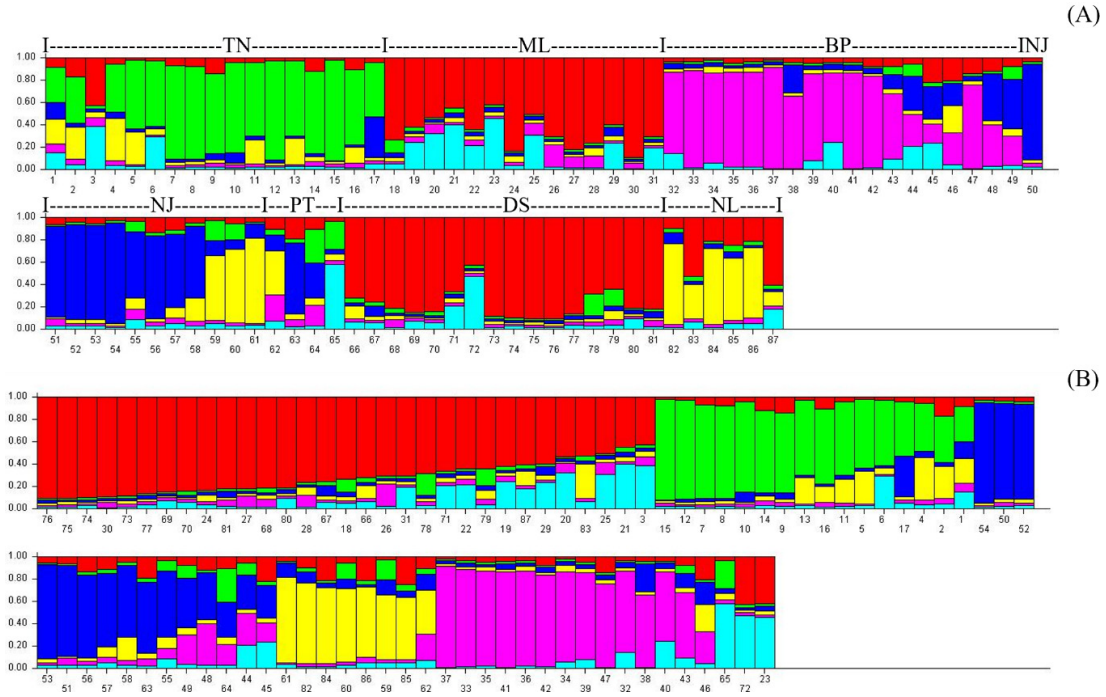

Fig. 3 Population structure of *Dipterocarpus alatus* based on combined data (K = 6): (A) original order based on locality; (B) sorted by Q value

Discussion

Genetic diversity of D. alatus

The Hardy-Weinberg equilibrium testing based on microsatellite data found that all populations were out of equilibrium ($p < 0.05$). The skewing of the equilibrium may have been due to several factors, such as a decrease in heterozygosity or the occurrence of sub-populations or selection (Duc et al., 2016; Huang et al., 2019). Additionally, there were high values from the homozygosity analysis. The examination of linkage disequilibrium showed that all the markers were not linked ($p > 0.05$) indicating that the locus positions selected for this study were independent of each other. The disequilibrium of homozygosity indicated a nonrandom pattern of sizable runs of homozygosity that occurred because of deviation from a random distribution of homozygotes and heterozygotes in the genome (Yang and Lin, 2016).

Analysis of the number of alleles from all samples using microsatellite markers (Table 4) showed that the *D. alatus* populations in this study had more alleles than *D. alatus* in Vietnam based on the same microsatellite markers (Tam et al., 2014; Duc et al., 2016). The total number of alleles per locus (N_A) varied from 4.86 to 9.00 with an average of 6.82, depending on the population. Such values were much higher than the corresponding values in Vietnam N_A = 2.20 (Tam et al., 2014) and N_A = 2.27 (Duc et al., 2016), using microsatellite markers with the same positions. However, most of allele frequencies were low indicating a chance loss of alleles from *D. alatus* populations. Additionally, the average value of H_o (0.237) for *D. alatus* in the current study was higher than the H_o values for Vietnam of 0.209, 0.130 and 0.223 reported by Tam et al. (2014), Duc et al. (2016) and Vu et al. (2019), respectively, based on microsatellite markers. However, the H_o and H_e values in all populations were also low, implying that there was low genetic diversity in the populations. In term of genetic diversity, both heterozygosity and the number of alleles per locus could indicate the potential for population evolution. Heterozygosity is correlated with responses to selection; thus, it is important for short-term adaptation, while allele diversity is associated with long-term adaptation (Allendorf, 1986). The main causes of the low genetic diversity in *D. alatus* could be deforestation, puncture wounds for oleoresin harvesting, urban expansion, increasing area of farmland, small population size and inbreeding within the population (Isagi et al., 2002; Deng et al., 2020). Considering the seven microsatellites, the average values of the effective population size (N_e) were between 3.27

and 6.01 with an overall average of 4.57, which was higher than for the corresponding value in Vietnam of N_e = 1.19 (Duc et al., 2016). These values implied that the number of members in a *D. alatus* population that are reproducible and pass the alleles to the next generation is greater for Thai than for the Vietnamese population.

The population from in TN had the minimum average value of F_{IS}, while the population from Don Sawan Island had the maximum average value of F_{IS}. The highly positive F_{IS} values suggested that all *D. alatus* populations were inbred within the study, which was a major cause of the decrease in the H_o values (Wang et al., 2020). Although the N_e value of the populations of *D. alatus* in this study were higher than for the *D. alatus* population in Vietnam, the genetic diversity of *D. alatus* in Thailand could decrease to a critical status if the resource management of *D. alatus* is haphazard with isolated areas preventing gene flow or pollen transfers (Carvalho et al., 2019). Furthermore, the population from Don Sawan Island had greater allele variation than any other population on the surrounding mainland, which was in agreement with García-Verdugo et al. (2015) who reported that high genetic diversity could be generalized to island plant populations due to their high dispersal ability, high potential for population establishment and persistence, with less human disturbance. The *D. alatus* population on the island are very tall with large stems, indicating they are old and possibly the ancestors of *D. alatus* populations downstream. Compared to the corresponding values for other populations, the H_o value for Don Sawan Island was lower and the F_{IS} value was higher, suggesting a decline in diversity within the population that had occurred from gene flows between the Island and the surrounding mainland, self-pollinating process, the small population and geographical isolation from the mainland (Frankham, 1997; Dwiyanti et al., 2014). Thus, *D. alatus* on Don Sawan Island would be more likely to become extinct in the future.

Genetic relationships and population

The genetic relationships of all the *D. alatus* samples were examined and classified into four major groups and two subgroups. Although Don Sawan Island is an isolated island in the middle of Nong Han Lake and surrounded by water, Ban Muang Lai is located closest to Nong Han being about 2.01 km distant (Fig. 1). Not surprisingly, most of the population on Don Sawan Island were more closely related to the Ban Muang Lai population than to other areas because Ban Muang Lai is located downstream of Nong Han Lake where the water flows into the Mekong River. This would provide an opportunity for some dipterocarp seeds to float along the water channels (Maury-Lechon and Curtet, 1998; Maki et al., 2003) or by distributed by wind up to 2 km (Ashton, 1982). Since the structure of the dipterocarp fruit has a modified wing to help in dispersal, wind is an important mechanism for dipterocarp distribution (Smith et al., 2016). In addition, dipterocarps can be cross-pollinated by various pollinators, resulting in pollen moving long distances (Maury-Lechon and Curtet, 1998). Therefore, two accessions on Don Sawan Island were also related to some populations from Ban Tao Ngoy. These relationships could have resulted from pollen and seed dispersal. However, the populations of Ban Nong Lad, Phutonphithak temple, Ban Phan and Ban Noi Jomsri were isolated from Don Sawan Island because these four populations are in different directions from Ban Muang Lai and Ban Tao Ngoy, at increased geographic distances (Franks, 2010).

Cluster analysis showed the relationship of populations from Ban Phan and Ban Noi Jomsri were closely related because these populations are in the same district, while the *D. alatus* populations from Ban Nong Lad are separated from any group because this area is located in a different district and a long distance from others. Geographically proximate populations are more efficiently connected by gene flow than populations separated by greater distances (Szczecińska et al., 2016). This result supported the research by Deng et al. (2020) that showed that an increase in geographical distance led to increased genetic distinction among populations. The *D. alatus* population at Ban Nong Lad has a relatively higher genetic distance than the other populations ($F_{ST} > 0.100$) except for Ban Muang Lai, because Ban Nong Lad is far away from the other locations and isolated, thus blocking gene transfer by pollen and seeds between areas, as was also reported by Tam et al. (2014) in Vietnam. Furthermore, genetic variation between *D. alatus* in Ban Muang Lai and Ban Nong Lad was also less than for the other studied populations, which could be explained by the increased areas of both *D. alatus* populations due to new plantings or community forests, making it possible for the species to spread by human intervention (Stuessy et al., 2014). These *D. alatus* populations have small, short trunks and hence are relatively younger. A survey in the vicinity of

Ban Nong Lad also found no large, tall *D. alatus* that would have been representative of older trees in the areas.

The results from the STRUCTURE analysis based on a combination of SRAP and SSR data were consistent with the dendrogram using NJ clustering because the STRUCTURE analysis result of $K = 6$ indicated that all populations in this research could be assembled into six groups. Populations on Don Sawan Island and at Ban Muang Lai were formed in the same group, while the five other populations were separated as an individual group. This analysis confirmed that isolation due to distance played a major role in the formation of the present genetic structure of populations (Szczecińska et al., 2016; Perez et al., 2018).

Conservation implication

The microsatellite and SRAP markers used in the current study on *D. alatus* diversity showed that *D. alatus* trees at each location were genetically related and there could have been gene transfer between populations. The average expected heterozygosity (H_o) in the *D. alatus* population on Don Sawan Island was the lowest compared to the surrounding mainland sites. Low heterozygosity decreases evolutionary potential and reproductive fitness (Spielman et al., 2004) and also would increase the risk of *D. alatus* extinction on Don Sawan Island. In addition, discrete geographical regions such as islands often have few species that are low in number, which might have resulted from a single ancestor producing many offspring. Genetic diversity within a small population or on a small island could be decreased due to the loss of rare alleles and an increase in common alleles (Burkey, 1995; Gijbels et al., 2015). Don Sawan is an old island where *Dipterocarp alatus* grows naturally. The older ages of *D. alatus* population on Don Sawan Island could also lead to the risk of original gene pool loss. Thus, these old dipterocarp trees are a valuable source for genetic conservation. Considering plant conservation, if the *in situ* resource cannot increase the genetic diversity its own, the relocation of *D. alatus* from other habitats to Don Sawan Island could provide an opportunity to increase genetic diversity or gene variation in this risk area (Boontawee, 2001). Additionally, an ecological model, such as MaxEnt (Kamyo and Asanok, 2020), could be utilized as a tool to evaluate the distribution, to seek appropriate habitat and to organize a planting project of *D. alatus* in the future.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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