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Population genetic structure and diversity of a critically endangered Ramin [*Gonystylus bancanus* Miq.(Kurz), Thymelaeaceae] from Kalimantan and Sumatra based on Sequence Random Amplified Polymorphism

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Abstract. *Gonystylus bancanus* (Miq.) Kurz (Thymelaceae) or ramin is an important species producing valuable timber. The existence of this species has now under severe threat due to illegal logging and habitat conversion. Ramin is found only on peat swamp forests, scatteredly distributed in Peninsular Malaysia, Sumatra, and Borneo. Genetic diversity of ramin in the Malesian region has been studied previously for Sumatran populations as well as East and West of Malaysian populations. This present study aimed to determine the genetic diversity and population structures of ramin from Sumatra and Kalimantan using Sequence Random Amplified Polymorphism (SRAP). Five combinations of SRAP primers used to amplify seventy seven samples of ramin from eight populations. Eighty-three putative loci of SRAP were scored and analysed using Popgene and MVSP software. Among eight populations studied, NPL were ranged between 14 – 68 with PPL varied at 16.87 – 81.93%. Number of observed alleles and effective allele numbers were ranged between 1.17 – 1.76 and 1.13 – 1.36 respectively. The lowest gene diversity was accounted for population 2 ($H_e = 0.08$) while the highest gene diversity was population 7 and 8 ($H_e = 0.22$). The Sumatran and Kalimantan populations were genetically structured at medium level ($G_{st} = 0.25$). The pairwise genetic distance among Sumatran and Kalimantan population was considered low (0.04-0.07), and is between Sumatran populations (0.01 – 0.17). The results from this study can be used as an essential information to design conservation strategy for ramin in Indonesia.

1. Introduction

Gonystylus bancanus (Miq.) Kurz, known as ramin (Indonesian, Malay), is one of important species of Thymelaeaceae family producing timber. Ramin is found only on peat swamp forests, scatteredly distributed in Brunei Darussalam, Malaysia and Indonesia. In Indonesia, ramin is only found in the island of Sumatra and Kalimantan (Indonesian part of Borneo island) covering 5 provinces of Jambi, Riau, South Sumatra, West and Central Kalimantan [1]. As with many timber species that have been heavily exploited for their economic value, ramin has suffered from the severe threat due to illegal logging and land conversion. Ramin was heavily exploited for its timber between the 1980s and early 2000s [2,1] and continue become target of illegal logging. Ramin has experienced poor natural



regeneration and slow growth rate [3,1]. The natural habitat of ramin is also being at risk due to land clearance and conversion for agriculture and infrastructure as well as forest fire. Nowadays the number of mature trees and natural population are decreasing. Ramin has categorised as critically endangered (CR A2cd, Barstow 2018) following the IUCN Redlist Category and Criteria. The international trade of ramin's timber is regulated under CITES Appendix II. It is estimated that in Indonesia *G. bancanus* timber stock declined by over 90% between 1991 and 2000. Also, across five provinces of Indonesia it was estimated that only 11.3% of the forests original 1983 standing remained. Further to this, forest in this area is estimated to have declines to 46.4% of its original area over the same time period [2]. At present, Ramin's trade is now under moratorium.

While the natural populations are decreasing, significant efforts on conservation of ramin have not yet been properly carried out. Advance studies involving molecular markers for ramin have been studied for the purpose of timber tracking [4, 5, 6]. However, information on the genetic diversity and structure of the species in Indonesia remains lacking except for Sumatra [7]. In fact, population genetic diversity and structure is a fundamental information for the development of appropriate conservation strategy and sustainable forestry management [8, 9]. One of the molecular markers that can be used to determine genetic diversity within and between populations is the Sequence-related amplified polymorphism (SRAP). SRAP is a PCR-based molecular marker targeting coding sequences in the genome and results in a moderate number of co-dominant markers [10, 11, 12], thus suitable for mutant detections as well as assessment on genetic diversity [13, 14]. The main advantage in using SRAP marker is that it can analyze many loci in a single reaction, thus very practical but scientifically reliable markers [15]. This present study was aimed to determine the genetic diversity and population structures of ramin from Sumatra and Kalimantan based on SRAP profiles.

2. Materials and Methods

The ramin samples used in this study that were collected from Sumatra and Kalimantan Island (Figure 1) consisting of seven populations from Sumatra and one population of Kalimantan with various numbers of individual samples (Table 1).

Table 1. List of samples and population of ramin from Sumatra and Kalimantan used in this study.

No	Population	Sample number
1	Palawan ^{*)}	1-4
2	Sungai Kutub Alam Lestari ^{*)}	5-7
3	Blok Palalawan, Blok Konservasi Palalawan, Batas Konservasi, Triomas Serapung ^{*)}	8-10, 26
4	Serapung A, Palalawan Batas ^{*)}	11, 13-19
5	Blok Palalawan ^{*)}	12, 20, 27-28
6	Serapung Tengah ^{*)}	21-25
7	PT Diamond Raya Timber	29-52
8	KDHTK Sungai Penuh, Central Kalimantan	53-77

^{*)}The same samples as Yulita *et al.* [7]

Total genomic DNA was isolated from collected DNA material using Genomic DNA Mini Kit (Plant) from GeneAid. PCR-SRAP amplification of ramin was generated using SRAP set of primers [10]. Ten combinations of SRAP primers were used to amplify the total genomic DNA (Table 2). A total volume of 15 µL PCR reactions consisted of 1x PCR master mix (Promega), ~10 ng DNA template, 2 µM of each primer. The optimum condition for PCR amplification was as followed: 94°C for 5 min, 30 amplification cycles containing 94°C for 1 min, 50°C for 45 second and 72°C for 2 min. The reaction was terminated by extension at 72°C for 5 min. Amplicons were visualized using 2% agarose gel run electrophoretically then stained in GelRed (Biotium) before photographed using gel documentation system (Atto Bioinstrument).

Observations of electrophoretic gel photos showed cleared observable bands to score. Present band was scored 1 and absent band was scored as 0. The data matrix was compiled in Excel software to perform cluster analysis using UPGMA (Unweighted Pair Group Method with Arithmetic Average) [16]. Cluster analysis was calculated using Nei and Li coefficient of distance [17]. UPGMA was performed using MVSP (Multi Variate Statistical Package) [18]. Analysis on population genetic was also performed using PopGen 1.3.1 [19].

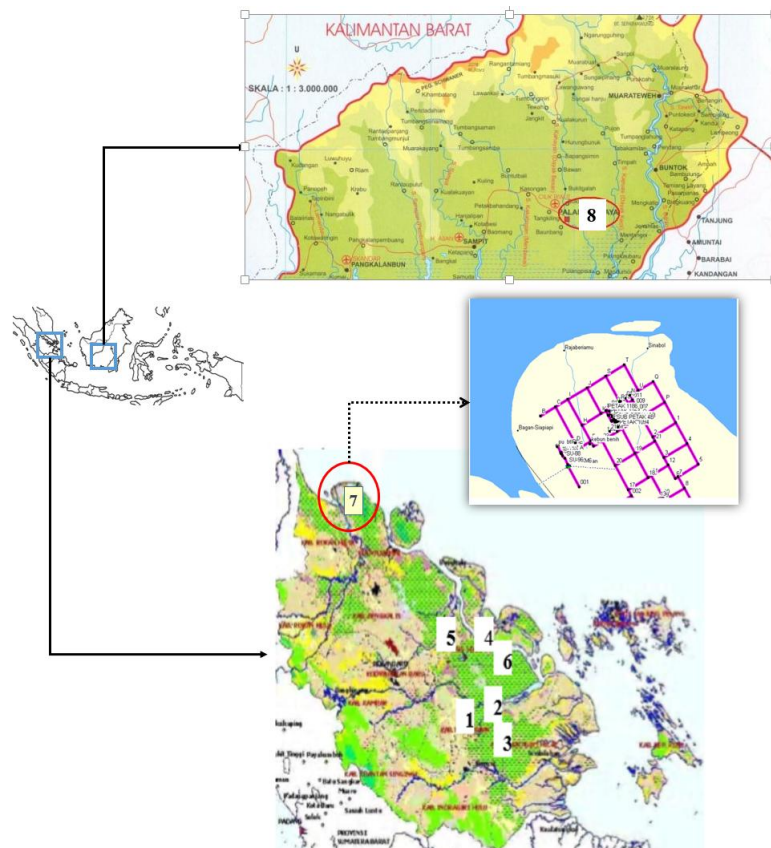


Figure 1. Map of the study site.

Table 2. DNA sequence of SRAP primers used in this study (Li and Quiros 2001).

Primer's name	Sequence
me 1F	TGAGTCCAAACCCGATA
me 2F	TGAGTCCAAACCGGAGC
me 3F	TGAGTCCAAACCGGAAT
me 4F	TGAGTCCAAACCGGACC
me 5F	TGAGTCCAAACCGCAAC
em 1R	GACTGCGTACGAATTAAT
em 2R	GACTGCGTACGAATTTGC
em 3R	GACTGCGTACGAATTGAC
em 4R	GACTGCGTACGAATTTGA
em 5R	GACTGCGCACGAATTGCA

3. Results and Discussion

3.1. Genetic diversity within populations

There was little difference in the observed number of alleles (N_a), effective number of alleles (N_e), percentage of loci polymorphic (PLP), expected heterozygosity (H_e), and Shannon's information index (I) between populations of *ramin* growing in Population 1-6, and population 7 and 8 (Table 3). When examining the value of PLP, N_a , N_e , H_e and I , the Sungai Kutub Alam (population 2) and Palalawan (population 1) had the lowest genetic diversity, H_e was 0.08 and 0.09 respectively. Meanwhile, the Central Kalimantan and PT DRT have the highest genetic diversity, accounted for 0.22 each. These differences may due to the differences of number of samples taken from population 1-6 were much lower than those of population 7 and 8.

Table 3. Genetic diversity parameters in populations of *Gonystylus bancanus* at eight populations of the study site

No.	Population	n^a	PLP ^b (%)	N_a^c	N_e^d	H_e^e	I^f
1	Palalawan	4	21.69	1.21	1.15	0.09	0.13
2	Sungai Kutub Alam Lestari Blok Palalawan, Blok Konservasi Palalawan, Blok Konservasi, Triomas	3	16.87	1.17	1.13	0.08	0.11
3	Serapung	4	31.33	1.31	1.22	0.13	0.19
4	Serapung A, Palalawan Batas	8	37.35	1.37	1.19	0.12	0.18
5	Blok Palalawan	4	25.30	1.25	1.18	0.10	0.15
6	Serapung Tengah	5	27.71	1.28	1.18	0.11	0.16
7	PT DRT KHDTK Sungai Penuh, Central	24	81.93	1.82	1.36	0.22	0.35
8	Kalimantan	25	75.90	1.76	1.36	0.22	0.37
	Mean	9.63	39.76	1.40	1.22	0.13	0.21

Note:

^a n = number of individual.

PLP = percentage of loci polymorphic.

^c N_a = observed number of alleles.

^d N_e = effective number of alleles.

^e H_e = expected heterozygosity.

^f I = Shannon's information index.

The mean PLP value for *ramin* was 39.76%. This number is lower than that of obtained using RAPD for population 1-6 [7] that is of 52.38%. A similar trend of lower PLP was also observed in another timber family like Dipterocarpaceae including *Vatica guangxiensis* (32.46%) [20], *Dipterocarpus retusus* and *D. hassletii* (56.06% and 63.63%, respectively) [21], and *Parashorea chinensis* (20.80%) [22] using RAPD markers. RAPD and SRAP have different nature of polymorphism. SRAP were only contained coding regions (genes) that were more conserved for having slow mutation rate. While amplicons of RAPD contained both coding and noncoding regions, to which the non-coding regions were generally have faster mutation rates than coding regions. Hence, amplicons from RAPD generally have greater polymorphisms than those of SRAP as shown by the other studies [15, 23]. However, the similar pattern of low genetic diversity obtained from this results and other studies using RAPD [20, 22] may also due to the imbalance number of sample size obtained between population 1-6 and population 7 and 8, in which the first populations have much lower number of samples than those of the population 7 and 8.

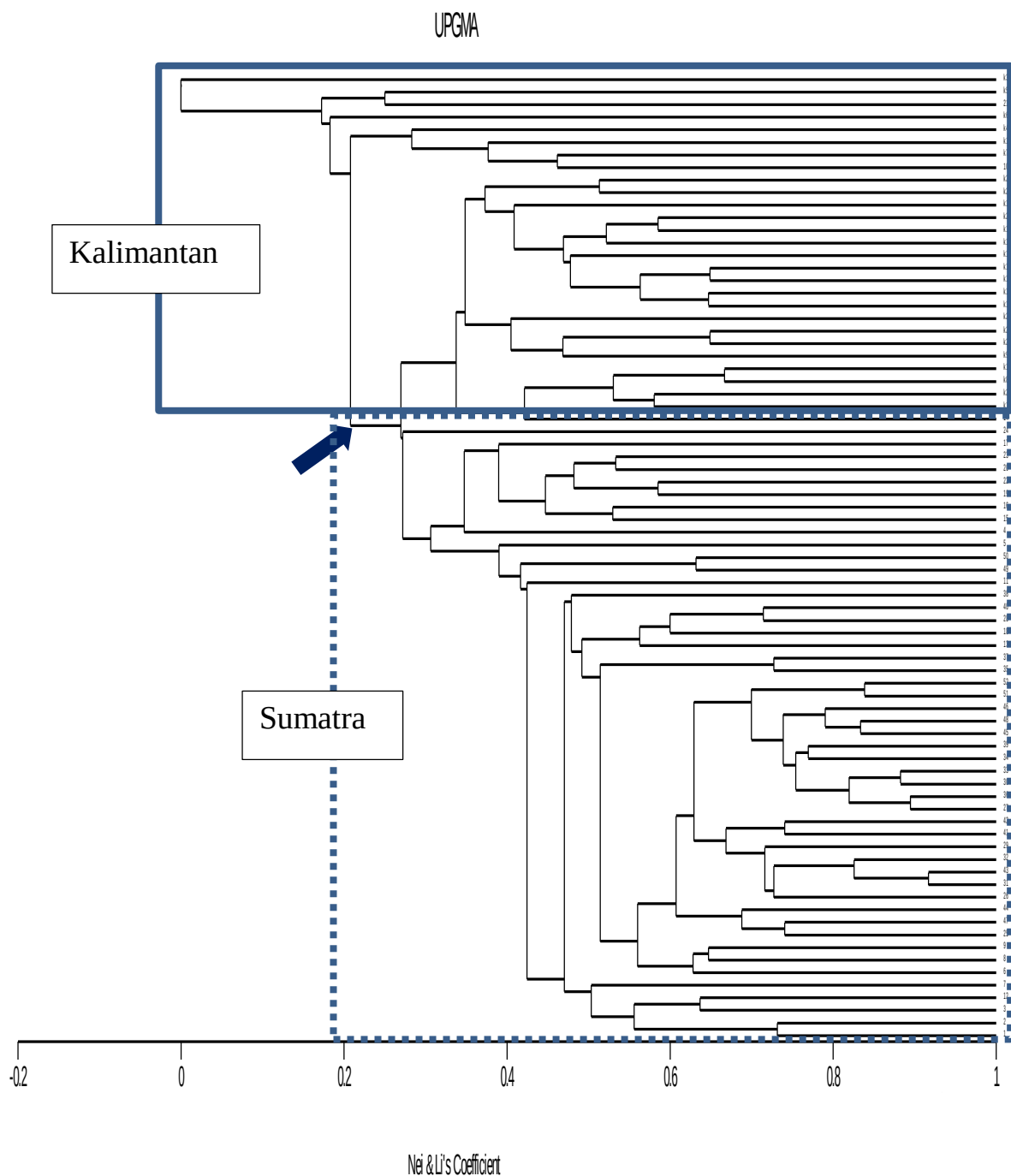


Figure 2. UPGMA dendrogram of 77 samples of ramina from Sumatra and Kalimantan based on SRAP profiles. Solid line box: samples from Kalimantan, dashed line box: samples from Sumatra. The arrow show the node where the Kalimantan and Sumatran populations separated.

Result from the cluster analysis showed that the individual sampel forming groups according to the islands (Figure 2 and 3). The range of similarity coefficient of all individual samples was up to 90% with one sample from Kalimantan (K3) being the most distinct sample. This value indicate a high genetic similarity among the ramina samples reflected from the SRAP profiles regardless the islands boundary.

The two groups (Kalimantan and Sumatra) was separated by the coefficient similarity of about 20%, this means they shared similarity of around 80% of their SRAP profiles (Figure 2). The grouping pattern drawn from the dendrogram indicated that SRAP profile resulted from this study is reliable enough to distinguish populations from the two islands. Meanwhile the grouping within the Sumatran populations showed that the individual samples did not form groups based on the sub-populations. Ramin is deciduous plants, in this case the grouping of the samples with high coefficient of similarity may come from the same mother trees.

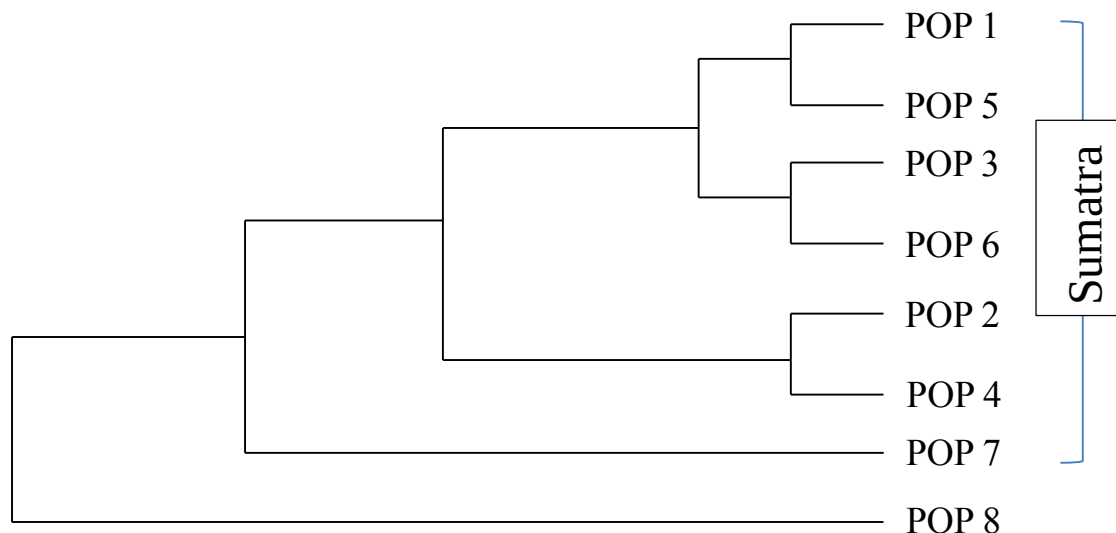


Figure 3. UPGMA dendrogram of eight populations of ramin from Sumatra (Pop 1-7) and Kalimantan (Pop 8) based on SRAP profiles using Nei and Li's coefficient.

3.2. Genetic differentiation among populations

The highest genetic distance observed between population 5 (Blok Palalawan) and 7 (PT DRT). These populations are located within the Sumatra island. Meanwhile, the genetic distance between Kalimantan population and Sumatran population showed considerably low level (Table 4). This can indicate that the genetic distance does not always correspond with the geographical distances as suggested by Schnabel and Hamrick [24] and Alpert et al. [25].

Table 4. Genetic identity (above diagonal) and genetic distance (below diagonal) based on Nei's unbiased measures.

Population	1	2	3	4	5	6	7	8
1	-	0.98	0.98	0.98	0.99	0.98	0.90	0.95
2	0.02	-	0.97	0.99	0.96	0.98	0.90	0.94
3	0.02	0.03	-	0.98	0.98	0.98	0.92	0.96
4	0.02	0.01	0.02	-	0.96	0.97	0.92	0.96
5	0.02	0.04	0.02	0.04	-	0.97	0.89	0.94
6	0.02	0.02	0.02	0.02	0.03	-	0.89	0.94
7	0.11	0.12	0.08	0.08	0.17	0.12	-	0.94
8	0.05	0.07	0.04	0.04	0.07	0.06	0.06	-

The total genetic diversity in all populations (H_t) was 0.18, whereas the average genetic diversity within populations (H_s) was 0.13 (Table 5). By contrast, the genetic diversity between populations (D_{ST}) was 0.05, which is much lower than both H_t and H_s . Genetic differentiation between the populations (G_{ST}) was 25%, which is considered intermediate, whereas gene flow (N_m) between the populations was as low as 1.48 that reflects the restricted pollination system of ramin. As a deciduous plant, ramin has small flowers located at the terminal of peduncle. The stamens have fine little hairs to allow ramin to perform outcrossing easily [3]. A low level of N_m value (1.8424) was also observed in *Dipterocarpus littoralis*, in which pollination is only assisted by insects [7]. Cross-pollination between plants having a small genetic distance or closed relationship can increase homozygosity, in contrast cross-pollination between plants with high genetic distance or distantly related can increase heterozygosity [26]. In addition, the genetic differentiation among populations is also affected by mutation as well as by migration, drift and selection [27].

Table 5. Analysis of gene diversity (Nei, 1987) in sub-divided populations

Sample size	H_t	H_s	D_{ST}	G_{ST}	N_m
77	0.18 ± 0.02	0.13 ± 0.01	0.05	0.25	1.48

In conclusion, ramin had an intermediate level of genetic diversity ($H_e = 0.13$), with the DRT and Central Kalimantan population having the highest levels ($H_e = 0.22$) and the Sungai Kutub Alam Lestari population having the lowest levels ($H_e = 0.08$). Ramin also had an intermediate level of genetic differentiation ($G_{ST} = 25\%$), with restricted genflow among the eight populations. These findings indicated that *in situ* management in combination with enrichment planting in population with a low genetic diversity using locally sourced seeds or other plant materials would be an effective genetic conservation strategy for ramin.

4. Conclusion

Ramin occurred in Indonesia, had an intermediate level of genetic diversity ($H_e = 0.13$), with the DRT and Central Kalimantan population having the highest levels ($H_e = 0.22$) and the Sungai Kutub Alam Lestari population having the lowest levels ($H_e = 0.08$). Ramin also had an intermediate level of genetic differentiation ($G_{ST} = 25\%$), with restricted genflow among the eight populations. These findings indicated that *in situ* management in combination with enrichment planting in population with a low genetic diversity using locally sourced seeds or other plant materials would be an effective genetic conservation strategy for ramin.

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