

Article

High Genetic Diversity of *Shorea acuminata* Dyer in the Rehabilitated Area of a Degraded Lowland Dipterocarp Tropical Rainforest

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Abstract: The United Nation's Decade on Ecosystem Restoration 2021–2030 aims to halt ecosystem degradation to achieve Sustainable Development Goals (SDGs) by 2030. In Malaysia, the concept of sustainable forest management (SFM) has been practiced since 1901. In this study, we evaluated the genetic diversity of the native dipterocarp timber tree *Shorea acuminata* in a rehabilitated area at Kenaboi Forest Reserve (Kenaboi FR). The rehabilitated area was formerly a degraded forest managed with the taungya restoration system for 50 years. All trees with diameter at breast height (DBH) of 5 cm and over were measured, tagged and identified in a one-hectare study plot. A total of 132 inner bark samples were collected for DNA extraction. Four SSR markers (Sle280, Sle392, Sle475 and Sle566) and two EST-SSR markers (SleE07 and SleE16) were used to analyse 95 good-quality DNA samples. Genetic diversity parameters including maternal contribution were determined for 75 samples. The genetic diversity of big trees ($H_e = 0.656 \pm 0.19$) and small trees ($H_e = 0.652 \pm 0.17$) were high and both were in genetic equilibrium, with F_{is} values of the big trees being 0.035 and small trees being 0.164. Clustering analysis based on Jaccard's similarity values (at 95% confidence level) confirmed that big trees in the Kenaboi FR rehabilitated area had originated from genetically diverse seed trees of the Sungai Menyala Forest Reserve which were used as the planting stock for the taungya restoration system. Maternal contribution showed that the allele contribution of the small trees came from the planted *S. acuminata* trees within the study area. The high genetic diversity of small trees in this study provides strong evidence that the existing big trees would be suitable for a genetically diverse seed collection to rehabilitate other degraded forests. Sustainable forest management must emphasise genetic diversity in order to ensure the resilience of rehabilitated forest ecosystems.

Keywords: rehabilitation; taungya restoration system; *Shorea acuminata*; microsatellite markers

1. Introduction

Forests around the world benefit humanity via a wide variety of functioning ecosystems that provide and regulate services such as mitigating climate change and water cycle and erosion control. Forest also provides cultural ecosystem services that people gain from their interaction with forest environmental spaces via activities such as tourism and recreation [1–3]. For Malaysia, the forest is not only an ecosystem service provider but it is also significant as an economic contributor. For example, the Malaysian timber industry is the third-ranked industry after palm oil and rubber in the primary commodities sector.

According to the Malaysian Timber Industry Board (MTIB), the Malaysian timber and timber-related products will achieve RM23 billion for timber export in 2021 [4].

Meanwhile, deforestation and forest degradation keep accelerating and sweeping the world's forest cover. For instance, the world population was estimated to reach 9.7 billion in 2050 and 11.2 billion in 2100 [5]. Five years prior to 2020, it was shown that the total area of tropical forests disappeared at a rate of 9.28 million hectares per year and only 25–30% of all existing tropical forests are old-growth forests [6,7]. In addition to conversion into agricultural land uses, deforestation is also a result of commodity-driven deforestation (27%), followed by logging activities (26%) and wildfire (23%) [8].

By recognising this situation, sustainable forest management is the best concept in balancing the environmental, social and economic objectives that are related to forests while meeting the needs of the present and future generations. Forest restoration and increase in forest areas are needed to meet the SDGs such as economic growth, poverty reduction and global environmental improvement.

Rehabilitation is one of the best silviculture practices in sustainable forest management. The important key to successful rehabilitation is the regeneration of the planting stocks in the degraded forest areas with high genetic diversity. Genetic diversity is an essential basis for the adaptation and resilience of tree species to environmental stress and change [9]. The effort on rehabilitating degraded areas is worthless if the planted trees have a low genetic diversity. According to Kettle [10], the establishment of plantations is increasing, but the genetic quality of the planting stocks has received little research attention. As a result, the rehabilitation areas will lose biodiversity, and the trees are not well adapted to such areas. No study has yet evaluated the genetic variation in restored tree populations [11]. The information about the genetic diversity of trees in the rehabilitated areas can be used as an indicator of long-term restoration success. Unfortunately, such studies are scarce [12–14].

About 250 acres of Kenaboi Forest Reserve (FR), Negeri Sembilan, Malaysia, is a rehabilitated area that has been treated with the taungya restoration system since 1969 [15,16]. The taungya restoration system is an agroforestry practice in Malaysia established since the 1950s to reduce the cost of rehabilitation of a degraded forest by using farmers who conducted forest plantations together with cash crops (banana, tapioca, papaya, pineapple, chili, pumpkin, maize, groundnut, sweet potato, watermelon, yam and ginger). Farmers gained benefits from the cash crops, while the forestry department obtained free labour services for forest plantations [17].

Compartment 107 is one of the degraded areas in Kenaboi FR that was treated by the taungya restoration system. The planting stocks were mainly indigenous species from the wildings of *Shorea* species taken from Sungai Menyala FR, Port Dickson, Negeri Sembilan. The planting stocks were *Shorea leprosula* Miq. (Meranti tembaga), *S. parvifolia* Dyer Ssp. *parvifolia* (Meranti sarang punai) and *S. acuminata* Dyer (Meranti rambai daun). Today, taungya is no longer practised in Malaysia after the new economic policies preferred a variety of industries that were more attractive than cash crop plantations in generating higher income with better assurance [17,18]. A previous study showed that compartment 107 was successfully rehabilitated with great potential for timber production of *Shorea* species [16]. Fatma et al. [19] found that the wildings of *Shorea* trees from Sungai Menyala FR were suitable planting stocks because the compartment was not only successfully rehabilitated but had established a forest structure similar to the primary forest. Fifty years of taungya restoration system showed that the highest tree density in compartment 107, Kenaboi FR was *S. acuminata* with 33.42% out of all trees in the compartment. However, the sustainability of the tree density relies on genetic diversity of the trees.

As mentioned earlier, genetic diversity is vital to ensure the success of the rehabilitation in the restored forest areas. In addition, the information on the genetic diversity can be used to evaluate the effectiveness of the taungya restoration system in compartment 107, Kenaboi FR. Low genetic diversity can threaten the long-term viability of the restored forests. If regeneration and spatial distributions of trees are indicators of a successful forest rehabilitation

in a short term time frame, then genetic diversity is a prediction and proof of the successful adaptation of planting stocks in the rehabilitated area in the long term time frame.

In this study, the genetic diversity of the *S. acuminata* in compartment 107, Kenaboi FR, was evaluated using six microsatellite markers developed for *S. leprosula*. We assumed that the small trees are mostly the regenerated trees from seeds of the reproductively active big *S. acuminata* trees in the study plot. Genetic relatedness between the big and small trees of *S. acuminata* was determined and discussed. Maternal contribution was used to determine the allele contributions of small *S. acuminata* trees in compartment 107, Kenaboi FR. We hope that the results of the study will be useful to improve the policy on silviculture practices for sustainable forest management.

2. Materials and Methods

2.1. Study Plot and Sample Collection

This study was conducted in compartment 107, Kenaboi FR (300–600 m a.s.l), in the district of Jelebu, Negeri Sembilan, Malaysia (Figure 1). The district lies between latitude $2^{\circ} 57' N$ and longitude $102^{\circ} 04' E$. Five subplots with dimensions of 20 m \times 100 m each in compartment 107, Kenaboi FR, were established. The inner bark tissues were sampled from *S. acuminata* trees because the height of the trees made accessibility to the leaves more difficult than the inner barks. The inner barks were wrapped in wet tissues and sealed in labelled plastic bags before being brought to the laboratory. The diameter at breast height (DBH) for each *S. acuminata* trees in one-hectare study plot of compartment 107, Kenaboi FR, was also recorded. In this study, the trees with a diameter of 30 cm DBH and above (≥ 30 cm) were considered as big *S. acuminata* trees, and the trees with diameters below 30 cm DBH (< 30 cm) were small *S. acuminata* trees.

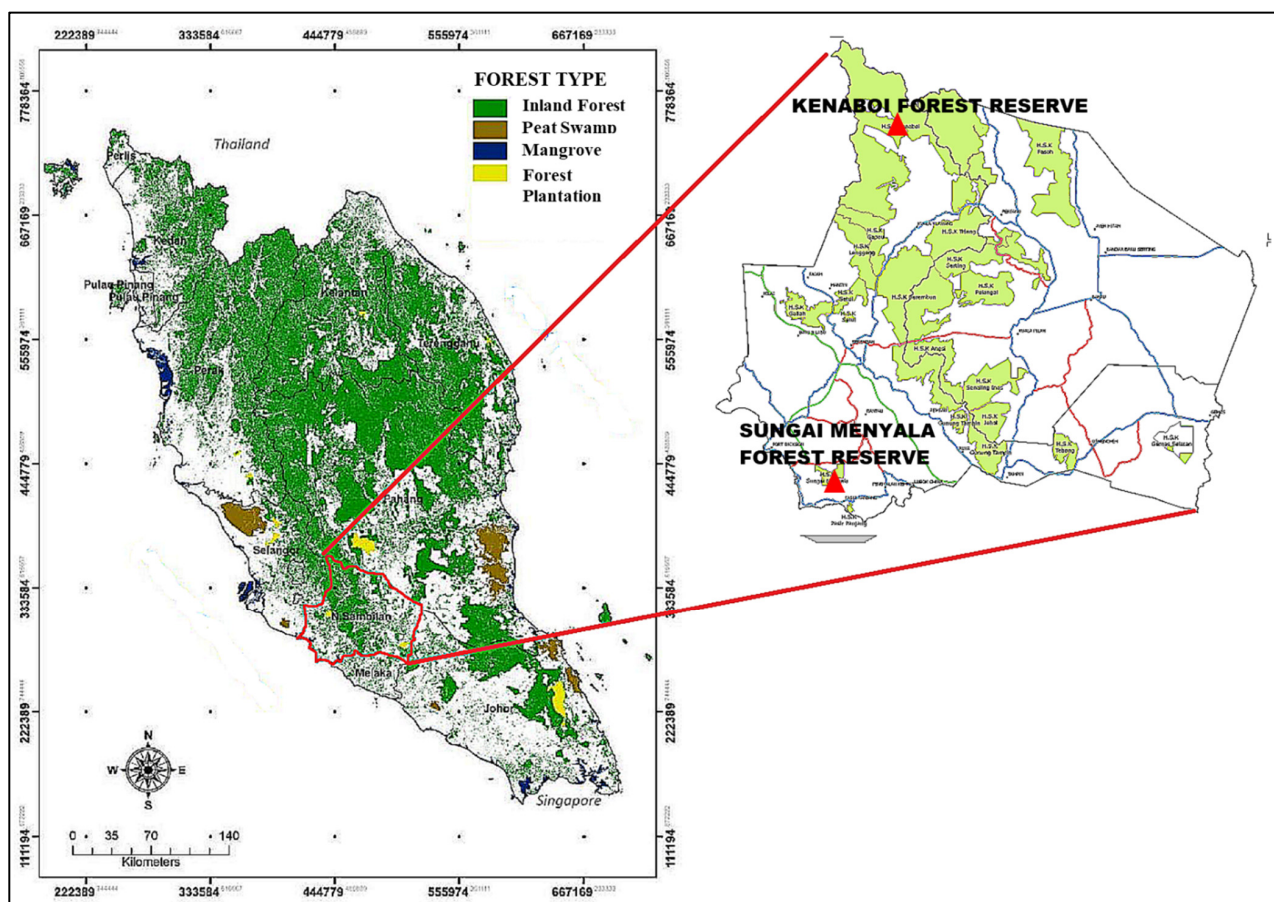


Figure 1. Location of Kenaboi Forest Reserve, Jelebu, Negeri Sembilan, Malaysia. Source: Modified from Forest Department of Negeri Sembilan.

2.2. DNA Extraction and Microsatellite Analysis

A total of 132 inner barks of *S. acuminata* were extracted by using the modified cetyltrimethylammonium bromide (CTAB) method [20]. About 3–5 g of inner bark tissues was ground by using a grinder (Millser IFM 66D, Iwatani, Japan). The samples were genotyped using seven microsatellite loci consisting of four SSR loci (Sle280, Sle392, Sle475 and Sle566 [21]) and three EST-SSR loci (SleE02, SleE07 and SleE16 [22]), all of which were developed for *S. leprosula* (Table 1).

Table 1. Characteristics of four SSR loci and three EST-SSR loci developed from *Shorea leprosula*.

Loci	Accession No.	Primer Sequence (5'-3')	Repeat	Allele Size Range (bp)	Annealing Temp. (°C)
Sle280	AJ616880	F: GCAACTAAAATGGACCAGA R: GAGTAAGGTGGCAGATATAGAG	(CT)7	107–137	52
Sle 392	AJ616886	F: ATGTCCTTGAAGATGTAAAGTGGGTG R: AATAATGGAAGTGAGACGAGGCTG	(GA)11	161–231	55
Sle 475	AJ616888	F: AGCGAAACCTTGTGGAGA R: GAGACTACGGTGGCGACGA	(GA)10	129–139	50
Sle 566	AJ616890	F: TGAGTAACAAGTAATGAGGG R: GCAGAGATTGAAACAGAAG	(GA)13	59–104	52
SleE02	DC649188	F: GGAGGAGAGAAACGAAG R: GTTTGAGGTAGTGAATAACGAGC	(AGC)9	142–160	45
SleE07	DC649404	F: AGAAGAATATGGGTACGACTG R: GTTTGAATCAACTGGCACCTCTAT	(GAA)7	175–190	45
SleE16	DC651058	F: TCGTCAACCTCCGTAGTCC R: GTTTGCGCAATAAATAGAGCAATCA	(CT)12	184–192	45

Microsatellite amplification was performed in a 10 µL reaction volume containing 10 ng DNA, 50 mM KCl, 20 mM Tris-HCl (pH 8.0), 1.5 mM MgCl₂ and 0.2 µM of each primer; 0.2 mM of each dNTP; and 0.5 U of Taq DNA polymerase (Bioline, Heidelberg, Germany). The PCR was carried out in a PCR MasterCycler® (Eppendorf, Germany). For SSR loci, an initial denaturing step at 94 °C for 3 min was carried out, followed by 35 cycles each at 94 °C for 1 min, 52–55 °C for 30 s and 72 °C for 45 s. A final extension step at 72 °C for 30 min was performed after the 35 cycles. For EST-SSR loci, an initial denaturing step at 94 °C for 3 min was conducted, followed by 40 cycles each at 94 °C for 1 min, 45 °C for 30 s and 72 °C for 30 s. A final extension step at 72 °C for 7 min was performed after the 40 cycles.

Genotyping was performed using the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystem/Hitachi, Foster City, CA, USA), and the ABI PRISM® GeneScan software was used to score allele sizes. The total volume of PCR reaction was 25 µL with the same PCR protocol and programme, but the annealing temperature was adjusted accordingly to each primer. Only forward primers of SSR and EST-SSR were selected for fluorescent labelling either with ROX, FAM, TAMRA or HEX. The PCR amplifications were conducted by using microplate 96 well Half Skirt PCR (Axygen, Glendale, AZ, USA) and then sealed by using Microseal 'A' (BIO-RAD, Hercules, CA, USA). Thus, the analysis was only limited to 95 PCR reactions of *S. acuminata* derived from 45 small and 50 big *S. acuminata* trees which were selected for the analysis. The fragment analysis was carried out by preparing the amplifications into two panels in order to avoid overlapping alleles of same size from different loci (Table 2).

Table 2. Two panels consisting of seven microsatellite loci in fragment analysis.

Panel	Loci	Allele Size	Fluorescent	Peak Colour
Panel 1	Sle280	107–137	TAMRA	Yellow
	Sle392	161–231	TAMRA	Yellow
	Sle566	59–104	FAM	Blue
Panel 2	Sle475	129–139	FAM	Blue
	SleE02	142–160	TAMRA	Yellow
	SleE07	175–190	ROX	Red
	SleE16	184–192	HEX	Green

2.3. Data Analysis

The level of genetic diversity in compartment 107, Kenaboi FR, was estimated for polymorphic information content (PIC), observed heterozygosity, expected heterozygosity, Hardy-Weinberg equilibrium and frequency of null allele with the assistance of CERVUS 3.0 [23]. The genetic relatedness of *S. acuminata* trees in compartment 107, Kenaboi FR, was determined by using PAST3 Version 3.22 [24]. The genotype data from seven microsatellite loci were used to estimate similarity index in matrix form ($n \times n$). Jaccard's similarity coefficient was employed to build a dendrogram based on unweighted pair group method with arithmetic means (UPGMA).

Shaharuddin et al. [16] has predicted that the average diameter is 21.21 cm, and the mean annual increment is 1.06 cm per year for *S. acuminata* in Kenaboi FR over the 20 years period of the taungya restoration system. Now, the planted *S. acuminata* is almost 50 years old, and this study has found that the biggest *S. acuminata* tree in compartment 107, Kenaboi FR, is 60.3 cm DBH [19], supporting Shaharuddin et al. [16].

Maternal contribution between big and small *S. acuminata* trees in compartment 107, Kenaboi FR, was estimated by comparing genotype data from four SSR loci and three EST-SSR loci. Alleles at each locus for small *S. acuminata* trees were compared with that of the big *S. acuminata* trees. The big trees which did not share any alleles at each locus were excluded as candidate parents.

The seed trees will contribute the allele for small trees. According to Appanah and Rasol [25], the small dipterocarps trees are able to produce a fruit at 22 cm DBH. However, the study identified that only one out of 22 dipterocarps trees produced fruit at the DBH size from 20 to < 30 cm DBH, which is lower compared to the dipterocarps trees that are 30–125 cm DBH and 11 out of 37 dipterocarps trees are fruiting trees. Therefore, in this study, the paternity study was conducted for big *S. acuminata* trees at three percentages of the biggest diameter size (DBH) trees, i.e., 10% of the biggest *S. acuminata* trees, 20% of the biggest *S. acuminata* trees and 30% of the biggest *S. acuminata* trees. Here, we assumed the biggest trees were the planted trees from the taungya restoration system. On the other hand, small trees of *S. acuminata* are the trees below 30 cm DBH, and they are mostly regenerated trees that were produced by the big *S. acuminata* trees in compartment 107, Kenaboi FR.

3. Results

A total of 123 genomic DNA were extracted from 132 inner barks of *S. acuminata* in compartment 107, Kenaboi FR. Nine of the inner barks were not of good quality due to poor handling in the field. About 48 genomic DNA were from small *S. acuminata* trees, whereas 75 genomic DNA were from big *S. acuminata* trees.

Out of the seven primers used, six primers were found to be polymorphic in the ABI PRISM® GeneScan software. The primers were four SSR loci and two EST-SSR loci. Only one primer showed monomorphic locus, which is SleE02. PCR was successfully amplified for 41 small *S. acuminata* trees and for 34 big *S. acuminata* trees with the mean of alleles being 6.7 and at 6.5 alleles, respectively (Table 3). SleE16 is the most efficient primer to detect polymorphism with the highest PIC, which is 0.813 for big *S. acuminata* and 0.786 for

small *S. acuminata* trees. The lowest PIC is locus Sle280 with 0.309 for big *S. acuminata* and 0.293 for small *S. acuminata* trees.

Table 3. Four microsatellite SSR primers and two microsatellite EST-SSR primers developed for *S. leprosula* and transferability to 75 *S. acuminata* in compartment 107, Kenaboi FR: expected heterozygosity (H_e); observed heterozygosity (H_o); inbreeding coefficient (F_{is}); polymorphic information content (PIC); Hardy–Weinberg (HW); not significant (NS). Figures in the brackets are the estimated standard errors of the mean for genetic diversity parameters.

Locus	Number of Alleles	Allele Size Range (bp)	H_e	H_o	F_{is}	PIC	Null Allele Frequency	HW
Big tree	$n = 34$							
Sle280	4	111–128	0.335	0.324	0.033	0.309	0.05	NS
Sle392	7	175–187	0.784	0.765	0.024	0.738	0.00	NS
Sle475	8	126–187	0.593	0.647	−0.091	0.557	−0.08	NS
Sle566	12	63–114	0.697	0.676	0.030	0.652	0.01	NS
SleE07	5	180–188	0.683	0.794	−0.163	0.606	−0.08	NS
SleE16	9	190–203	0.845	0.529	0.374	0.813	0.23	NS
Mean	6.5		0.656 (± 0.180)	0.623 (± 0.174)	0.035 (± 0.184)	0.613 (± 0.178)		
Small tree	$n = 41$							
Sle280	3	111–128	0.319	0.268	0.160	0.293	0.06	NS
Sle392	6	175–187	0.773	0.78	−0.009	0.728	−0.01	NS
Sle475	7	126–187	0.533	0.39	0.268	0.505	0.13	NS
Sle566	10	63–114	0.765	0.634	0.171	0.725	0.09	NS
SleE07	5	180–188	0.700	0.561	0.199	0.635	0.11	NS
SleE16	9	190–203	0.820	0.659	0.196	0.786	0.10	NS
Mean	6.7		0.652 (± 0.191)	0.549 (± 0.188)	0.164 (± 0.09)	0.612 (± 0.186)		

The expected heterozygous (H_e) was higher than the observed heterozygous (H_o) for both big and small *S. acuminata* trees. The expected heterozygous for big *S. acuminata* is 0.656 and small *S. acuminata* trees is 0.652, whereas the observed heterozygous value for big *S. acuminata* is 0.623 and small *S. acuminata* trees is 0.549. This comparison was not significantly different from the Hardy–Weinberg equilibrium at $p < 0.01$ after sequential Bonferroni correction via CERVUS. The inbreeding coefficient (F_{is}) for the 34 big *S. acuminata* trees is 0.035 lower than F_{is} of the 41 small *S. acuminata* trees, which was 0.164. All of the six microsatellite loci did not significantly deviate from the Hardy–Weinberg equilibrium for both big and small *S. acuminata* trees. Null allele was detected in all loci except for Sle392 for big *S. acuminata* with a frequency range of −0.08 to 0.23. The highest null allele frequency was displayed by locus SleE07, and the lowest null allele frequency was shown by Sle475. For small *S. acuminata* trees, the highest null allele frequency was displayed by locus Sle475 at 0.13, and the lowest was shown by Sle392 at −0.01. According to Marshall et al. [26], the negative value for null allele frequency can be assumed as no existence of null allele. The null allele frequency that is below 0.2 can be used in the analysis of genetic diversity and paternity [27,28]. In this study SleE16 yielded null allele frequency of 0.23, but the locus SleE16 was not prone as null allele in the previous study [22]. On the contrary a high frequency of null allele indicates that the primer used has failed to detect loci in the PCR assay and, thus, cannot be used in genetic diversity and paternity study.

A dendrogram based on UPGMA revealed that 75 individuals of *S. acuminata* in compartment 107, Kenaboi FR, are divided into two main clusters, which are I and II (Figure 2). In cluster I, about 46% of *S. acuminata* consisted of big trees and 54% of *S. acuminata* consisted of small trees, whereas cluster II comprise only a big tree, A11 and two small trees, which are R4 and R98. The Jaccard's similarity coefficient ranged from 0 to 0.889. The highest similarity of 89% was observed, and it was between R52 and R149. No similarity was observed between A11, A37, A40, A67 and A73, respectively, implying that these trees are taken from different seed trees with no shared pollen donors in Sungai Menyala FR. The small tree R4 also does not have similarity with R26, R28, R92 and R148 and also with two big trees, which are A63 and A80. The highest Jaccard's similarity coefficient for regenerated trees is 0.385 for R4 and R98. Therefore, the *S. acuminata* trees in compartment 107, Kenaboi FR are genetically diverse as illustrated in the dendrogram. Figure 3 shows the distribution for all *S. acuminata* trees in compartment 107, Kenaboi FR. The ten big

S. acuminata trees with DBH 50 cm and above are dispersed in the compartment, and the trees could be potential seed trees in this rehabilitated area.

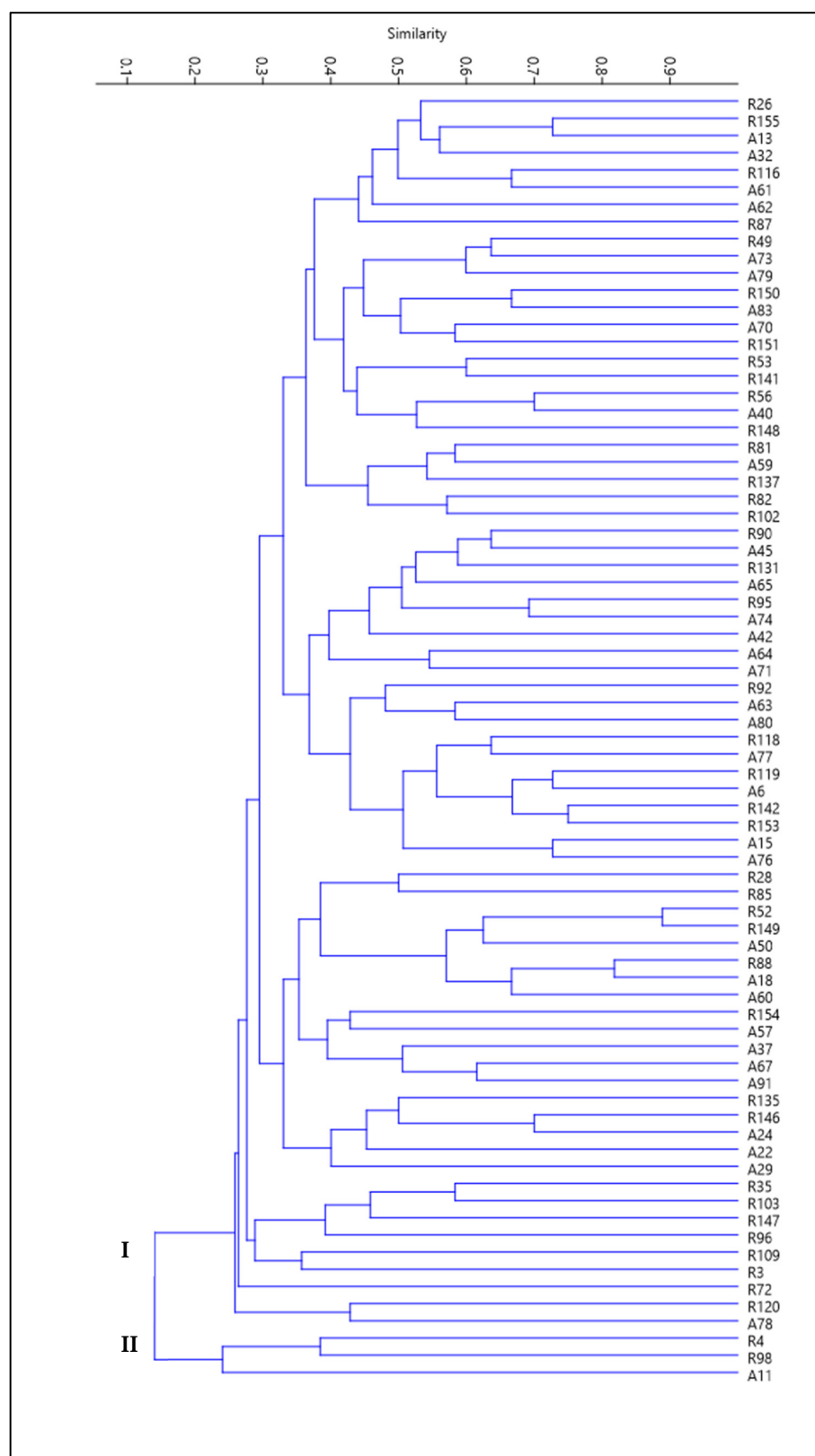
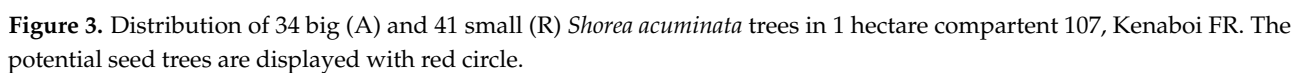


Figure 2. UPGMA cluster based on Jaccard for 34 big *S. acuminata* trees (A) and 41 small *S. acuminata* trees (R) in compartment 107, Kenaboi FR.



The total exclusion probability (the exclusion probability in the case of both parents being unknown [26]) over four SSR loci and two EST-SSR loci for *S. acuminata* is 0.849. From the maternal contribution, when the percentage of the biggest big tree of *S. acuminata* increased, the percentage of allele contribution of small *S. acuminata* from the planted trees in the study plot decreased (Table 4). The mean for percentage of allele contribution from big *S. acuminata* trees outside of the study plot is 7.8%. The allele contribution of small *S. acuminata* from big *S. acuminata* outside of the study plot was 14%, if the big *S. acuminata* is 10% of the biggest trees. The DBH for 10% of the biggest tree was between 52 and 60 cm, which is a small amount of a big tree, and only eight big *S. acuminata* trees were in the study plot. If the big *S. acuminata* was 20% of the biggest trees, i.e., trees between 46 and 60 cm DBH (15 trees), the allele contribution of small *S. acuminata* trees was 9.5% from big *S. acuminata* outside of the study plot. If the big *S. acuminata* was 30% of the biggest tree, i.e., trees between 42 and 60 cm DBH (24 trees), the allele contribution of small *S. acuminata* trees comes from the planted trees in the study plot.

Table 4. Maternal contribution for *S. acuminata* according to percentage of the biggest tree in compartment 107, Kenaboi FR. The underlined allele is probably the allele from big *S. acuminata* in the study plot.

Diameter	Locus	Observed Allele		Tree < 30 cm DBH *	
		Tree ≥ 30 cm DBH	Tree < 30 cm DBH	In Study Plot (%)	Outside Study Plot (%)
Biggest 10% (52–60 cm) <i>n</i> = 8	SLEE07	A, B, C, D	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u>	100	0
	SLEE16	A, B, C, D, E, F	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u> , <u>F</u> , G	86	14
	SLE280	A, B, C	<u>A</u> , <u>B</u> , <u>C</u>	100	0
	SLE392	A, B, C, D, E	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u>	100	0
	SLE475	A, B, C, D	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , E, F, G	57	43
	SLE566	A, B, C, D, E	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u> , F, G	71	29
	Mean			86	14
Biggest 20% (46–60 cm) <i>n</i> = 15	SLEE07	A, B, C, D	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u>	100	0
	SLEE16	A, B, C, D, E, F	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u> , <u>F</u> , G	86	14
	SLE280	A, B, C	<u>A</u> , <u>B</u> , <u>C</u>	100	0
	SLE392	A, B, C, D, E	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u>	100	0
	SLE475	A, B, C, D, E, F	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u> , <u>F</u> , G	86	14
	SLE566	A, B, C, D, E	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u> , F, G	71	29
	Mean			90.5	9.5
Biggest 30% (42–60 cm) <i>n</i> = 24	SLEE07	A, B, C, D	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u>	100	0
	SLEE16	A, B, C, D, E, F, G, H	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u> , <u>F</u> , <u>G</u> , <u>H</u>	100	0
	SLE280	A, B, C	<u>A</u> , <u>B</u> , <u>C</u>	100	0
	SLE392	A, B, C, D, E	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u>	100	0
	SLE475	A, B, C, D, E, F, G	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u> , <u>F</u> , <u>G</u>	100	0
	SLE566	A, B, C, D, E, F, G	<u>A</u> , <u>B</u> , <u>C</u> , <u>D</u> , <u>E</u> , <u>F</u> , <u>G</u>	100	0
	Mean			100	0

* Small *S. acuminata* trees that received alleles.

4. Discussion

The microsatellite markers that were developed for *S. leprosula* were applicable to amplify 75 *S. acuminata* in compartment 107, Kenaboi FR. Other studies mentioned that the markers are able to resolve the genetic parameters in other *Shorea* species as well [21,22,29,30]. The SSR markers are derived from genomic libraries. On the contrary, EST-SSR markers are the sequence derived from expressed and functional sequences that are present in the genome [31]. Thus, this study suggests that the transferability of genomic SSRs and EST-SSRs to closely related species is high due to the conservation of flanking DNA sequences of SSR motifs between closely related species.

The mean expected heterozygosity (H_e) detected in big and small tree *S. acuminata* was 0.656 and 0.652, respectively. These values are almost similar to that reported in a previous study on the same species but in a primary forest where the same SSR markers, i.e., SLE280, SLE392, SLE475 and SLE566, were used, providing a mean expected heterozygosity of 0.676 [29].

Genetic diversity for big and small trees of *S. acuminata* was high although the big trees were wildings from one population in Sungai Menyala FR. This value is considered high compared to other studies, which are 0.616 [28] and 0.811 [32]. The high genetic diversity of *S. acuminata* in the rehabilitated area of Kenaboi FR draws a few possibilities that need to be understood. The possibilities are either that the Sungai Menyala FR is a good seed production area for enrichment planting, or because *S. acuminata* is a desirable species to be used as a planting stock or the effects of the type of silviculture practices may influence the genetic diversity of the rehabilitated area.

Zeng and Fisher [33] claimed that the genetic diversity for native tropical oak in Hong Kong, *Quercus bambusifolia*, is the highest (0.69) when the sources of seeds for the trees are from multiple populations rather than the trees which are naturally regenerated in one rehabilitated forest area. Ang et al. [34] found that the genetic diversity of *S. leprosula* used for enrichment planting was low compared to natural regenerated stands. More reduction

happens if the seedlings were taken from single-species plot compared to mixed-species plot. The genetic diversity value is not significantly different for enrichment planting of *S. parvifolia* either in the permanent forest or other silviculture systems. However, the details of the planting stocks used in this enrichment planting programme are not described [35]. A reasonable explanation for the high genetic diversity of *S. acuminata* in the rehabilitated area of Kenaboi FR is that the planted *S. acuminata* were taken from different seed trees in Sungai Menyala FR. Therefore, when the planting stocks were planted in a degraded area during the taungya system, the area was successfully rehabilitated and contained high genetic diversity after 50 years. The idea that the planting stocks of *S. acuminata* were taken from different seed trees in Sungai Menyala FR was supported by cluster analysis (Figure 2). The average of similarity index of 0.329 indicates that all 75 *S. acuminata* trees are highly diverse. Trees A11, A37, A40, A67 and A73 are the trees that originated from different seed trees in Sungai Menyala FR. Tree A67 is the biggest *S. acuminata* tree in compartment 107, Kenaboi FR; therefore, the tree is the planted tree from the taungya restoration system. This tree also can be suggested as a potential seed tree in the rehabilitated area in addition to the other nine big *S. acuminata* trees, which are A15, A18, A32, A40, A42, A63, A64, A79 and A91 (Figure 3). The highest similarity observed for small trees of *S. acuminata* was between R52 and R149 with DBH of 28 cm and 11 cm, respectively. Tree R52 is most likely a planted *S. acuminata* during the taungya restoration system with stunted growth. According to Shaharuddin et al. [16], the taungya plot was not treated with proper silvicultural treatment until the study was conducted. Therefore, interspecies and intraspecies competition could occur and affect the growth of some planted trees, resulting in some stunted trees. Moreover, a previous study observed that small size dipterocarps are also able to produce fruits [25], and R149 could be a progeny of R52 with 89% similarity between each other.

A maternal study discovered that there are allele contributions in small *S. acuminata* from the big trees that were not in the one-hectare study plot. There is a probability that during the early days of the taungya system, the number of big *S. acuminata* tree in the study plot was limited. Furthermore, the *S. acuminata* species is a predominantly outcrossing species. Thus, mating was between big *S. acuminata* tree in the study plot and outside of the study plot due to less big *S. acuminata* tree in the study plot. Decreased population size may decrease the effective population size; hence, it may limit mating opportunities [36]. The results fit the study by Sujii et al. [14] in which the private alleles in juveniles in the restoration areas were found and claimed as an evidence of gene flow between restored and neighbouring natural populations. After 50 years, the rehabilitated area of Kenaboi FR contains high tree density of the big *S. acuminata* tree, and outcrossing events may occur between the big trees in the study plot. Thus, all allele contributions of small *S. acuminata* trees come from the planted trees in the study plot. Although the allele contributions of small *S. acuminata* trees originate within a study plot, the genetic diversity of the *S. acuminata* population in the rehabilitated area remains high. This is because the source of planting stocks used to rehabilitate the forest includes seedlings from different seed trees in Sungai Menyala FR.

The genetic diversity of future generations in the rehabilitated area depends on the diversity of remnant seed trees in the study plot and gene flow from neighbouring stands. According to Shaharudin [37], the residual stocking after harvesting should be at least 32 commercial trees per hectare of different species from the DBH class between 30 and 45 cm. This study found ten highly diverse big *S. acuminata* trees to produce very highly diverse seedlings for future enrichment planting program. Hence, more trees of similar species should be retained after harvesting for successful regeneration of high quality tree species for future harvest.

Compartment 107 is a successfully rehabilitated area that preserved the high genetic diversity of important commercial tree species such as *S. acuminata*. Therefore, the compartment should be protected as a seed production area. Furthermore, the compartment is also the oldest rehabilitated forest area in Malaysia providing very valuable knowledge on

sustainable forest management. This forest will be best protected by gazettement the area as one of the High Conservation Value Forest (HCVF) in Malaysia.

5. Conclusions

The taungya restoration system that was practised by the Forestry Department of Negeri Sembilan, Malaysia, since 1969 has successfully rehabilitated a forest area that was once a poorly degraded area in Kenaboi FR. The structural function of this forest is now similar to primary forest, and the genetic diversity of *S. acuminata* is high. As this species is recommended for enrichment planting programmes, seed collection should follow the best practice protocol of collecting from many seed trees. The big trees in compartment 107, Kenaboi FR, and the area where their seed was collected in Sungai Menyala FR should be marked and protected. Both forests are suggested as ideal seed production areas to produce good quality planting stock. It is highly recommended that compartment 107, Kenaboi FR, should be proposed as a protected area and classified as a High Conservation Value Forest (HCVF).

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