

## GENETIC DIVERSITY ANALYSIS OF KEMIRI SUNAN POPULATION IN EAST NUSA TENGGARA BASED ON RAPD MARKERS

### *Analisis Keragaman Genetik Populasi Kemiri Sunan di Nusa Tenggara Timur Berdasarkan Marka RAPD*

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#### ABSTRACT

Genetic diversity analysis is the first step to determine the level of genetic diversity of kemiri sunan accessions. High genetic diversity in generative propagated germplasm collection is a basic foundation to develop new high-yielding varieties. The research aimed to detect the genetic diversity of kemiri sunan population in NTT based on RAPD markers. The study was conducted at the Integrated Laboratory, Balitri, Sukabumi from May to July 2019. A total of 32 kemiri sunan accessions were obtained from a private plantation owned by PT BHLI in Bajawa, Flores, NTT analyzed its genetic diversity using 40 RAPD markers. Markers that produced polymorphic bands were scored in binary data format and were then used for genetic diversity analysis using the NTSYS program. Results showed that 11 RAPD markers were polymorphic and generated a total of 41 bands consisting of 32 polymorphic bands (78.05%) and 9 monomorphic bands (21.95%). The genetic diversity analysis with a genetic similarity value of 0.737 showed that the 32 kemiri sunan accessions were divided into 2 clusters. Among those, two accessions (T2 and T4) can be selected as candidates for new high-yielding varieties, because they are located in different groups and showed high per plant fruit number. The result of the study also obtained the genetic distance value between kemiri sunan accessions varies from 0.00 to 0.46. The combination of two kemiri sunan accessions with a high genetic distance value should be useful as parents to obtain F1 progeny with a high heterosis effect.

**Keywords:** Accession, genetic diversity, parental selection, RAPD marker, *Reutealis trisperma* (Blanco) Airy Shaw

#### ABSTRAK

Analisis keragaman genetik merupakan langkah awal untuk mengetahui tingkat keragaman genetik dan hubungan kekerabatan dari setiap aksesi kemiri sunan. Keragaman genetik yang tinggi pada koleksi plasma nutfah yang diperbanyak secara generatif merupakan modal dasar dalam upaya untuk merakit varietas unggul baru. Penelitian bertujuan untuk mendeteksi keragaman genetik populasi kemiri sunan (*Reutealis trisperma* (Blanco) Airy Shaw) di Nusa Tenggara Timur (NTT) berdasarkan marka RAPD. Penelitian dilaksanakan di Laboratorium Terpadu, Balitri, Sukabumi mulai bulan Mei sampai Juli 2019. Sebanyak 32 aksesi kemiri sunan yang diperoleh dari perkebunan swasta milik PT BHLI di Bajawa, Flores, NTT, dianalisis keragaman genetiknya menggunakan 40 marka RAPD. Marka yang menghasilkan pita *polimorphic* diskoring dalam

format data biner dan digunakan untuk analisis keragaman genetik menggunakan program NTSYS. Hasil penelitian menunjukkan bahwa 11 marka RAPD bersifat polimorfik dengan jumlah pita sebanyak 41 yang terdiri dari 32 pita polimorfik (78,05%) dan 9 pita monomorfik (21,95%). Sementara, hasil analisis keragaman genetik membagi 32 aksesi kemiri sunan menjadi 2 kelompok pada nilai kesamaan genetik 0,737. Berdasarkan hasil analisis diperoleh dua aksesi kemiri sunan (T2 dan T4) yang dapat dipilih sebagai kandidat varietas unggul baru, karena terdapat pada kelompok yang berbeda dan mempunyai jumlah buah yang banyak. Dari hasil penelitian juga diperoleh nilai jarak genetik antar aksesi kemiri sunan yang bervariasi, yaitu 0,00-0,46. Kombinasi dari dua aksesi kemiri sunan dengan nilai jarak genetik yang tinggi dapat digunakan sebagai tetua persilangan untuk mendapatkan keturunan F1 yang lebih unggul.

**Kata kunci :** Aksesi, keragaman genetik, marka RAPD, *Reutealis trisperma* (Blanco) Airy Shaw, seleksi tetua.

#### INTRODUCTION

Kemiri sunan [*Reutealis trisperma* (Blanco) Airy Shaw] is a non-food vegetable oil that is currently being developed in Indonesia. In comparison to other non-food vegetable oils, kemiri sunan seed oil can produce biodiesel up to 88% of the oil volume (Aunillah and Pranowo 2012). Biodiesel produced from kemiri sunan seed oil has a quality that meets the requirements of biodiesel standards (Hendra 2014). In addition, kemiri sunan seed oil can be further processed into oleochemical products and their derivatives, as well (Herman *et al.* 2013). On the other hand, kemiri sunan can also be used as a conservation plant on marginal land and/or degraded land, because of its wide adaptability, which can grow well at an altitude of 0 to 1200 m above sea level with various types of soil and climate, has a root system that is capable of binds the soil and holds water tightly, and has a very shaded canopy that can

withstand splashing rain (Hendra 2014; Pranowo *et al.* 2016).

Considering the high potency of kemiri sunan for biodiesel and its derivative products, the development of a new high-yielding variety is relevant to carry-out in the future kemiri sunan breeding program. To date, four varieties of kemiri sunan have been released, i.e. Kemiri sunan-1, Kemiri sunan-2, Kermindo-1, and Kermindo-2 (Pranowo *et al.* 2016). These varieties have been widely developed in dry climates, including Bajawa, Flores, East Nusa Tenggara (NTT). Of which, kemiri sunan planted in that region usually derived from two types of propagation, including vegetatively through the grafting method and generatively (seeds) resulting from open pollination. The characteristics of kemiri sunan has separate male and female flowers in the same plant, thus indicating an open pollination system (Allogamy) (Ajjjah, Wicaksono, & Syafaruddin, 2009). The open pollination system on kemiri sunan led to segregation in their progeny. Plants that are propagated through seeds (illegitimate seeds) on cross-pollinated system, such as kemiri sunan, demonstrated relatively higher diversity compared to those from vegetative propagation. This is due to plant genes derived from seed propagation will be segregated, which makes the gene arrangement heterozygous. Úbeda and Haig (2005) emphasized that the segregation process produced individual plants in a population with heterozygous gene composition. Furthermore, Oktavianti and Soegianto (2019) reported that in artificial pollination of kenaf, segregation patterns can be used as an estimator of trait inheritance and the number of genes involved in controlling a trait.

The high phenotypic diversity in the generatively propagated plant population (seeds) provides a high opportunity to obtain site-specific superior genetic material through selection activities. However, selection activities based solely on phenotypic performance have not progressed significantly, because they are influenced by environmental factors. Theoretically, it is known that phenotypic diversity is the result of genotypic diversity and environmental diversity (Falconer and Mackay, 1996). Therefore, to obtain significant genetic progress, phenotypic selection needs to be followed by genotypic selection through analysis of genetic diversity and relationship based on DNA markers such as Randomly Amplified Polymorphic DNA (RAPD). DNA marker-based analysis is expected to be able to answer these problems. In this regard, it is emphasized that a high level of genetic diversity is a major requirement in assembling new high-yielding varieties (Dani *et al.*

2012), and genetic diversity of plant populations is one of the determining factors for the success of breeding and conservation strategies (Sulistyawati *et al.* 2014). It is important to note that genetic diversity is a key component of biodiversity, thus each species can evolve and adapt in the face of environmental changes (Putranto 2016).

The advantage of DNA-based markers in the analysis of genetic diversity and relationships is their sensitivity and ability to identify different species, as well as to distinguish closely related individuals (Ilbi, 2003). RAPD markers which have the advantage of low technical input and a large number of markers have proven to be useful in analyzing the genetic diversity of woody plants, genetic relationships, and identification studies (Chen *et al.* 2005). Previous studies have used RAPD markers to identify genetic diversity in oil palm (Basyuni *et al.* 2018), tea (Martono and Udarno 2014) and, suren (Restu *et al.* 2012).

This study aimed to determine the genetic diversity of 32 accessions of kemiri sunan from East Nusa Tenggara (NTT) based on RAPD markers. It is expected that the results of present study would be useful as basic information in assembling new high-yielding varieties in the future kemiri sunan breeding program.

## MATERIALS AND METHODS

The research was carried out at the Molecular Laboratory of the Integrated Laboratory, Indonesian Industrial and Beverage Crops Research Institute (Balittri), Sukabumi, from May to July 2019.

### Plant Materials

A total of 32 kemiri sunan accessions which were propagated generatively (seeds) and vegetatively (grafting) were used as genetic material in the analysis of genetic diversity using RAPD markers. Kemiri sunan accessions used in this study were collected from three locations, namely: Poma, Kurubhoko, and Wolokuku at Bajawa region, Ngada Regency, East Nusa Tenggara. These 32 accessions were obtained from phenotypic selection conducted in 2018-2019 and were divided into 6 groups based on varieties, productivity, and leaf color (Table 1).

Table 1. Kemiri sunan accessions collected from Bajawa, NTT, used for genetic diversity and relationship analysis  
 Tabel 1. Akses kemiri sunan koleksi dari Bajawa, NTT, yang digunakan untuk analisis keragaman dan kekerabatan genetik

Accession code	Number of plants	Observed phenotypic characters	Origin
T1 – T10	10	Derived from the seeds of Kemiri Sunan 2 varieties and has high productivity (4 – 13.5 kg dry seeds/plant/harvest)	Block F15, Poma
R1 – R5	5	Derived from the seeds of Kemiri Sunan 2 varieties and has low productivity (< 1 kg dry seeds/plant/harvest)	Block F15, Poma
KT1 – KT4	4	Derived from the seeds of Kermino varieties, which has a production potential of $\pm$ 3 kg of dry seeds/plant/harvest	Kurubhoko
WM1 – WM8	8	Propagated by grafting from Kemiri Sunan 2 varieties and has red leaves	Block B25, Wolokuku
WH1 & WH2	2	Propagated by grafting from Kemiri Sunan 2 varieties and has green leaves	Block B25, Wolokuku
WK1 – WK3	3	Derived from the seeds of Kemiri Sunan 2 varieties and has yellow leaves	Block A25, Wolokuku

### Isolation of Genomic DNA

Isolation of genomic DNA was carried out using 3 g of young and healthy leaf. The DNA isolation process was conducted following the modified *Cetyltrimethylammonium Bromide* (CTAB) method by adding *Polyvinyl Pyrrolidone* (PVP) and  $\beta$ -mercaptoethanol without using liquid Nitrogen (Allen et al. 2006). The kemiri sunan leaf were cut into small pieces and ground using a mortar and pestle by adding 3 mL of extract buffer, 0.05 g of PVP and 5  $\mu$ L of  $\beta$ -mercaptoethanol. The extracted DNA was then measured for its quality and quantity using the NanoDrop ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE, USA). Furthermore, the resulting DNA concentration was used as a reference for diluting the DNA using 1x TE buffer solution to a concentration of 10 ng/ $\mu$ L for PCR analysis.

### PCR for RAPD Markers Amplification

The components for PCR reaction consisted of 10 ng of DNA, 0.2  $\mu$ M forward and reverse primers as well as 1x PCR mix (MyTaq Red Mix from Bioline) with a final volume of 15  $\mu$ L. A total of 40 RAPD primers were used in this study (Table 2). PCR program used for amplification was: pre-denaturation at 94 °C for 3 minutes, followed by 45 cycles of denaturation at 94 °C

for 1 minute, annealing at 37 °C for 1 minute, extension at 72 °C for 2 minutes, and a final extension at 72 °C for 1 minute. PCR reaction was carried out using labcyler machine (SensoQuest, Germany). Afterwards, the amplified DNA were separated on 1% agarose gel using 0.5x TBE buffer solution. The separation process was conducted using horizontal electrophoresis at a voltage of 100 volts for 75 minutes. The next step was staining the gel using Ethidium bromide solution for 20 minutes and visualizing it on a Keta UV transilluminator (Wealtec, Taiwan) using a gel documentation system.

### Data Analysis

The polymorphic RAPD marker were scored in a binary data format, which was scored “1” to indicate the presence of a band and “0” to indicate the absence of a band. The scoring data were then analyzed using Numerical Taxonomy and Multivariate Analysis System (NTSYS-Pc) program version 2.10 (Rohlf 2000). Clustering analysis was conducted using Unweighted Pair Group Method with Arithmetic Average (UPGMA) method in the Sequential Agglomerative Hierarchical Nested Cluster Analysis (SAHN) subprogram based on the genetic similarity matrix. Meanwhile, genetic distance value among kemiri sunan accessions were calculated as 1 – genetic similarity value.

Table 2. RAPD primers used in this study  
 Tabel 2. Primer RAPD yang digunakan pada penelitian ini

No	Primer name	The order of nucleotide bases (5' - 3')	Number of bases	T <sub>m</sub> (°C)
1	OPA-01	CAGGCCCTTC	10	36.4
2	OPA-02	TGCCGAGCTG	10	40.7
3	OPA-03	AGTCAGCCAC	10	34.3
4	OPA-04	AATCGGGCTG	10	35.1
5	OPA-05	AGGGGTCTTG	10	32.6
6	OPA-06	GGTCCCTGAC	10	35.2
7	OPA-07	GAAACGGGTG	10	33.2
8	OPA-08	GTGACGTAGG	10	31.1
9	OPA-09	GGGTAACGCC	10	37.4
10	OPA-10	GTGATCGCAG	10	33.1
11	OPA-11	CAATCGCCGT	10	36.7
12	OPA-12	TCGGCGATAG	10	34.0
13	OPA-13	CAGCACCCAC	10	37.7
14	OPA-14	TCTGTCCTGG	10	34.3
15	OPA-15	TTCCGAACCC	10	34.2
16	OPA-16	AGCCAGCGAA	10	38.3
17	OPA-17	GACCGCTTGT	10	35.7
18	OPA-18	AGGTGACCGT	10	36.2
19	OPA-19	CAAACGTCGG	10	34.2
20	OPA-20	GTTGCGATCC	10	33.5
21	OPB-01	GTTTCGCTCC	10	33.4
22	OPB-02	TGATCCCTGG	10	32.2
23	OPB-03	CATCCCCCTG	10	35.1
24	OPB-04	GGA CTGGAGT	10	32.2
25	OPB-05	TGCGCCCTTC	10	41.1
26	OPB-06	TGCTCTGCCC	10	39.8
27	OPB-07	GGTGACGCAG	10	38.1
28	OPB-08	GTCCACACGG	10	37.3
29	OPB-09	TGGGGGACTC	10	37.0
30	OPB-10	CTGCTGGGAC	10	36.6
31	OPC-01	TTCGAGCCAG	10	34.6
32	OPC-02	GTGAGGCGTC	10	37.6
33	OPC-03	GGGGGTCTTT	10	33.1
34	OPC-04	CCGCATCTAC	10	31.9
35	OPC-05	GATGACCGCC	10	37.6
36	OPC-06	GAACGGACTC	10	31.6
37	OPC-07	GTCCCGACGA	10	38.9
38	OPC-08	TGGACCGGTG	10	39.1
39	OPC-09	CTCACCGTCC	10	36.2
40	OPC-10	TGTCTGGGTG	10	33.4

## RESULTS AND DISCUSSION

### Polymorphism of RAPD Markers on Kemiri Sunan Accessions

The results of DNA amplification of 32 kemiri sunan accessions using 40 RAPD markers showed that 11 markers (27.5%) produced polymorphic DNA bands, 27 markers (67.5%) generated monomorphic DNA bands and 2 markers failed to produce DNA bands. The low polymorphism level of RAPD markers probably due to most of the markers used have not been able to distinguish the 32 kemiri sunan accessions used in this study. The possible explanation is each RAPD primer has its own annealing site; thus, the resulting bands can be either polymorphic or monomorphic with different numbers of bands. Polymorphic DNA bands are absolutely required in the study of genetic diversity analysis because they demonstrated the differences in the genetic characteristics of each individual (Langga et al. 2012). In this study, we found that primer OPA-19 produced the highest number of polymorphic bands (six bands) (Figure 1). All of polymorphic DNA bands were then scored in the binary format for further analysis.

Eleven polymorphic RAPD markers produced 41 bands, consisting of 32 polymorphic bands (78.05%) and 9 monomorphic bands (21.95%). The average band

generated from each primer was 3.73 bands or 1.28 bands for each kemiri sunan accession (Table 3). Previously, genetic diversity analysis of 9 tea accessions using 6 RAPD markers generated 54 bands consisting of 47 polymorphic bands (87.04%) and 7 monomorphic bands (12.96%) (Martono and Udarno 2014). The results showed that each type of plant had a different level of polymorphism.

Regarding the number of bands, each RAPD marker produced a varying number of bands, ranging from 2 bands (OPA-08 primer, OPB-06, OPC-04) to 7 bands (OPA-19 primer). In addition, 5 markers produced bands that were all polymorphic (100%), namely primers OPA-05, OPA-10, OPA-11, OPA-16, and OPB-06. In contrast, primer OPC-07 only produced one polymorphic band, out of 4 resulting bands (25%) (Table 3). Thus, each RAPD marker probably detects a different location of DNA fragments in the genome of each kemiri sunan accession. This is in accordance with the research conducted by Gusmiaty et al. (2016) who reported that each RAPD primer has its own annealing site on genomic DNA, therefore the polymorphic DNA bands generated from each primer differ in terms of band size and the number of DNA bands.

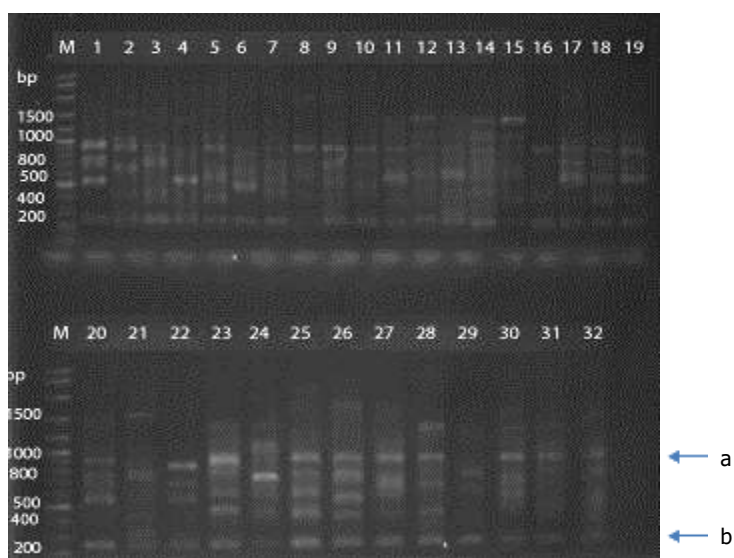


Figure 1. Example of PCR products resulted from amplification of genomic DNA of 32 kemiri sunan accessions using primer OPA-19. 1 – 32 = 32 kemiri sunan accessions (T1 - WK3), M = 100 bp DNA ladder, a = polymorphic band, b = monomorphic band.

Gambar 1. Contoh hasil PCR 32 aksesi kemiri sunan yang diamplifikasi menggunakan primer OPA-08. Keterangan: 1 – 32= sampel kemiri sunan T1 – WK3, M= 100 bp DNA ladder, a= pita polimorfik, b= pita monomorfik

Table 3. Number of polymorphic and monomorphic bands resulted from each polymorphic RAPD markers used in this study

Tabel 3. Jumlah pita polimorfik dan monomorfik yang dihasilkan dari setiap marka RAPD polimorfik yang digunakan pada penelitian ini

No.	Primer name	Allele		Total	Polymorphic (%)
		Polymorphic	Monomorphic		
1	OPA-05	3	0	3	100
2	OPA-08	1	1	2	50
3	OPA-10	3	0	3	100
4	OPA-11	3	0	3	100
5	OPA-16	5	0	5	100
6	OPA-17	4	1	5	80
7	OPA-19	6	1	7	85.71
8	OPB-03	3	2	5	60
9	OPB-06	2	0	2	100
10	OPC-04	1	1	2	50
11	OPC-07	1	3	4	25
Total		32	9	41	78.05

### Genetic Diversity of Kemiri Sunan Accessions

Genetic diversity was analyzed based on the genetic similarity coefficient of 0.737 as the threshold for classifying all kemiri sunan accessions using the UPGMA method. The grouping based on the genetic similarity value successfully divided 32 kemiri sunan accessions into 2 groups (Figure 2). **Group I**, consisted of 23 accessions including T1, KT3, WK2, T7, T8, WM8, WH1, WH2, KT2, WK3, WK1, WM4, T3, WM1, T5, T9, T10, WM2, WM5, WM6, WM7, and WM3, whereas **group II** contained nine accessions including T2, KT1, T6, R1, R3, R4, KT4, R5, and R2.

The clustering results of kemiri sunan accessions revealed in the present study were mostly grouped on the basis of their origin of the population. In the other words, the clustering of kemiri sunan were not related to the sampling location. Group I consisted of all kemiri sunan accessions derived from vegetative propagation (grafting) and some accessions that were propagated generatively (seeds), while group II contained all kemiri sunan accessions derived from generative propagation (seeds). Genetic diversity analysis using RAPD markers was proven to be able to group all kemiri sunan accessions derived from grafting in the same group. This is in accordance with the characteristics of plants propagated by grafting which are genetically similar to each other. Although group I also contained population derived from seeds, most of the population members have the same parents (Kemiri Sunan 2 varieties). Of these, only two accessions in group I were derived from seeds of Kermindo varieties. Meanwhile, the two

varieties (Kemiri sunan 2 and Kermindo) may have a close genetic relationship. This is indicated by the high value of genetic similarity in all kemiri sunan accessions used in the study (0.73-1.00) (Figure 2).

The results of genetic diversity analysis showed that two kemiri sunan accessions, T4 and T2, which have high productivity (Table 1) could be selected as candidates for new high-yielding varieties. Although these two accessions were in the same group and location (Table 1), the clustering results were separated into different groups (Figure 2), hence they were assumed to have different characteristics. This condition exhibited the presence of gene segregation from each individual, and showed the advantage of RAPD markers which are can detect plant genetic differences without being influenced by environmental factors. On the other hand, R1-R5 accessions that had low production were grouped together in group II with a threshold value of 0.786, thus they could be categorized as genetic groups of low-producing plants.

Genetic diversity analysis exhibited that kemiri sunan accessions with different leaf colors (green, red, and yellow) belonged to the same group (group I). We assumed that the difference in leaf color was caused by environmental factors that affect the changes in the anthocyanin levels in the leaves. Therefore, the difference in leaf color cannot be concluded as a difference in genetic factors, and the leaves color (especially the red and yellow ones) gradually turns green with good cultivation techniques.

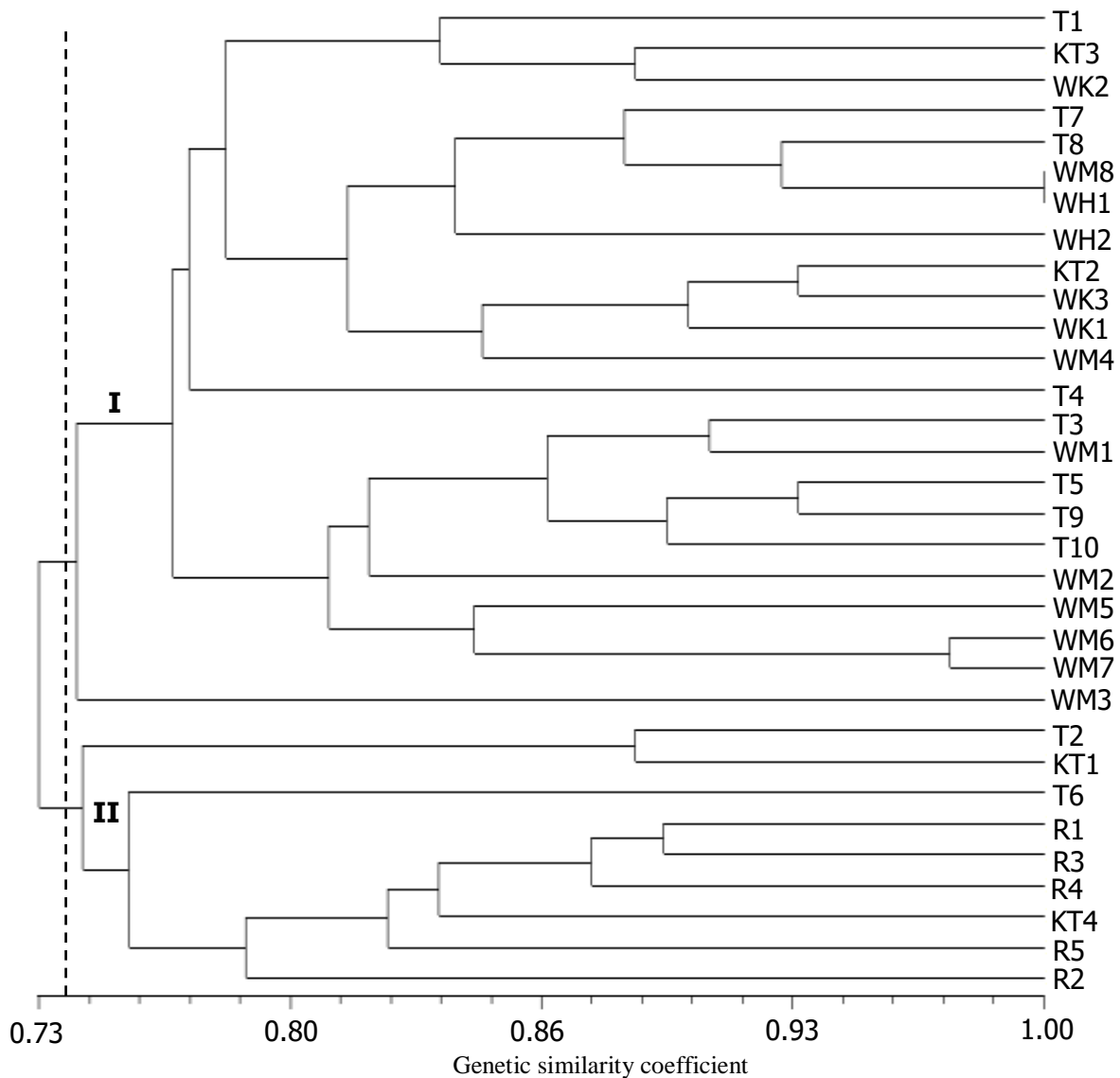


Figure 2. Phylogenetic tree of 32 kemiri sunan accessions collected from Bajawa, NTT analyzed using 11 RAPD markers

Gambar 2. Pohon filogenetik 32 aksesi kemiri sunan yang dikoleksi dari Bajawa NTT dianalisis menggunakan 11 marka RAPD

### Genetic Distance of Kemiri Sunan Accessions

In addition to having the advantage of not being influenced by environmental factors, RAPD markers are also able to detect the relationships between accessions. Hence, they can be used as a basis for selecting parents to improve plant genetic traits (Izzah et al. 2018). The genetic similarity value describes the genetic relationship between accessions. Of which, a high value

of genetic similarity coefficient indicates a close genetic relationship between accessions, and vice versa.

Even though kemiri sunan accessions had a high genetic similarity value (0.73-1.00), each kemiri sunan accession was genetically different, except for WH1 and WM8 accessions which were 100% similar (Figure 2). The results obtained in this study are in accordance with previous research conducted by Budiani et al. (2014), who reported that plants with the same morphology but

genetically different were different individuals, and vice versa. Therefore, it is important to analyze plant genetic diversity using DNA markers, particularly in plants that are morphologically difficult to distinguish. This is because DNA markers can detect variations in genomic DNA sequence.

The genetic distance value between kemiri sunan accessions was 0.00-0.46 (Table 4). The highest genetic distance value (0.46) was found between T7 and T2 as well as between WH2 and R5 accessions, while the lowest genetic distance value (0) was found between WH1 and WM8 accessions. The combination of kemiri sunan accessions that have the highest genetic distance

value can be used as a parent in order to obtain superior progenies. The results showed that T2 accession had the highest genetic distance value with T4 and T7 accessions (0.43 and 0.46), thus it had the opportunity to be crossed to obtain F1 progenies with high heterosis in the number of fruits (production).

On the other hand, even though R5 accession belonged to the low-producing group, it had a high genetic distance value (0.43-0.46) with WM8, WH 1 and WH 2 accessions (Table 4). These accessions have the opportunity to be crossed in an effort to obtain F1 with superior characteristics compared to their parents.

Table 4. Genetic distance values of 32 kemiri sunan accessions based on RAPD markers

Tabel 4. Nilai jarak genetik 32 akses kemiri sunan berdasarkan marka RAPD

Akses	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	R1	R2	R3	R4	R5	KT1	KT2	KT3	KT4	WM1	WM2	WM3	WM4	WM5	WM6	WM7	WM8	WH1	WH2	WK1	WK2	WK3		
T1	0																																	
T2	0.29	0																																
T3	0.23	0.23	0																															
T4	0.29	<b>0.43</b>	0.29	0																														
T5	0.2	0.27	0.09	0.2	0																													
T6	0.31	0.31	0.19	0.24	0.16	0																												
T7	0.38	<b>0.46</b>	0.24	0.23	0.21	0.26	0																											
T8	0.26	0.33	0.2	0.19	0.17	0.21	0.1	0																										
T9	0.21	0.29	0.10	0.21	0.07	0.17	0.15	0.11	0																									
T10	0.29	0.29	0.10	0.29	0.13	0.24	0.23	0.19	0.07	0																								
R1	0.21	0.21	0.16	0.21	0.13	0.24	0.31	0.19	0.14	0.14	0																							
R2	0.38	0.38	0.31	0.31	0.23	0.27	0.33	0.29	0.31	0.31	0.24	0																						
R3	0.24	0.31	0.19	0.17	0.16	0.2	0.26	0.21	0.17	0.17	0.10	0.2	0																					
R4	0.29	0.36	0.23	0.21	0.13	0.24	0.23	0.26	0.14	0.21	0.14	0.17	0.10	0																				
R5	0.33	0.26	0.33	0.33	0.31	0.36	0.4	0.38	0.33	0.33	0.19	0.29	0.14	0.19	0																			
KT1	0.26	0.11	0.13	0.33	0.17	0.21	0.4	0.23	0.19	0.19	0.11	0.29	0.21	0.26	0.31	0																		
KT2	0.17	0.31	0.19	0.24	0.23	0.33	0.26	0.14	0.24	0.24	0.17	0.33	0.2	0.31	0.36	0.21	0																	
KT3	0.19	0.26	0.2	0.19	0.10	0.14	0.3	0.23	0.19	0.26	0.19	0.21	0.14	0.19	0.31	0.15	0.21	0																
KT4	0.23	0.23	0.24	0.23	0.14	0.19	0.33	0.3	0.23	0.31	0.15	0.19	0.19	0.15	0.2	0.2	0.33	0.1	0															
WM1	0.2	0.27	0.09	0.27	0.13	0.23	0.36	0.24	0.2	0.2	0.13	0.29	0.16	0.27	0.31	0.17	0.16	0.17	0.21	0														
WM2	0.26	0.33	0.13	0.26	0.24	0.21	0.3	0.23	0.19	0.19	0.19	0.36	0.14	0.26	0.31	0.23	0.21	0.23	0.3	0.17	0													
WM3	0.26	0.33	0.27	0.26	0.17	0.29	0.4	0.23	0.19	0.26	0.19	0.36	0.21	0.26	0.38	0.31	0.29	0.23	0.3	0.24	0.31	0												
WM4	0.26	0.41	0.27	0.26	0.17	0.36	0.2	0.15	0.19	0.26	0.26	0.36	0.29	0.26	0.38	0.31	0.14	0.23	0.4	0.31	0.38	0.31	0											
WM5	0.33	0.4	0.15	0.33	0.3	0.29	0.21	0.24	0.2	0.2	0.27	0.35	0.23	0.27	0.38	0.31	0.23	0.31	0.36	0.3	0.17	0.38	0.31	0										
WM6	0.27	0.39	0.17	0.33	0.2	0.35	0.29	0.3	0.21	0.21	0.35	0.24	0.27	0.38	0.31	0.18	0.31	0.35	0.2	0.3	0.31	0.25	0.14	0										
WM7	0.24	0.35	0.14	0.29	0.17	0.31	0.3	0.21	0.18	0.18	0.18	0.31	0.2	0.24	0.33	0.27	0.14	0.27	0.31	0.17	0.21	0.27	0.21	0.17	0.03	0								
WM8	0.31	0.31	0.19	0.24	0.16	0.27	0.11	0.07	0.10	0.17	0.24	0.33	0.27	0.24	<b>0.43</b>	0.29	0.2	0.29	0.33	0.29	0.29	0.21	0.14	0.23	0.24	0.2	0							
WH1	0.31	0.31	0.19	0.24	0.16	0.27	0.11	0.07	0.10	0.17	0.24	0.33	0.27	0.24	<b>0.43</b>	0.29	0.2	0.29	0.33	0.29	0.29	0.21	0.14	0.23	0.24	0.2	0							
WH2	0.26	0.33	0.27	0.26	0.31	0.29	0.2	0.15	0.26	0.33	0.33	0.36	0.29	0.33	<b>0.46</b>	0.31	0.14	0.23	0.4	0.31	0.23	0.31	0.23	0.24	0.31	0.27	0.14	0.14	0					
WK1	0.27	0.27	0.2	0.3	0.35	0.29	0.17	0.27	0.27	0.2	0.35	0.23	0.33	0.38	0.24	0.10	0.24	0.36	0.3	0.31	0.24	0.17	0.31	0.26	0.22	0.16	0.16	0.17	0					
WK2	0.14	0.29	0.23	0.21	0.13	0.24	0.23	0.11	0.14	0.21	0.14	0.24	0.17	0.21	0.33	0.19	0.10	0.11	0.23	0.2	0.26	0.19	0.11	0.27	0.21	0.18	0.17	0.17	0.19	0.13	0			
WK3	0.24	0.31	0.3	0.24	0.23	0.33	0.26	0.14	0.24	0.17	0.14	0.33	0.2	0.31	0.36	0.21	0.07	0.21	0.33	0.23	0.29	0.29	0.14	0.29	0.24	0.2	0.2	0.2	0.21	0.10	0.10	0		

Notes : Numbers in bold typed indicate the genetic distance >0.40.

Keterangan: Angka-angka yang dicetak tebal menunjukkan nilai jarak genetik >0,40.

### CONCLUSION AND SUGGESTION

The results in present study demonstrated that 11 RAPD markers were polymorphic and produced 41 bands consisting of 32 polymorphic bands (78.05%) and 9 monomorphic bands (21.95%). Genetic diversity analysis based on polymorphic RAPD markers divided 32 kemiri sunan accessions into 2 groups at a genetic similarity value of 0.737. Two kemiri sunan accessions (T2 and T4) could potentially be selected as candidates for new high-yielding varieties with the advantage of having high productivity (4-13.5 kg of dry seeds/plant/harvest) and phylogenetically their locations

were dispersed in different groups. Based on the genetic distance value, T2 accessions can be selected as parents to be crossed with T4 and T7 accessions in obtaining high-producing F1 progenies. Meanwhile, R5 accession has the opportunity to be crossed with WM8, WH1 and WH2 accessions to obtain F1 which has superior characteristics other than production.

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In this paper, Nur Kholilatul Izzah acted as the main contributor, whereas Maman Herman, Edi Wardiana, and Dibyo Pranowo as member contributors.

#### REFERENCES

- Ajjah, N., Wicaksono, I. N. A., & Syafaruddin (2009) Karakteristik Morfologi Bunga. In U. Daras, Syafaruddin, N. Ajjah, Y. Ferry, G. Indriati, A. M. Hasibuan, ... A. M. Rivai (Eds.), *Kemiri Sunan Penghasil Biodiesel Solusi Masalah Energi Masa Depan* (p. 162). Unit Penerbitan dan Publikasi Balittri.
- Allen, G. C. *et al.* (2006) A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nature protocols*. [Online] 1 (5), 2320–2325. Available from: doi: 10.1038/nprot.2006.384.
- Aunillah, A. & Pranowo, D. (2012) Karakteristik Biodiesel Kemiri Sunan [*Reutealis trisperma* (Blanco) Airy Shaw] Menggunakan Proses Transesterifikasi Dua Tahap. *Jurnal Tanaman Industri dan Penyegar*. [Online] 3 (3), 193–200. Available from: doi: 10.21082/jtidp.v3n3.2012.p193-200.
- Basyuni, M. *et al.* (2018) RAPD markers on genetic diversity in three populations of pisifera type of oil palm (*Elaeis guineensis*). IOP Conference Series: Earth and Environmental Science. [Online] 130 (1). Available from: doi: 10.1088/1755-1315/130/1/012050.
- Budiani, A. *et al.* (2014) Evaluasi 18 primer Simple Sequence Repeats untuk pengembangan sidik jari DNA tanaman karet (*Hevea brasiliensis* Muell. Arg.). *Menara Perkebunan*. [Online] 82 (2), 81–93. Available from: doi: 10.22302/ppbbi.jur.mp.v82i2.23.
- Chen, L. *et al.* (2005) The use of RAPD markers for detecting genetic diversity, relationship and molecular identification of Chinese elite tea genetic resources [*Camellia sinensis* (L.) O. Kuntze] preserved in a tea germplasm repository. *Biodiversity and Conservation*. [Online] 14 (6), 1433–1444. Available from: doi: 10.1007/s10531-004-9787-y.
- Dani, Syafaruddin & Supriadi, H. (2012) ‘Karakter morfometrik pembeda antartiga populasi kemiri sunan (*Reutealis trisperma*). *Widyariset*. 15 (2), 343–348.
- Falconer, D. S. & Mackay, T. F. C. (1996) *Introduction to Quantitative Genetics*. 4th Edition, Addison Wesley Longman, Harlow. Harlow: Addison Wesley Longman.
- Gusmiaty *et al.* (2016) Polimorfisme penanda RAPD untuk analisis keragaman genetik *Pinus merkusii* di Hutan Pendidikan Unhas. *Jurnal Natur Indonesia*. [Online] 16 (2), 47–53. Available from: doi: 10.31258/jnat.16.2.47-53.
- Hendra, D. (2014) Pembuatan biodiesel dari biji Kemiri Sunan. *Jurnal Penelitian Hasil Hutan*. [Online] 32 (1), 37–45. Available from: doi: 10.20886/jphh.2014.32.1.37-45.
- Herman, M., Tjahjana, B. E. & Dani (2013) Prospek pengembangan tanaman kemiri minyak (*Reutealis trisperma* (Blanco) Airy Shaw) sebagai sumber energi terbarukan. *Sirinov*. 1 (1), 1–10.
- Ilbi, H. (2003) RAPD markers assisted varietal identification and genetic purity test in pepper (*Capsicum annum*). *Scientia Horticulturae*. [Online] 97 (3–4), 211–218. Available from: doi: 10.1016/S0304-4238(02)00158-9.
- Izzah, N. K. *et al.* (2018) Keragaman genetik klon kakao lokal Sulawesi Tenggara berdasarkan marka SSR dan karakter morfologi. *Jurnal Tanaman Industri dan Penyegar*. [Online] 5 (3), 95–104. Available from: doi: http://dx.doi.org/10.21082/jtidp.v5n3.2018.p95-104.
- Langga, I. F., Restu, M. & Kuswinanti, T. (2012) Optimalisasi suhu dan lama inkubasi alam ekstraksi DNA tanaman Bitti (*Vitex cofassus* Reinw) serta analisis keragaman genetik dengan teknik RAPD-PCR. *J Sains & Teknologi*. 12 (3), 265–276.
- Martono, B. & Udarno, L. (2014) Keragaman genetik beberapa genotipe teh berdasarkan penanda RAPD (Random Amplified Polymorphic DNA). *Jurnal Tanaman Industri dan Penyegar*. [Online]

- 1 (1), 1-6. Available from: doi: 10.21082/jtidp.v1n1.2014.p1-6.
- Oktaviyanti, R. N. & Soegianto, A. (2019) Pola segregasi pada Beberapa karakter tanaman Kenaf (*Hibiscus cannabinus* L.) Generasi F<sub>2</sub> Hasil Persilangan HC48 dan SM004. *Jurnal Produksi Tanaman*. 7 (8), 1393–1400.
- Pranowo, D., Herman, M. & Syafaruddin (2016) Potensi pengembangan Kemiri Sunan (*Reutealis trisperma* (Blanco) Airy Shaw) di lahan terdegradasi. *Perspektif*. [Online] 14 (2), 87. Available from: doi: 10.21082/p.v14n2.2015.87-101.
- Putranto, R. A. (2016) Penanda molekuler dalam biologi konservasi: dari DNA barcoding hingga next-generation sequencing. in *Biokonservasi: Penelitian, Penerapan, dan Pembelajarannya Untuk Menjawab Tantangan dan Isu Global*. Prosiding Seminar Nasional Pendidikan Biologi dan Biologi Jurusan Pendidikan Biologi, Yogyakarta.
- Restu, M., Mukrimin, M. & Gusmiaty, G. (2012) Optimalisasi teknik ekstraksi dan isolasi DNA tanaman Suren (*Toona Sureni* Merr.) untuk analisis keragaman genetik berdasarkan Random Amplified Polymorphic DNA (RAPD). *Jurnal Natur Indonesia*. [Online] 14 (1), 138. Available from: doi: 10.31258/jnat.14.1.138-142.
- Rohlf, F. (2000) NTSYSpc, numerical taxonomy and multivariate analysis system version 2.1. Exeter Software. New York: Applied Biostatistics Inc, 1–44.
- Sulistiyawati, P., Widyatmoko, A. & Nurtjahjaningsih, I. (2014) Keragaman genetik anakan *Shorea leprosula* berdasarkan penanda Mikrosatelit. *Jurnal Pemuliaan Tanaman Hutan*. 8 (3), 171–183.
- Úbeda, F. & Haig, D. (2005) On the evolutionary stability of Mendelian segregation. *Genetics*. [Online] 170 (3), 1345–1357. Available from: doi:10.1534/genetics.104.036889.