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GENETIC VARIATION OF FEI BANANA (*MUSA TROGLODYTARUM* L.) IN MALUKU ISLANDS USING RAPD MARKERS

R.L. KARUWAL¹, R. KASIAMDARI², and B.S. DARYONO^{2*}

¹Program of Biology Education, Universitas Pattimura, Ambon 97233 Indonesia

²Biology Faculty Universitas Gadjah Mada, Yogyakarta 55281 Indonesia

*Corresponding author's email: bs_daryono@mail.ugm.ac.id

Email addresses of co-authors: karuwalritha8@gmail.com, rkasiamdari@ugm.ac.id

SUMMARY

Fei banana (*Musa troglodytarum* L.) is the only species found in the Maluku and Papua islands of East Indonesia. Distribution in Maluku is throughout Ambon, Haruku, Saparua, Nusalaut, and Seram islands. This banana is unique because it has an erect bunch, is classified as a cooking banana, and serves as a medicine. Genetic variation determination is the chief parameter for the conservation of genetic resources of Fei banana and its utilization in hybridization programs. However, there exists limited available genetic data on Fei banana in Maluku. Therefore, the study is crucial for gathering such information to help its improvement in the future. This research purposed to analyze the genetic variation of Fei bananas using RAPD markers. The RAPD profiles for eight different populations, generated with 12 random primers, revealed various levels of polymorphism. The results showed these primers generated 128 DNA fragments, where 16 were polymorphic, averaging 90–900 pb. The overall range of similarity among eight banana populations was narrow, ranging from 8.385 to 9.692, indicating a low genetic variation among Fei banana populations under study.

Keywords: Fei banana (*Musa troglodytarum* L.), genetic variation, genetic resources, hybridization, RAPD markers, polymorphism, Maluku Island

Key findings: The 12 primers used in eight different populations of Fei banana produced 27 monomorphic (21.09%) and 101 polymorphic (78.91%) DNA bands. The Fei banana populations, prevalent in the Maluku Islands, incur clustering in one group, with the Sangga Buana banana as an outgroup from Java, Indonesia.

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INTRODUCTION

Banana is valuable as a staple food and export commodity in many tropical and subtropical countries (Kharadi *et al.*, 2014). Banana is a climacteric flora that varies widely due to numerous cultivars developed in Indonesia. According to Singh *et al.* (2014), banana ranks first in production and trade volume of fresh fruit worldwide and the fourth food source after rice, wheat, and corn. The total banana production globally reached 119.8 million tons in 2020 (FAOSTAT, 2022). Banana is a commodity crop in tropical and subtropical countries, including Indonesia. The demand for bananas in the global market reached 19.6 million tons (FAO, 2022). More than 200 banana cultivars underwent cultivation throughout Indonesia (Poerba *et al.*, 2018).

In Indonesia, the central production areas of bananas are the islands of Sumatra, Lampung, Java, Kalimantan, Sulawesi, Bali, West Nusa Tenggara, and Maluku (Suyanti and Supriyadi, 2008). According to Poerba and Ahmad (2013), banana belongs to the genus *Musa* and consists of the Callimusa and Musa sections. Callimusa is a combination of the sectors Australimusa with chromosome numbers ($x = 10$), Ingentimusa ($x = 7$), and Callimusa ($x = 10$), while in Musa, Eumusa ($x = 11$) and Rhodochlamys ($x = 11$) (Hakkinen, 2013). In the genus *Musa*, several species have seeds and are a source of genetic diversity, and these genotypes' development can further continue through plant breeding for food purposes (Hakkinen and Wallace, 2011).

Fei (Fe'i or Fehi) is one of the banana species with the name given to a group of bananas easily recognized by their erect bunch. Its origin is not well-known; however, its ancestors belong to the former Australimusa section, which has merged with the Callimusa section (Ploetz *et al.*, 2007). Fei bananas are believed to originate in the New Guinea area but have also occurred in Molluccas, Indonesia, from Tahiti, South Pacific Ocean. Based on morphological characters, the Fei banana originated from *Musa maclayi*, while from DNA studies, it showed relations to *Musa lolodensis*. These two species may be the parents of the Fei banana. These bananas are also unique and

have incurred domestication independently from bananas related to *Musa acuminata* and *Musa balbisiana* in the section Musa (Ploetz *et al.*, 2007). According to Dwivany *et al.* (2020), Fei banana has a T genome and vast beta-carotene content.

According to Macdaniels (1947), the Fei banana is also named "borabora or polapola." It is widespread in Hawaii Island and the South Pacific. It differs from *Musa sapientum* with striking characteristics, including upright inflorescence, bright violet sap, and copper red color on ripe fruits. Fei bananas, as a staple carbohydrate food, require cooking before eating. Ploetz *et al.* (2007) mention that the Fei banana is an Indonesian banana found in eastern Indonesia, and in Maluku, its name is "Tongka Langit." The Fei banana was an essential staple food; however, in Maluku (Louhenapessy 2009), it became rare due to the introduction of other types of bananas. With its distinct feature of an upright bouquet, Rumphius (1750) named it *Musa uranoscopus*. Stover and Simmonds (1987) used the name *Musa troglodytarum* in the Fe'i banana group that grows in Eastern Indonesia.

In the Eumusa section, the Fei banana is distinct from the cultivars of *M. acuminata* and *M. balbisiana*. According to Karuwal *et al.* (2012), the morphology of the Fei banana includes an upright tree with a height of 5 m. The leaves are light or dark green. The fruit skin color is yellow, orange, or red-orange, with a length of 6–23.5 cm. The color of the flesh is yellow and, generally, does not have seeds. This type of banana is a commercial banana often found in the market and can be beneficial after being processed first. This banana also serves as a medicine to cure jaundice. Currently, in Maluku, the potential of the Fei banana is under development as an alternative food ingredient. Fei bananas' general cultivation is traditional and thrives in people's gardens (Louhenapessy, 2009).

Fei banana spreads across five islands separated by the sea, which possibly causes phenotypic and genotypic variations. These variations and genetic parameters can be measurable using specific methods. Based on the morphological characterization by Karuwal

et al. (2012), the genetic variations were visible among the Fei banana populations, like the shape, size, color, and appearance of each organ on stems, leaves, flowers, and fruits in the Mollucas islands. Rodrigues *et al.* (2012) also revealed that analyzing the genetic variation helps to manage and use the genetic resources for further improvement in the various plant breeding programs. *M. acuminata* and *M. balbisiana* are wild-type bananas that have been widely developed in Indonesia, causing the bananas' high diversity (Mukunthakumar *et al.*, 2013; Cizkova *et al.*, 2015; Martanti *et al.*, 2015; Sardos *et al.*, 2016).

Genetic variation scrutiny is mostly by DNA probing through molecular studies. Given its nature, DNA is more stable than environmental factors and their interactions that can change spatially and temporally. However, DNA polymorphisms can indicate the genetic variation in concerned individuals. Several developed genetic tools have risen to detect differences in DNA sequences and continued application to numerous organisms. Molecular markers' use, i.e., Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), and Amplified Fragment Length Polymorphism (AFLP), can help detect and study the genetic variations in various organisms (Zamani *et al.*, 2008). The RAPD technique has several advantages, showing a high polymorphism of amplified DNA. Compared with other methods, it requires a small amount of DNA and is fast, inexpensive, and easy to use. The weaknesses of the RAPD marker are unreproducible, producing different bands when repeated with the same primers (Jain *et al.*, 2007).

In the last decade, the genetic variation study with RAPD markers has been effectively analyzing genetic diversity in bananas (Poerba and Ahmad, 2013; Lamare and Rao, 2015). However, research on the Fei banana still requires broad implementation due to a lack of information about the banana's existence, an endemic plant, especially in Maluku, Indonesia (Karuwal *et al.*, 2012). Primarily, an analysis of the DNA profile of the Fei banana plant using molecular markers is nonexistent. Therefore, the relevant research

hopes to contribute to developing, utilizing, and conserving the Fei banana germplasm resources in Maluku, Indonesia.

MATERIALS AND METHODS

Sample collection

The sampling for eight different Fei banana populations proceeded in five islands, i.e., Ambon, Haruku, Saparua, Nusalaut, and Seram (Figure 1). The collected samples were in sapling form, measuring 50 cm, and then grown in polybags using sandy soil media. For comparison, four other types of bananas included Raja, Tanduk, Ambon, and Sangga Buana bananas. These also existed in the Maluku, except Sangga Buana, in Yogyakarta. Besides that, Ambon and Tanduk bananas have fruit sizes similar to the Fei banana, while Sangga Buana has an upright bunch like the Fei banana. The fourth banana belonged to the *Musa* sp. with a different genome composition, i.e., AAB genome (Raja and Tanduk bananas), AAA genome (Ambon banana), while Sangga Buana is unknown. The collected samples continued for use in the DNA isolation.

DNA isolation

DNA isolation commenced according to the Phytopure Illustrated Protocol Kit, with modifications to the buffer concentration. By adding phytopure reagents I and II to 0.1 g of crushed leaves, the samples proceeded incubation at 65 °C for 10 min in a water bath, then adding reagent III, the mixture incurred centrifugation at 3,000 rpm for 10 min. The resulting supernatant received a cold isopropanol, then sustained centrifugation at 10,000 rpm for 10 min to obtain DNA pellets. Drying the pellet ensued on a tissue, then added with 100 L of 70% ethanol was before centrifuging at 10,000 rpm for five minutes and added with the Tris ETA solution. After that, the isolated sample proceeded to storage in the freezer. Testing continued on the quantity of DNA with a spectrophotometer at an absorbance of 260/280 nm for RAPD analysis.



Figure 1. Map of sampling locations of Fei banana in the Maluku Island, Indonesia.
 ● : Sampling location

RAPD analysis

At this stage, performing PCR amplification used 12 RAPD primers (Lakshmanan *et al.*, 2007; Nsabimana and Van-Staden, 2007). The volume of the PCR reaction was 25 μL consisting of 2.5 μL DNA template, 2.5 μL RAPD primer, and 20 μL Mega Mix blue PCR kit. The PCR reaction commenced for 45 cycles consisting of pre-denaturation at 94 $^{\circ}\text{C}$ for five minutes, denaturation at 94 $^{\circ}\text{C}$ for 30 seconds, annealing at 36 $^{\circ}\text{C}$ for one minute, elongation at 72 $^{\circ}\text{C}$ for two minutes, and post-elongation at 72 $^{\circ}\text{C}$ for seven minutes. Electrophoresis followed on 1.5% agarose for 40 minutes, with the results photographed on a UV transilluminator.

Data analysis

The DNA band profile formed from the amplification results received a score of 1 (if there is a band at the same migration level) and 0 (if there is no band). The genetic variation measurement calculated DNA bands consisted of monomorphism and polymorphism. The DNA band calculation progressed by counting the DNA bands for monomorphic (those always present in all the

banana samples compared) and polymorphic (DNA bands found only in some of the compared specimens). The calculations continued by comparing the DNA bands found in each population and then calculating the percentage. The total number of monomorphic DNA bands, polymorphisms, and the number of all bands' computations also occurred for each primer. The data analysis used the NTSys-PC version 2.01 program. The grouping analysis of RAPD data employed the Sequential Agglomerative Hierarchical and Nested (SAHN)-UPGMA (Unweighted Pair Group Method Arithmetic Average), producing the form of a dendrogram.

RESULTS

Quantification of DNA

Based on the spectrophotometric results, DNA has relatively varied purity. The highest DNA ratio resulted in the Fei banana population from Nusalaut (1,932). The highest DNA concentration emerged in the Ambon population (53.4 $\mu\text{g}/\text{mL}$) and the lowest in the Seram III (5.3 $\mu\text{g}/\text{mL}$). The results of DNA quantification appear in Table 1.

Table 1. Spectrophotometric absorbance readings and concentration of DNA of Fei banana populations.

No.	Sample Code	Absorbance reading at (λ 260/ λ 280)	Concentration of DNA (μ g/mL)
1	Saparua 1	1.789	22.3
2	Haruku 1	1.645	14.1
3	Nusalaut 1	1.932	11.8
4	Ambon I.2	1.899	53.4
5	Ambon II.1	1.531	47.7
6	Seram I.1	1.911	31.0
7	Seram II.1	1.516	11.2
8	Seram III.1	1.635	5.3
9	Raja 1	1.519	8.7
10	Tanduk 1	1.913	126.0
11	Ambon 1	1.983	50.4
12	Sanga Buana 1	1.211	20.8

Table 2. Number of DNA bands and percentage polymorphic of the Fei banana using RAPD markers.

No.	Primers	Sequence (5'-3')	Size range (pb)	Total bands	Monomorphic bands	Polymorphic bands	Polymorphic bands (%)
1	OPA-18	AGGTGACCGT	150-750	9	2	7	77.78
2	OPA-19	CAAACGTCGG	150-620	9	1	8	88.89
3	OPB-18	CCACAGCAGT	100-500	6	3	3	50.00
4	OPC-07	GTCCCGACGA	190-900	6	3	3	50.00
5	OPC-11	AAAGCTGCGG	100-900	13	4	9	69.23
6	OPC-13	AAGCCTCGTC	120-900	12	-	12	100
7	OPD-16	AGGGCGTAAG	150-800	12	4	8	66.67
8	OPE-01	CCCAAGGTCC	90-750	16	-	16	100
9	OPF-01	ACGGATCCTG	100-750	8	3	5	62.5
10	OPJ-09	TGAGCCTCAC	120-320	6	5	1	16.67
11	OPN-10	ACAACCTGGGG	180-700	15	2	13	86.67
12	OPN-11	TCGCCGCAA	100-850	16	-	16	100
Total				128	101	868.41	27.00
Means				10.67	8.42	72.36	2.25

Data scoring of RAPD PCR amplification

Electrophoresis of RAPD PCR amplification, using 12 primers in eight Fei banana populations of Maluku islands and four other bananas for comparison, produced 128 DNA bands (Table 2). The electrophoresis results showed that each primer formed various amplified DNA bands. Generally, there were 6–16 bands per primer at 90–900 bp in size. Based on the DNA band scoring of the 12 primers, it produced 27 monomorphic (21.09%) and 101 polymorphic DNA bands (78.91%). Primers OPE-01 and OPN-11 produced the most polymorphic DNA bands (16), while primer OPJ-09 produced the lowest polymorphic DNA band (1). On the contrary, the three primers, OPC-13, OPE-01, and OPN-11, did not develop monomorphic DNA bands.

The most abundant monomorphic DNA bands were evident in primer OPJ-09. DNA bands that have polymorphism occur in Figure 2.

Similarity analysis of Fei banana using RAPD markers

Similarity analysis of 128 DNA bands resulted in similarity coefficients with values from 4.846 to 9.692 (Table 3). The highest similarity coefficient (9.692) appeared between the Fei banana populations Ambon I and Seram II. However, the lowest similarity coefficient value (4.846) occurred between Fei banana populations obtained from Haruku and Ambon, Indonesia. All the analysis results became the basis for forming the dendrogram in the population grouping analysis.

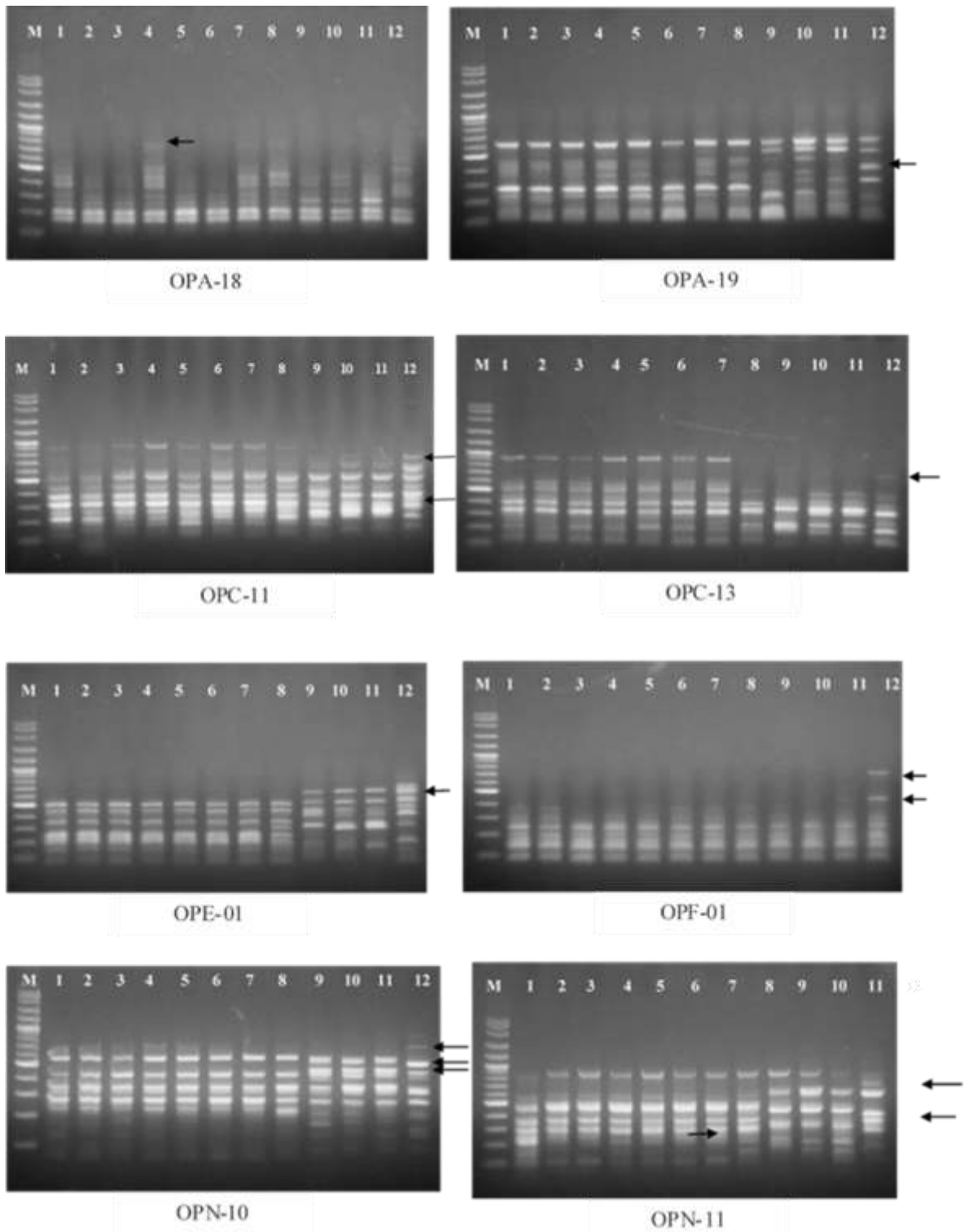


Figure 2. DNA bands of the Fei banana using RAPD markers.

Note: M = DNA Marker; 1 = Saparua; 2 = Haruku; 3 = Nusalaut; 4 = Ambon I; 5 = Ambon II; 6 = Seram I; 7 = Seram II; 8 = Seram III; 9 = Raja banana; 10 = Tanduk banana; 11 = Ambon banana; 12 = Sangga Buana banana.

Table 3. Similarity coefficient of the Fei banana using RAPD markers.

Populations	SPR	HRK	NSL	AM I	AM II	SRM I	SRM II	SRM III	R	TND	A	SB
SPR	1.00											
HRK	0.94	1.00										
NSL	0.92	0.95	1.00									
AM I	0.86	0.90	0.94	1.00								
AM II	0.89	0.91	0.93	0.95	1.00							
SRM I	0.91	0.94	0.96	0.95	0.96	1.00						
SRM II	0.88	0.90	0.94	0.97	0.93	0.95	1.00					
SRM III	0.85	0.84	0.88	0.91	0.86	0.89	0.91	1.00				
R	0.59	0.59	0.61	0.58	0.60	0.60	0.58	0.59	1.00			
TND	0.52	0.50	0.54	0.52	0.53	0.52	0.54	0.62	0.81	1.00		
A	0.50	0.49	0.52	0.49	0.52	0.52	0.49	0.59	0.82	0.87	1.00	
SB	0.55	0.54	0.58	0.56	0.59	0.59	0.57	0.58	0.56	0.52	0.56	1.00

Note: SPR = Saparua; HRK = Haruku; NSL = Nusalaut; AM I = Ambon I; AM II = Ambon II; SRM I = Seram I; SRM II = Seram II; SRM III = Seram III; R = Raja banana; TND = Tanduk banana; A = Ambon banana; SB = Sanga Buana banana.

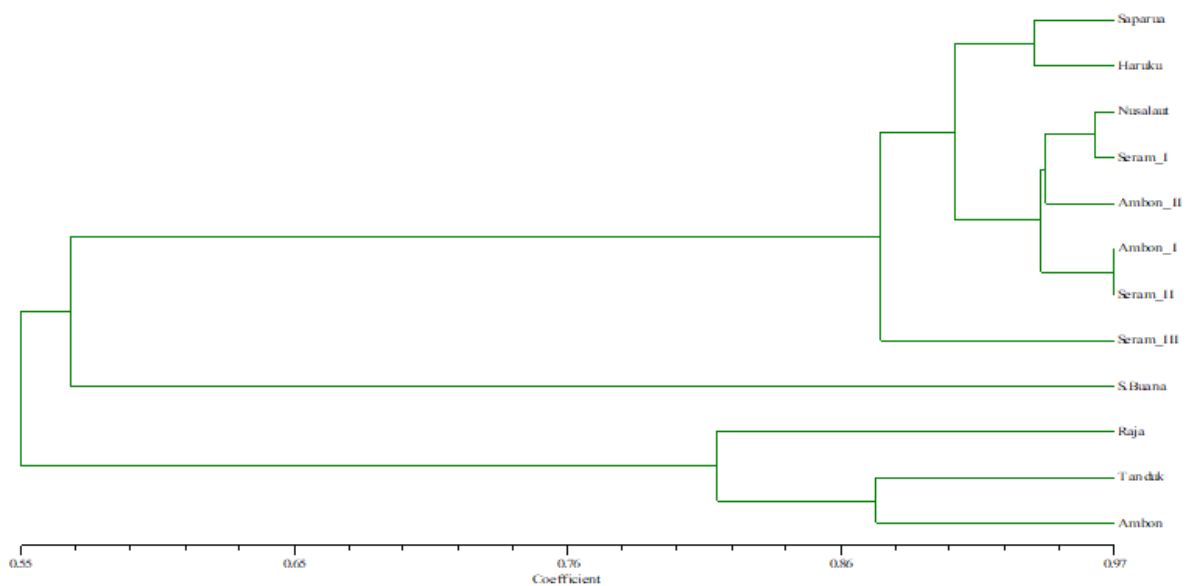


Figure 3. Dendrogram of the Fei banana using RAPD markers.

Clustering analysis of Fei banana using RAPD markers

Based on the clustering analysis of all the molecular data using RAPD markers, it produced a dendrogram with similarity coefficient values ranging from 0.55 to 0.97 (Figure 3). The presented study ably divided the dendrogram formed based on the molecular characters into two clusters. Eight Fei banana populations attained grouping in cluster I, comprising Saparua and Haruku, Nusalaut and Seram I, Ambon II, Ambon I and

Seram II, Seram III, and Sanga Buana banana. Cluster II consisted of three genotypes, i.e., Raja, Tanduk, and Ambon bananas as an outgroup.

In cluster I, Fei bananas from Saparua and Haruku islands were part of one group with a similarity coefficient of 0.93, and Fei bananas from Nusalaut, Ambon, and Seram had the group with a similarity coefficient of 0.94, except Seram III at a similarity coefficient of 0.86. Next, the Sanga Buana banana gained clustering with the Fei bananas' population on a similarity coefficient (0.57).

Tanduk and Ambon bananas' grouping had a similarity coefficient (of 0.87), and incurred clustering with the Raja banana with a similarity coefficient (of 0.81). Based on the similarity index, Fei bananas from several populations in the Maluku Islands showed few variations. If comparable with Raja, Tanduk, Ambon, and Sangga Buana bananas, Fei bananas displayed a wide variation.

DISCUSSION

Molecular markers can benefit the detection of polymorphisms at the DNA level to analyze genetic diversity, including RFLP, RAPD, AFLP, SSR, ISSR, and SNP (Nadeem *et al.*, 2018). The use of genetic diversity assessment in a study of plant evolution and their comparative genomics and the structure of the different populations succeeded (Liu *et al.*, 2015; Nawaz *et al.*, 2017). In addition, molecular markers can also help analyze the genetic variation among and within cultivars of different species (Solouki *et al.*, 2008; Martins-Lopes *et al.*, 2009; Poerba *et al.*, 2019). Kiran *et al.* (2015) mentioned that molecular markers are unaffected by environmental factors and plant developmental stages. This study showed that the results of the DNA quantification varied. According to Daryono and Natsuaki (2005), isolated DNA has a high purity degree between ratios of 1.8–2.0. In addition, the minimum concentration of the sample DNA required is 10 ng/ μ L.

In this study, the analysis for genetic variation proceeded on eight Fei banana populations using RAPD markers. Such studies can help conserve the Fei banana germplasm resources and the hybridization program of the endemic plant in the Maluku area, Indonesia. Of the eight Fei banana populations studied, 12 RAPD primers helped analyze the presence of genetic variability. RAPD as dominant markers aided assessing genetic variations and Inter- and intra-specific identification of banana cultivars because polymorphic bands' production can take place in a short time without prior genome sequence information (Kiran *et al.*, 2015; Lusiyanto *et al.*, 2021). The pertinent research produced 27

monomorphic DNA bands (21.09%) and 101 polymorphic DNA bands (78.91%). The highest polymorphic percentage was evident in the primers, viz., OPC-13, OPE-01, and OPN-11, with 12 to 16 bands. However, these results contradicted the findings of Rodrigues *et al.* (2012), who used 14 RAPD primers, producing a total of 176 strips consisting of 116 monomorphic (88%) and 60 polymorphic bands (20%). The average number of polymorphic bands per primer was 4.28, and the primers OPP 14, OPB 10, OPG 17, OPE 12, and OPU 10 formed the maximum number of polymorphic bands, i.e., 7, 6, 6, 6, and 6, respectively.

The DNA bands' appearance may be related to various types of mutations and evolution (Dhakshanamoorthy *et al.*, 2015). Based on the study, the polymorphism among the populations ranged from 50%–100%, and these differences could serve to analyze the kinship relationship within varieties. The genetic variation in the Fei banana was low based on the similarity value; it could be due to significant influences by the primers and the diversity of the populations in the Fei banana variety. Mukunthakumar *et al.* (2013) also observed similar polymorphism bands, with as much as 87.5% RAPD, and Lamare and Rao (2015) obtained 85.09% of RAPD polymorphic bands. RAPD is easy, fast, affordable, and widely advantageous for banana diversity studies (Poerba and Ahmad, 2010, 2013; Poerba *et al.*, 2012, 2018; Martanti *et al.*, 2022).

According to Williams *et al.* (1990), the polymorphism level incurs adjustment to primer sequences and the number of annealing regions in the template sequences. In the same geographic zone, isolation caused the lower genetic distance in the populations, which drives the evolution of a particular trait with similar genetic properties. In contrast, distinct geographical conditions also lead to other adaptation patterns and genetic properties. Therefore, using more advanced molecular markers is essential in future studies, i.e., multi-locus indicators, which can correlate alleles between domesticated bananas and their wild relatives (Volkaert, 2011).

The dendrogram shows the division and clustering of Fei banana populations in two clusters. Kiran *et al.* (2015) explained that differences in geographic origins resulted in the introduction and naturalization of cultivars from their origin areas. Higher polymorphism causes easier and faster analysis of the genetic variation and can develop cultivar-specific markers. Conversely, lower polymorphism can also contribute to an accurate and reliable estimate of genetic variability, thereby helping determine the nature of the existing gene pool of a genotype. The Fei banana population of Maluku island was small, and genetic variation in such small populations is often lower compared with large ones.

Mutation is also a chief source of genetic variation. Another factor that causes genetic variation based on molecular characteristics is the population fragmentation caused by the total reduction of the habitat area and the separation of some portions from a broad habitat. Population fragmentation leads to reduced population size for species and migration (gene flow) among the population areas (Frankham *et al.*, 2002). Padmesh *et al.* (2012) also stated that habitat fragmentation due to natural or human disturbances can lead to population isolation. In addition, the features of different areas as a medium for pollen transfer in pollination mechanisms can cause low gene flow rates and lead to genetic differentiation among the populations.

The Maluku Islands constitute a small population, with an estimated geologically united and experienced population fragmentation. Its explanation can also be that way because, on the Webber and Wallace line, the Maluku region has flora and fauna similar to the Pacific islands, and one is the Fei banana. Low similarity indicates a genetic diversity caused by isolation, resulting in the evolution of a specific trait. Diverse environmental conditions also lead to adaptation patterns and other genetic features (Poerba *et al.*, 2018, 2019). However, vegetative selection and propagation are also significant constraints on why banana cultivars have such high diversity. On the other hand,

diverse environmental factors and geographical conditions also influence the genetic variation among and within the species (Padmesh *et al.*, 2012).

In the similarity index, the Sangga Buana banana clustered with eight Fei banana populations, which are also genetically unique and may have similar genetic material. Karuwal *et al.* (2012) also mentioned that the Fei and Sangga Buana banana populations have the same upright bunch position. Rumphius (1750) was the first author, before Linnaeus, to describe in detail bananas in his book 'Herbarium Amboinense.' The Fei banana received the Latin name *Musa uranoscopus*. According to him, in Maluku, Fei bananas rarely existed in Ambon and only grew in home gardens. However, bananas are more commonly available in the North.

Morphologically, the Fei banana has the following characteristics: small fruit size, irregular fruit, erect sign, red fruit color, tough flesh, and sweet taste. The presence of RAPD markers in the Fei banana variety can be beneficial and applicable in developing the conservation of various germplasm resources for future hybridization programs. The progressive study is only the first step in using RAPD markers to assess genetic diversity in the Fei banana populations in Maluku Islands, Indonesia. The presented results can also provide an invaluable contribution to the future strategy of breeding and conserving genetic resources of the Fei banana in the Maluku Islands. These results were in analogy with the findings of Lamare and Rao (2015), which stated that genetic variability is imperative in developing and conserving genetic resources.

CONCLUSIONS

Based on the results of PCR amplification using 12 RAPD primers, the research obtained 101 polymorphic DNA bands and 27 monomorphic DNA bands. The similarity value ranged from 0.55–0.97 and comprised a grouping into eight clusters. Thus, genetic variations existed among populations of Fei bananas in the Maluku Islands using RAPD markers.

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