

# Allelic Variation of Tongka Langit Banana (*Musa troglodytarum* L.) in the Aru and Kei Islands Based on SSR Markers

Alveus Saloy MOSE

**Abstract**— Tongka Langit banana (*Musa troglodytarum* L.) is distributed across several islands in Maluku Province, including the Aru and Kei Islands. This banana cultivar exhibits variations in fruit color, such as yellow, reddish-yellow, and orange-yellow. However, the extent of its genetic variation has not been clearly characterized. Therefore, this study aimed to analyze the allelic variation of Tongka Langit banana using Simple Sequence Repeat (SSR) markers. A total of six Tongka Langit banana samples were successfully amplified using SSR primers, producing diverse allelic variations. The amplification results showed polymorphic bands generated by each primer, with a total of 48 polymorphic bands observed. The allele sizes ranged from 122 to 436 bp, indicating considerable genetic variability among the samples. Genetic distance analysis revealed values ranging from 0.6 to 0.8. The highest genetic distances were observed between the following sample pairs: Kilo 8 and Fangamas, Kilo 8 and Rahareing, Kilo 8 and Ngefuit Atas, Kilo 9 and Kilo 10, Kilo 9 and Fangamas, Kilo 9 and Ngefuit Atas, Kilo 10 and Fangamas, Kilo 10 and Rahareing, Fangamas and Rahareing, Fangamas and Ngefuit Atas, and Rahareing and Ngefuit Atas. In contrast, the lowest genetic distance was found between Kilo 10 and Ngefuit Atas samples. A phenogram constructed using MEGA version 10.0.4 software grouped the samples into two major clusters. Cluster I consisted of Tongka Langit banana samples from Kilo 8, Kilo 10, and Ngefuit Atas, while Cluster II included samples from Kilo 9, Rahareing, and Fangamas. The allelic variation observed in this study was not influenced by the geographical distribution of the samples. The results provide valuable information for the selection of Tongka Langit banana individuals for germplasm conservation and banana breeding programs in Maluku.

**Keywords**— Allelic variation, Tongka langit banana (*Musa troglodytarum* L.), Aru and Kei Islands, Simple Sequence Repeat (SSR).

## I. INTRODUCTION

In Maluku, Indonesia, there is a unique and potentially valuable banana variety known as Tongka Langit banana (*Musa troglodytarum* L.), which belongs to the Callimusa group [1]. In Maluku and North Maluku Provinces, Tongka Langit bananas are distributed across several islands, including Ambon, Nusalaut, Saparua, Haruku, Seram, Buru, Dobo, Wokam, Tual, Elat (Kei Besar), and Halmahera.

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Mose A.S. is with the School of Life Sciences and Technology, Institute Teknologi Bandung, Bandung 40132, Indonesia

This banana exhibits high morphological variation, including differences in fruit size (short to long), plant height ranging from approximately 3–5 meters, and variation in leaf orientation. Unripe fruits are green, while ripe fruits display various colors such as orange, yellow, yellowish-orange, reddish-orange, brownish-red, and brownish-orange [2], [3].

A 2018 survey reported that Tongka Langit bananas are widely found in forest areas on the Kei and Aru Islands [4]. However, land-use changes, uncontrolled logging, and forest fires may reduce the population of Tongka Langit bananas, thereby narrowing the genetic base of this species. In addition, local communities rarely cultivate Tongka Langit bananas in large quantities due to their relatively low market value, despite their high nutritional content, including beta-carotene [5], [6] as well as vitamins A, B, and C [6].

Genetic conservation of bananas requires information on genetic diversity both within and among populations. Banana germplasm resources represent valuable genetic material for banana breeding programs. Characterizing germplasm based on important traits can reveal genetic variation within populations, where each individual may possess distinct genotypic values. Therefore, understanding genetic diversity is an essential first step in plant breeding and germplasm conservation [7].

Genetic diversity is often assessed through morphological traits; however, such approaches may be less accurate because they are influenced by environmental factors. More reliable results can be obtained through PCR-based molecular marker analysis, as genetic markers are generally stable and less affected by environmental conditions [8]. Among molecular markers, Simple Sequence Repeat (SSR) markers are widely used due to their high polymorphism, locus specificity, ease of amplification, requirement for only small amounts of DNA, distribution throughout the genome, high reproducibility, and codominant inheritance [9]–[11].

The availability of reliable molecular markers such as SSRs can accelerate banana conservation and breeding programs. Various strategies have been used to develop SSR markers [12]–[14], yet the number of SSR loci available for genetic analysis remains limited. Therefore, more validated and highly polymorphic SSR markers are needed [13]. This study aimed to analyze the allelic variation of Tongka Langit banana from the Aru and Kei Islands, which represent the outer islands of Maluku, using SSR markers.

## II. METHODS

### A. Sample Collection

Leaf samples of Tongka Langit banana (*Musa troglodytarum* L.) were collected from the Aru Islands (Dobo) and Kei Island, Maluku, Indonesia. The leaf samples were wrapped with moist cotton, sealed with tape, and placed in envelopes before being transported to the Indonesian Research Institute for Biotechnology and Bioindustry in Bogor for further analysis.

### B. DNA Extraction and Purification

Genomic DNA was extracted from Tongka Langit banana (*Musa troglodytarum* L.) leaf samples. Approximately 0.1 g of leaf tissue was cleaned, cut into small pieces, and ground in liquid nitrogen with polyvinylpyrrolidone (PVP) using a mortar and pestle. The powdered sample was transferred into microcentrifuge tubes and mixed with preheated extraction buffer containing 1%  $\beta$ -mercaptoethanol, followed by incubation at 65 °C for 30 min. The mixture was extracted with chloroform: isoamyl alcohol (24:1) and centrifuged to obtain the aqueous phase. DNA was precipitated with cold isopropanol and centrifuged to obtain the DNA pellet. The extracted DNA was further purified using phenol–chloroform–isoamyl alcohol

(PCI) and chloroform: isoamyl alcohol (CI). The aqueous phase was collected, and DNA was reprecipitated with isopropanol, washed with 70% ethanol, and air-dried. RNase treatment was performed at 37 °C for 1 h to remove RNA contamination. The purified DNA was then resuspended in TE buffer. DNA concentration and purity were determined by measuring absorbance at 230, 260, and 280 nm, and the purity was evaluated based on the  $A_{260}/A_{280}$  and  $A_{260}/A_{230}$  ratios [15].

### C. DNA Amplification

DNA amplification was performed using PCR (Primers profiles in Table I) with a total reaction volume of 25  $\mu$ L containing 12.5  $\mu$ L KAPA2G Fast Ready Mix, 1  $\mu$ L primer (10  $\mu$ M), 2  $\mu$ L DNA template, and 9.5  $\mu$ L nuclease-free water. PCR amplification was conducted with an initial denaturation at 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 25 s, annealing at 55 °C for 15 s, and extension at 72 °C for 15 s, with a final extension at 72 °C for 7 min.

The amplified PCR products were separated using 6% polyacrylamide gel electrophoresis with SB 1 $\times$  buffer and visualized by silver nitrate staining. Fragment separation and documentation were performed using a Cole-Parmer Dedicated Height Sequencer [16].

TABLE I: POLYMORPHIC PRIMERS USED FOR DNA AMPLIFICATION OF BANANA SAMPLES

No	Gene Target	Primer Name	Sequence (5'-3')	Length (bp)	Annealing Temperature (°C)
1	Ma-1-27	F- Ma-1-27	TGAATCCCAAGTTTGGTCAAG	21	55
2		R- Ma-1-27	CAAAACACTGTCCCCATCTC	20	
3	Ma-1-17	F- Ma-1-17	AGGCGGGGAATCGGTAGA	18	55
4		R- Ma-1-17	GGCGGGAGACAGATGGAGT	19	
5	MaCIR108	F- MaCIR108	TTTGATGTACAATGGTGTTC	22	55
6		R- MaCIR108	TTAAAGGTGGTTAGCATTAGG	22	
7	MaCIR327b	F- MaCIR327b	AAGTTAGGTCAAGATAGTGGGATTT	25	50
8		R- MaCIR327b	CTTTTGCACCAGTTGTTAGGG	21	

### D. Data Analysis

Amplified DNA bands were scored manually, and alleles were coded numerically (1, 2, 3, etc.) according to their fragment sizes. The scoring results were converted into allelic data and compiled to generate a genetic distance matrix and estimate genetic parameters, including the average number of alleles, heterozygosity, and the percentage of polymorphic loci using GenAlEx software [17]. The polymorphism information content (PIC) values were calculated using PowerMarker v3.25. A phylogenetic tree was constructed using MEGA X software.

## III. RESULTS AND DISCUSSIONS

The quality of genomic DNA was evaluated using 0.8% agarose gel electrophoresis (Fig. 1), which showed clear and distinct DNA bands in all six Tongka Langit banana samples. Samples 1–5 exhibited brighter band intensity compared to sample 6, indicating differences in DNA concentration among samples. Distinct and clearly defined band patterns indicate good DNA quality, although slight smearing was observed, the DNA bands remained visible in all samples [18]. Smearing below the DNA bands may indicate partial DNA degradation,

whereas accumulation at the bottom of the lane suggests RNA contamination [19]. In this study, no accumulation was observed at the bottom of the lanes, indicating the absence of RNA contamination.

DNA quantity and purity were further analyzed using a NanoDrop spectrophotometer by measuring absorbance at 260, 280, and 230 nm. The  $A_{260}/A_{280}$  ratios for the six samples ranged from 1.92 to 2.00, indicating good DNA purity and the absence of protein contamination [20]. Meanwhile,  $A_{260}/A_{230}$  ratios ranged from 1.80–1.99, suggesting the possible presence of minor contaminants such as phenolic compounds or carbohydrates, as the ideal  $A_{260}/A_{230}$  ratio is approximately 2.0–2.2 [21]. These results indicate that the extracted DNA was of sufficient quality for downstream molecular analysis, although minor smearing was observed in the gel, which may be related to residual contaminants.

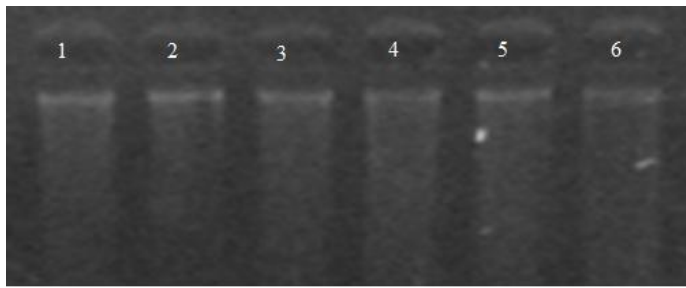


Fig. 1. Visualization of DNA band patterns from genomic DNA isolation of six Tongka Langit banana samples: (1) Tongka Langit banana Kilo 8; (2) Tongka Langit banana Kilo 9; (3) Tongka Langit banana Kilo 10; (4) Tongka Langit banana Fangamas; (5) Tongka Langit banana Rahareing; and (6) Tongka Langit banana Ngefuit Atas.

The DNA concentration obtained in this study varied among samples, with the lowest concentration observed in the Ngefuit Atas sample (1048 ng/ $\mu$ L) and the highest in the Kilo 8 sample (1533 ng/ $\mu$ L) (Table 2). Since the optimal DNA concentration for PCR amplification ranges from 10–100 ng/ $\mu$ L, dilution was required to standardize the DNA concentration across samples. The dilution process was calculated carefully to ensure uniform template concentration for subsequent analysis. In this study, SSR markers were used for DNA amplification, which typically requires at least 10  $\mu$ L of DNA template for PCR reactions. The recommended DNA template concentration for PCR amplification ranges from 10–100 ng/ $\mu$ L, indicating that the DNA concentrations obtained from the six Tongka Langit banana samples were adequate to be used as stock DNA for amplification [22]. This result aligns with previous findings showing that SSR markers require only small amounts of DNA and can still be effectively amplified even when the DNA quality is moderate [23].

TABLE II: QUANTITATIVE ANALYSIS RESULTS USING A NANODROP SPECTROPHOTOMETER

No	Sample	DNA Concentration (ng/ $\mu$ L)	DNA Purity $A_{260}/A_{280}$	DNA Purity $A_{260}/A_{230}$
1	Kilo 8	<b>1533</b>	1,98	<b>1,80</b>
2	Kilo 9	1247	<b>1,92</b>	1,90
3	Kilo 10	1396	1,97	1,85
4	Fangamas	1298	1,94	1,89
5	Rahareing	1455	<b>2,00</b>	1,95
6	NgefuitAtas	<b>1048</b>	1,96	<b>1,99</b>

Amplification of Tongka Langit banana DNA using 6% polyacrylamide gel electrophoresis showed clear separation of DNA fragments, allowing the banding patterns to be easily visualized and scored (Fig. 2). This method is suitable for separating DNA fragments ranging from 80–800 bp [24]–[27].

Four SSR primers (Ma-1-27, Ma-1-17, MaCIR108, and MaCIR327b) generated highly polymorphic banding patterns across the six samples, producing a total of 48 DNA bands. Each locus produced 12 polymorphic bands with a polymorphism level of 100%. The fragment sizes ranged from 122 to 436 bp and varied in band intensity, which may be influenced by primer binding sites, genome purity, and DNA

concentration. According to [28], a single band indicates a homozygous allele, whereas two bands indicate heterozygosity. The presence of multiple polymorphic bands suggests a high level of genetic diversity among the Tongka Langit banana samples, consistent with previous studies using microsatellite markers [29].

Genetic distance analysis was used to assess the genetic variation among the six Tongka Langit banana samples using four SSR primers, with values ranging from 0.6 to 0.8. According to [30], genetic diversity is categorized as low (0.1–0.4), moderate (0.5–0.7), and high (0.8–1.0).

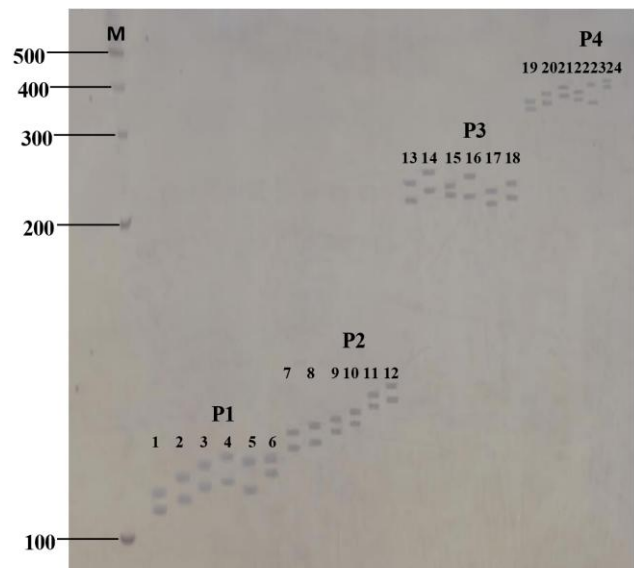


Fig. 2. Visualization of DNA amplification of Tongka Langit banana using four polymorphic SSR primers on 6% polyacrylamide gel electrophoresis. M = 100 bp DNA ladder; P1 = primer Ma-1-127; P2 = primer Ma-1-17; P3 = primer MaCIR108; P4 = primer MaCIR327b. Tongka Langit banana samples: Kilo\_8 (lanes 1, 7, 13, 21); Kilo\_9 (lanes 2, 8, 14, 20); Kilo\_10 (lanes 3, 9, 15, 21); Fangamas (lanes 4, 10, 16, 22); Rahareing (lanes 5, 11, 17, 23); and Ngefuit Atas (lanes 6, 12, 18, 24).

Based on this classification, the samples in this study showed moderate to high genetic diversity. The highest genetic distance value (0.8) was observed among several sample pairs, including Kilo 8–Fangamas, Kilo 8–Rahareing, Kilo 8–Ngefuit Atas, Kilo 9–Kilo 10, Kilo 9–Fangamas, Kilo 9–Ngefuit Atas, Kilo 10–Fangamas, Kilo 10–Rahareing, Fangamas–Rahareing, Fangamas–Ngefuit Atas, and Rahareing–Ngefuit Atas, indicating low similarity among these samples. The lowest genetic distance (0.6) was found between Ngefuit Atas and Kilo 10, indicating higher genetic similarity [31]. In general, greater genetic distance reflects higher genetic diversity among individuals or populations [32], [33]. Therefore, genetic distance analysis is important for supporting both in situ and ex situ conservation strategies to maintain genetic variation [34].

The genetic distance data obtained were used to construct a phylogenetic tree (phenogram) using MEGA version 10.0.4 with the neighbor-joining method (Table III). This method estimates relationships among sequences by selecting branch lengths that best represent the actual genetic distances [35].

Cluster analysis was used to group samples into clusters and subclusters (Fig. 3) [36], [37].

TABLE III: GENETIC DISTANCES AMONG SIX *MUSA TROGLODYTARUM* SAMPLES

	Kilo 8	Kilo 9	Kilo 10	Fangamas	Rahareing	Ngefuit Atas
Kilo 8	0					
Kilo 9	0.8	0				
Kilo 10	0.7	0.8	0			
Fangamas	0.8	0.8	0.8	0		
Rahareing	0.8	0.7	0.8	0.8	0	
Ngefuit Atas	0.8	0.8	0.6	0.8	0.8	0

The analysis produced two main clusters with a genetic similarity coefficient of 1. Cluster I consisted of two subclusters: subcluster IA contained the Kilo 8 sample, while subcluster IB included Kilo 10 and Ngefuit Atas, which showed relatively high similarity. Cluster II was also divided into two subclusters: subcluster IIA consisted of Kilo 9 and Rahareing, which showed high similarity, while subcluster IIB contained only the Fangamas sample.

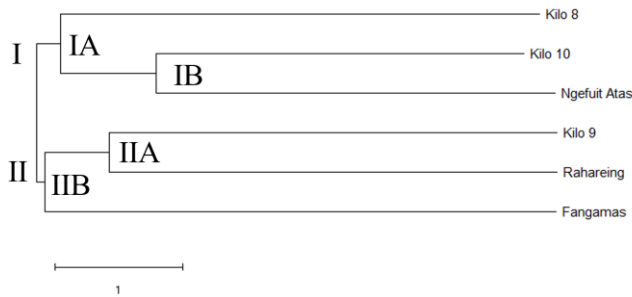


Fig. 3. Phenogram of six tongka langit banana (*Musa troglodytarum* L.) samples based on four SSR primers generated using MEGA version 10.0.4.

These results indicate that clustering patterns are not necessarily related to geographic distribution but are influenced by genetic factors [38]. For example, the Kilo 8 and Fangamas samples formed separate subclusters, indicating low similarity. Similarity levels observed among individuals in a phenogram represent their genetic distance, where higher similarity corresponds to closer genetic relationships and reduced genetic diversity, potentially increasing the risk of inbreeding. In contrast, lower similarity indicates greater genetic distance and higher genetic diversity. Such high genetic diversity is advantageous in plant breeding, as it supports the selection of suitable parental lines for crossing and improves plant adaptability to varying environmental conditions [26], [37]–[39].

#### IV. CONCLUSION

The analysis of Tongka Langit banana using four polymorphic SSR primers produced a total of 48 polymorphic bands with genetic distances ranging from 0.6 to 0.8, indicating moderate to high genetic diversity among the samples. The highest genetic distance (0.8) was observed among several sample pairs, including Kilo 8 with Fangamas, Rahareing, and Ngefuit Atas; Kilo 9 with Kilo 10, Fangamas, and Ngefuit Atas;

Kilo 10 with Fangamas and Rahareing; Fangamas with Rahareing and Ngefuit Atas; and Rahareing with Ngefuit Atas. Phenogram construction based on genetic distance formed two major clades, where samples Kilo 8 and Kilo 10 in clade I showed high similarity comparable to Kilo 9 and Rahareing in clade II, while Kilo 8 and Ngefuit Atas in clade I, as well as Kilo 9 and Fangamas in clade II, showed lower similarity. These results indicate that Tongka Langit banana populations possess considerable genetic variation, which is important for germplasm conservation and can support future breeding programs, particularly by utilizing individuals with the highest genetic distances.

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**Alveus Saloy Mose** was born in Maluku, Indonesia. He received the B.Sc. degree in biology from Pattimura University, Ambon, Indonesia, and is currently pursuing the M.Sc. degree in biotechnology at the School of Life Sciences and Technology, Institut Teknologi Bandung (ITB), Bandung, Indonesia. His major field of study focuses on plant biotechnology, molecular genetics, and plant molecular biology. He is currently a graduate researcher at the School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, Indonesia.

His research focuses on molecular marker analysis, plant molecular genetics, and plant breeding using molecular approaches. He has been involved in several research projects related to plant genetic resources and molecular characterization using SSR markers. His research interests include plant molecular genetics, plant biotechnology, genetic diversity analysis, and molecular approaches for crop improvement.

Mr. Mose is a recipient of the Indonesia Endowment Fund for Education (LPDP) Scholarship from the Ministry of Finance of the Republic of Indonesia. His current research focuses on the regulation of miRNA and target genes in banana fruit associated with the delay of fruit ripening.