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The Molecular Characterization of Local Bengkulu Ambon Banana Through Chloroplast Simple Sequence Repeats (SSR) Markers

Rini Suryani[1*], Irfan Suliansyah[2], Warnita Warnita [2], Aprizal Zainal[2] & Sukartini Sukartini[3]

[1]Agricultural Science at University of Andalas, Kampus Limau Manis Padang, Indonesia 25163
[2]Department of Agronomy, college of Agriculture, University of Andalas, Kampus Limau Manis Padang, Indonesia 25163
[3]National Research and Innovation Agency, Jakarta, Indonesia

***Corresponding author email**: **R.S.**: azmi.10juni@gmail.com ; **I.S.**: irfan.suliansyah@yahoo.com ; **W.W.**: warnita@agr.unand.ac.id; **A.Z.**: ap_zainal@yahoo.com; **S.S.**: sukartini.kasmidjan@gmail.com

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Abstract: This study aimed to explore the genetic diversity and molecular relationships of different genotypes of locally cultivated Bengkulu Ambon bananas. The investigation involved the utilization of chloroplast SSR markers and PCR techniques with 12 primers to examine 29 samples of Ambon bananas. The findings revealed monomorphic (uniform pattern) and polymorphic (varying patterns) DNA bands within the samples. In almost all samples, the monomorphic band pattern was found to be dominant at around 450 base pairs. Meanwhile, in 500 base pairs (primer 4), two different monomorphs were found.Additionally, primers 8 and 11 demonstrated multiple fragment band patterns, indicating the presence of polymorphism among the samples. The results of the dendogram analysis show that the level of similarity of all samples is relatively high. However, there are groups that represent genetic diversity. At a similarity level of 0.82 there are three clusters, four clusters at 0.85, seven clusters at 0.9 and so on. Of the 29 accessions studied, it is known that there are 11 samples that are completely genetically similar. This study provides valuable insights into the genetic diversity and relationships among locally cultivated Bengkulu Ambon bananas. The findings contribute to the existing knowledge regarding the molecular characteristics of these bananas and hold significance for conservation and breeding initiatives.

Keywords: Amplification, Genetic diversity, Molecular analysis, Polymorphic.

Introduction

Understanding the origins of plant species is very important, therefore large-scale exploitation is needed to preserve local varietal diversity. It is hoped that the banana germplasm conservation program can reduce the rate of extinction of banana varieties. Identification and exploration are the first steps for banana conservation purposes. Exploration activities are aimed at finding and collecting several local genotypes to enrich existing genetic diversity (Salgotra & Chauhan, 2023).

As is known, bananas are a food source that is rich in nutrients and are very popular in developed and developing countries.

Therefore, it seems that in the future the need for bananas is expected to continue to increase as well as their availability on the market. Apart from that, something that needs attention is the continuity of production (Backiyarani *et al*., 2022).

Bengkulu is one of the provinces in Indonesia that produces quality bananas, especially local Ambon. Banana production in Bengkulu since 2020-2022 has recorded an increase, namely 18,153 tons, 22,492 tons and 91,550 tons. The three areas in Bengkulu that produce the largest bananas are Rejang Lebong, North Bengkulu and Kepahiang. Even though banana production in each province has increased, in fact there are fluctuations in production in each region (Rahayuniati *et al*., 2021).

In Bengkulu, Curup Ambon (one of local Ambon) is a very famous high quality table banana. Bananas (with the AAA genome) originating from Curup in the Rejang Lebong area have high market prospects as regional souvenirs. Ambon Curup bananas have a sweet taste, soft flesh and are only found in the Rejang Lebong area (Efendi & Hidayat, 2018). This large market share opens up opportunities for exploration activities of bananas like Curup Ambon or other AAA genomes. However, it is necessary to ascertain the level of similarity and/or dissimilarity in the characteristics of several bananas of the Curup Ambon type found.

The main target of exploration activities is to determine similarities and/or differences in plant morphological characters. Molecular analysis is used to ensure there are similarities and/or differences in the characters obtained from the exploration results. This is because molecular characterization analysis can be used to determine similarities and/or differences in banana characters with high accuracy (Kalapchieva *et al*., 2020). According to Agarwal, *et al.* (2008), SSR can be used to analysis DNA and their used is very easy. Beside that markers SSR can be detect genetic diversity based on differences in DNA polimorphisme level (Andarini *et al*, 2023). Siew *et al* (2018) said that moleculer markers data is more accurate and stable because DNA is not influenced by the environment. SSR markers have locus thant do not require primer design (Cui, *et al*, 2017; Khalil *et al*., 2020), and according to Zhao *et al.* (2014) the advantage of SSR is that the process is fast spread throught out the genome.

In general, local Ambon farmers cultivate it not in a monoculture manner. Therefore, it is very possible for cross-pollination to occur with other cultivated bananas or even wild bananas growing nearby. In bananas, cross-pollination can not only change genome number but also genome composition. On the other hand, Chloroplasts have semi-independent, circular, double-stranded DNA (Dobrogojski *et al*., 2020; Kalapchieva *et al*., 2020). The size of the chloroplast genome (plastome) ranges from 110 to 200 kb (Takamatasu *et al*., 2018). The use of chloroplast DNA (cpDNA) sequences makes it possible to differentiate banana accessions without worrying about the effects of cross-pollination. SSR (simple sequence repeat) DNA markers are widely used to identify DNA genotypes. SSR markers have tandem repeats of 2–6 bp in the core sequence, which are highly repeatable, and are rich in the genome, and the number of repeats at a given location is highly variable (Pei *et al*., 2023).

This study aims to achieve two main objectives: (i) to explain the extent of genetic diversity in the local banana population of Ambon Bengkulu using chloroplast SSR

markers, and (ii) to assess the molecular relationships between various local banana genotypes of Ambon Bengkulu.

Materials & Methods

Molecular analysis started from July 2021 to January 2022. Twenty-nine bananas from exploration were used to determine their molecular characters. Twelve pairs of primers were diluted to a final volume of 100 ml, with 10X dilution achieved by adding 90 ml of ddH2O. DNA extraction using young banana leaf tissue. DNA quantity and quality testing follows these steps:

1. Primer dilution. Twelve pairs of primers were diluted using TE buffer. The dilution was performed to a final volume of 100 ml, with 90 ml of ddH2O added to 10X dilution.
2. DNA extraction: Tissue samples of desired organs, specifically young leaves, were collected as they are easier to extract and yield more precise DNA bands.
3. DNA quantity and quality testing. The accuracy of the spectrometer was tested using 1 µl ddH2O as a blank sample. DNA quantity ($mg.ml^{-1}$) was determined by measuring 1 µl of DNA solution. The quality of the DNA samples was assessed by visualizing and capturing the resulting bands on the gel (1%). DNA ladder was utilized as a reference to assess the quality and quantity of DNA. The power supply was connected to the electrophoresis apparatus, and the gel was run at a voltage of 50 V for 30 minutes or until the DNA migrated. After electrophoresis, the gel was stained by soaking it in 1% Ethidium bromide (40 µl 1% Ethidium bromide/1 liter of water) for 10 minutes. Subsequently, the gel was rinsed in distilled water for 15 minutes. Finally, the gel was placed on a UV transilluminator, and an image was captured. Good-quality DNA will exhibit clear bands, and DNA quantity can be determined by comparing the position of DNA bands with the DNA ladder. If the bands appear unclear, it indicates impure DNA and requires further purification.
4. Temperature gradient analysis was conducted preceding the PCR to determine the optimal annealing temperature for the forward and reverse primers. (Table 1).

Table (1): Base Sequences and Temperature Gradient of the 12 primers.

P	NP	Base Sequence + Primer Temperature	GS
1	mMaCIRcp01	F _ CCC CCA GAA ACG TAT AGG (54,3) R_ TTT CCC TTC GAA T TG TGT (51,5	52,9
2	mMaCIRcp02	F _ TAA CCT CCC CAA CCC TTC (55,6) R_ GTG AAT CCA TGG AGG GTC (54,4)	55,5
3	mMaCIRcp19	F_ GGA CCG TAT CGT GGA ACA (54,7) R_ GCG GAT TCT TTT CAT GTT (51,2)	53
4	mMaCIRcp20	F_ CGA AAC GGG TGG TGA TCT (54,5) R_ GGG GAA TGA ACA TTT GTT TGA (51,5)	53
5	mMaCIRcp25	F_ AAT AAC GGG ACC AAA ACC (50) R_ TCC TTC CTT CGA TTC TCA (49,8)	50
6	mMaCIRcp27	F_ CGG TTC AGG GTA CGA ATA (51,1) R_ CCC CAA AAG TAA AAA GTG (49,0)	50
7	mMaCIRcp29	F_ AGT TGG TAC CAC CCA ACC (54,6) R_ GGC GGA AAT CCA ATA TCT (49,3)	52
8	mMaCIRcp30	F_ AAC AAA CAT TGG GTT TGG (46,5) R_ AGT CCC TCC CTA CAA CTC (54,6)	51,5

9	mMaCIRcp31	F_ TCA ACG AAT GAA GCA GGT (51,6) R_ TAT ATG CGT TTC CGG GTA (50,3)	51
10	mMaCIRcp32	F_ ACC CCC GAC ACA TAA AAT (51,1) R_ CCG CTT CTA TGG GAT GTT (51,5)	51,2
11	mMaCIRcp 33	F_ GGA TGC ATA CGG TTC AAA (50,1) R_ AAA GGC CCA TTC AGA AAC (50,3)	50,2
12	mMaCIRcp 34	F _ TGG TGC GTC CTA ATT TTG (49,7) R_ CGG GAA TTG AGA CAG TTG (50,6)	50,2

Remarks: P = Primer, NP = Name of Primer, GS = Temperature Gradient

Table (1) presents data for each primer, including the base sequences, forward and reverse designations and the corresponding temperature gradients. The temperature gradients help determine the optimal annealing temperature for the primers during the PCR process.

5.Microsatellite amplification using Polymerase Chain Reaction (PCR). The method for amplifying genomic DNA through amplification is presented in the following table (Table 2). Table (2) shows the details of the genomic DNA amplification process. The table outlines the composition of the mixture contained within each PCR tube. Amplification uses a Polymerase Chain Reaction (PCR) machine. Table (2) shows details of the genomic DNA amplification process. One reaction per PCR tube is 25 µl (9 µl of distilled water, 2.5 µl of a 10 µM solution, 12.5 µl of Red mix mig tag, and 1 µl of 20 ng DNA). Furthermore, Table 2 also shows the composition of the mixture in the half reaction. This information will make it easier to carry out amplification.—These measurements illustrate the precise composition of the PCR mixture for both full and half-reactions. This information allows researchers to accurately prepare the PCR tubes for genomic DNA amplification based on the desired reaction volume.

Table (2): Genomic DNA amplification.

1 Reaction					½ reaction				
a.	Distilled water	=	9	µl	a	Distilled water	=	4,5	µl
b.	10 pM	=	2,5	µl	b	10 pM	=	1,25	µl
c.	Red mix mig tag	=	12,5	µl	c	Red mix mig tag	=	6,25	µl
d.	20 ng DNA	=	1	µl	d	20 ng DNA	=	0,5	µl
	Total volume		25	µl		Total volume		12,5	µl

Electrophoresis (Genomic DNA amplification results from PCR machine)

The following the process of microsatellite amplification, the subsequent phase involves the analysis of the amplified DNA fragments, wherein electrophoresis assumes critical role. Electrophoresis, technique employed for segregating DNA fragments according to size and charge, is utilized within microsatellite analysis. The electrophoresis process involved in genomic DNA amplification results from the PCR machine is as follows: Firstly, a 1.2% agarose gel was prepared by weighing 0.96 mg of agarose for an 80 ml SB 1x buffer requirement. The agarose was thoroughly mixed using a heating device, and the mixture was then poured into a mold and allowed to solidify. Next, the prepared gel was placed into the Mufid electrophoresis apparatus, following these steps: (1), the gel was positioned in the center of the buffer tag, (2)

The buffer tag was filled with SB 1x buffer until the gel was fully submerged, (3) The gel wells were filled, starting either from the left or right, with a mixture of 2 µl of 1 Kb ladder reference marker and 1 µl of loading dye. Subsequently, the electrophoresis lid was closed, and (4) 3 µl of the amplified DNA sample was added to the gel. (5) The power was turned on, set at 50 volts, and allowed to run for 60 minutes. The system was then left undisturbed for an additional hour (6). The lid of the container was opened, and the gel was lifted. Those two steps are to facilitate visualization.

Staining and visualization

Staining and visualization involve a series of steps to enhance the visibility of a gel sample. First, the gel is carefully detached from the electrophoresis apparatus and then submerged in an Ethidium bromide solution. This solution combines 40 µl of 1% Ethidium bromide with 1 liter of water. The gel is left to soak in this solution for 10 minutes, allowing the Ethidium bromide to interact with the gel and bind to the DNA molecules within. After the staining process, the gel is removed from the Ethidium bromide solution and placed in a container filled with distilled water.

This water bath serves to remove any excess staining solution from the gel and helps to clear away any unwanted residue. The gel is left in the distilled water for 15 minutes, allowing sufficient time for thorough rinsing. Once the gel has been adequately rinsed, it is carefully transferred to a UV transilluminator, a specialized device to visualize DNA bands. The gel is positioned within the transilluminator, and when the UV light is turned on, it illuminates the gel, causing the Ethidium bromide-bound DNA bands to fluoresce. This fluorescence enables the capture of an image of the gel, facilitating further analysis and interpretation of the DNA sample.

Interpretation results

The captured gel image is inverted to facilitate the scoring process. Scoring is done by assigning a value of 1 for clear DNA bands and 0 if there is no DNA band in the same horizontal line. The higher the similarity of DNA band patterns in the same horizontal line, the lower the diversity between plant samples. Scoring uses an Excel program containing plant sample codes and binary data (1 and 0).

Performing acrylamide gel electrophoresis.

Acrylamide gel electrophoresis separates and analyzes biomolecules, such as proteins and nucleic acids (DNA and RNA), based on their size and charge. It is a versatile and widely used technique that offers researchers a powerful tool for separating, characterizing, and quantifying biomolecules. It is crucial in various biological and biochemical research areas, including molecular biology, genetics, proteomics, and diagnostics (Ross *et al*., 2008; Hajba *et al*., 2023).

Results

This study found that molecular characterization of 29 samples of local Ambon bananas from Bengkulu exhibited monomorphic and polymorphic DNA patterns. Twenty-nine sample types were tested using the PCR technique to determine the resulting fragment band patterns. Each sample was analyzed using 12 primers. The amplification results showed both monomorphic and polymorphic patterns per primer used. The obtained size range was between 450 and 3000 base pairs. Amplification with all primers showed a single band with the same monomorphic design, ranging around 450 base pair.

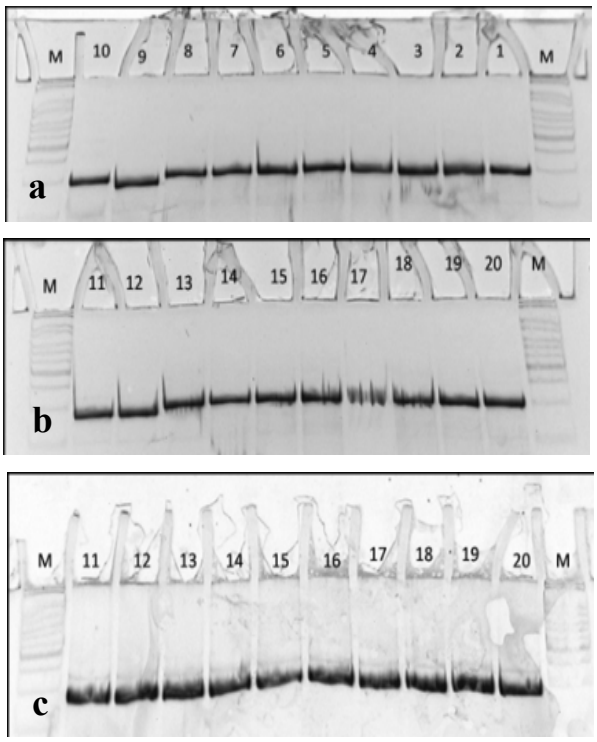

Fig. (1): The Result of Primer Acrylamide 8 and 11 were polymorphic. (a) & (b) Primer 8 sample no. 1-20. (c) Primer 11 smaple no 11-20

However, amplification with primer 4 produced two monomorphic patterns for all samples. At 500 base pairs—amplification of 29 samples using primers 8 and 11 produced a polymorphism band pattern. Primer 8 showed a linear pattern for samples 1 to 8, while samples 9 (Ambon Lumut) and 10 (Ambon Short) showed different designs at 500 base pairs. Primer 11, on sample 15 (Ambon putih), displayed a distinct fragment band pattern compared to other banana samples (Fig. 1). The amplification results, and fragment band scoring can be seen in table (3).

Table (3): Visualization results of amplification for 29 Samples with 12 primers.

Primer	Size (bp)	Monomorphic	Polymorphic
1	450-500	1	1
2	450-500	1	1
3	450-1250	1	2
4	450-3000	2	2
5	450	1	0
6	450	1	0
7	450	1	0
8	450-500	2	3
9	450	1	0
10	450	1	0
11	450-1250	1	3
12	450-1250	1	1

Discussion

Table (3) represented a matrix of similarity levels among the 29 banana samples. This similarity matrix is used to determine the genetic similarity relationships obtained from the scoring of PCR fragment data. The scoring data is based on the presence or absence of bands in the amplified results using 12 primers. Samples with fragments are assigned a value of 1, while models without components are 0. Based on this data, the analysis results indicate that the 29 models have similarity levels ranging from 0.73 to 1. The result shows a high similarity percentage ranging from 73% to 100%.

Genetic diversity values from generation to generation are separated into 3 categories, including low ranging from 0.1 - 0.4, medium ranging from 0.5 - 0.7, and high ranging from 0.8 - 1.0 (Sulistyawati & Widyatmoko, 2017). The smaller (approaching 0) the genetic similarity coefficient value, the further or greater the genetic distance (approaching 1) of the kinship relationship between accessions (Reichert Júnior *et al*., 2021). Samples with high similarity indicate that the growing conditions are the same or similar (Hosseini KorehKhosravi *et al*., 2018).

Genetic similarity can be seen from the coefficient value. The larger (closer to 1) the coefficient value, the more genetically similar they are. (Ccoefficient of gene similarity is 1. Samples 2, 4, 7, 8, 12, 13, 14, 15, 17, 18, and 19 have a coefficient of genetic similarity of 0.96, followed by samples 1, 3, 5, 6, 11, 16, 20, 21, 22, 23, 27, and 29 with a coefficient of genetic similarity of 0.96. The lowest coefficient of gene similarity is 0.81 for samples 9 and 10 compared to all other models. The dendogram (Fig. 3) below shows the relationships of 29 banana accessions based on their genetic similarity. Sample grouping is based on the coefficient value of gene diversity (KG). Three groups are formed at a coefficient of similarity of 0.82. The first cluster consists of 19 samples, namely samples number 1, 3, 2, 4, 5, 6, 7, 8, 11, 16, 12, 13, 14, 15, 17, 18, 19, 20, 26. In this first cluster there are several samples that are exactly the same as a similarity value of 1. These samples are sample number 2 which is exactly the same as sample number 4; sample number 7 with number 8; sample number 12 with 13, 14, 15, 17, 18, and 19. The second cluster consists of samples number 21, 23, 22, 24, 27, 29, 25, and 28. Meanwhile samples number 9 and number 10 are members of the third cluster. The number of clusters is determined by the selected level of similarity. The greater the level of similarity chosen, the greater the number of clusters obtained. For example, if the genetic similarity level is 0.82, then the number of clusters = 3 clusters; at genetic similarity 0.85 = 4 clusters; at 0.9 = 7, and so on. A high level of character similarity (above 0.8) indicates that the 29 samples are almost similar to each other. This is possible because all 29 samples have the same genome, namely AAA. Thus, it can be said that the diversity of the 29 samples is not high. The popularity of Ambon Curup as a high quality table banana should be able to be substituted with other types of Ambon banana. However, in reality, organoleptically, Ambon Curup bananas are of higher quality than the others.

This shows that the difference in quality is not caused by genetic factors. Environmental factors or perhaps the interaction of environment and genetics is what causes Ambon Curup bananas to be superior to other types of Ambon.

For collection and conservation purposes, samples will be more meaningful if they have a large degree of difference in genetic characteristics. If the sample obtained has low genetic diversity, then the sample is selected partly as a representative of the cluster. Cluster analysis was performed to group the samples based on their genetic similarity. Three clusters were formed based on the coefficient of gene diversity. The first cluster consisted of 19 samples, further divided into two sub-clusters and two additional groups. The second cluster included eight samples that formed a sub-cluster, and the third cluster comprised two samples. These clusters and sub-clusters indicate varying genetic similarity and distinct genetic relationships among the banana samples.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	1.00																												
2	0.92	1.00																											
3	0.96	0.96	1.00																										
4	0.92	1.00	0.96	1.00																									
5	0.88	0.96	0.92	0.96	1.00																								
6	0.92	0.92	0.96	0.92	0.88	1.00																							
7	0.88	0.96	0.92	0.96	0.92	0.96	1.00																						
8	0.88	0.96	0.92	0.96	0.92	0.96	1.00	1.00																					
9	0.81	0.88	0.85	0.88	0.85	0.88	0.92	0.92	1.00																				
10	0.77	0.85	0.81	0.85	0.81	0.77	0.81	0.81	0.88	1.00																			
11	0.92	0.85	0.88	0.85	0.81	0.85	0.81	0.81	0.73	0.77	1.00																		
12	0.88	0.88	0.92	0.88	0.92	0.88	0.85	0.85	0.77	0.81	0.88	1.00																	
13	0.88	0.88	0.92	0.88	0.92	0.88	0.85	0.85	0.77	0.81	0.88	1.00	1.00																
14	0.88	0.88	0.92	0.88	0.92	0.88	0.85	0.85	0.77	0.81	0.88	1.00	1.00	1.00															
15	0.88	0.88	0.92	0.88	0.92	0.88	0.85	0.85	0.77	0.81	0.88	1.00	1.00	1.00	1.00														
16	0.88	0.88	0.92	0.88	0.85	0.88	0.85	0.85	0.77	0.81	0.96	0.92	0.92	0.92	0.92	1.00													
17	0.88	0.88	0.92	0.88	0.92	0.88	0.85	0.85	0.77	0.81	0.88	1.00	1.00	1.00	1.00	0.92	1.00												
18	0.88	0.88	0.92	0.88	0.92	0.88	0.85	0.85	0.77	0.81	0.88	1.00	1.00	1.00	1.00	0.92	1.00	1.00											
19	0.88	0.88	0.92	0.88	0.92	0.88	0.85	0.85	0.77	0.81	0.88	1.00	1.00	1.00	1.00	0.92	1.00	1.00	1.00										
20	0.85	0.85	0.88	0.85	0.88	0.85	0.81	0.81	0.73	0.77	0.92	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	1.00									
21	0.81	0.81	0.85	0.81	0.85	0.81	0.77	0.77	0.77	0.81	0.81	0.92	0.92	0.92	0.92	0.85	0.92	0.92	0.92	0.88	1.00								
22	0.73	0.73	0.77	0.73	0.77	0.73	0.69	0.69	0.77	0.88	0.73	0.85	0.85	0.85	0.85	0.77	0.85	0.85	0.85	0.81	0.92	1.00							
23	0.77	0.77	0.81	0.77	0.81	0.77	0.73	0.73	0.73	0.77	0.77	0.88	0.88	0.88	0.88	0.81	0.88	0.88	0.88	0.85	0.96	0.88	1.00						
24	0.77	0.77	0.81	0.77	0.81	0.77	0.73	0.73	0.81	0.85	0.77	0.88	0.88	0.88	0.88	0.81	0.88	0.88	0.88	0.85	0.96	0.96	0.92	1.00					
25	0.77	0.77	0.81	0.77	0.81	0.77	0.73	0.73	0.73	0.77	0.77	0.88	0.88	0.88	0.88	0.81	0.88	0.88	0.88	0.85	0.88	0.81	0.85	0.85	1.00				
26	0.85	0.85	0.88	0.85	0.88	0.85	0.81	0.81	0.81	0.85	0.85	0.96	0.96	0.96	0.96	0.88	0.96	0.96	0.96	0.92	0.96	0.88	0.92	0.92	0.92	1.00			
27	0.77	0.77	0.81	0.77	0.81	0.77	0.73	0.73	0.81	0.85	0.85	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.92	0.88	0.88	0.85	0.92	0.85	0.92	1.00		
28	0.69	0.69	0.73	0.69	0.73	0.69	0.65	0.65	0.73	0.77	0.77	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.85	0.81	0.81	0.77	0.85	0.92	0.85	0.92	1.00	
29	0.73	0.73	0.77	0.73	0.77	0.73	0.69	0.69	0.77	0.88	0.81	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.88	0.85	0.92	0.81	0.88	0.81	0.88	0.96	0.88	1.00

Fig. (2): Matrix of genetic similarity relationships for 29 samples.

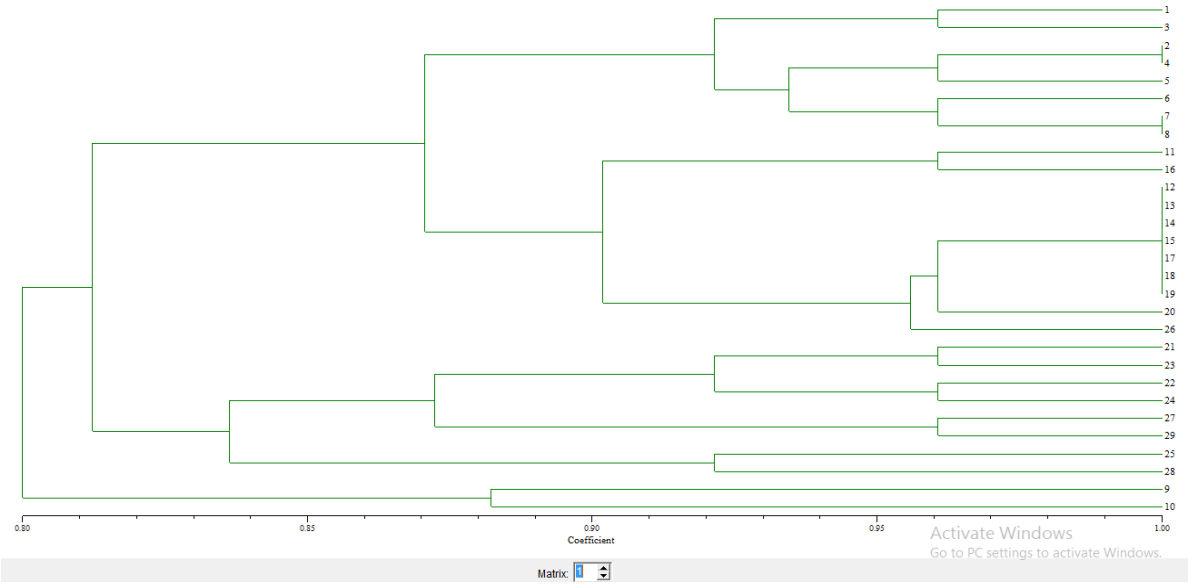

Fig. (3). Dendrogram of genetic similarity relationships for 29 Samples

Remarks: 1: Yellow sambe lama), 2: Yellow lubuk kembang), 3: kuning air bang, 4: kuning talang benih), 5: kuning sukarami, 6: kuning lubuk ubar, 7: kuning PUT, 8: dingin tempel rejo, 9: lumut PUT, 10: pendek dwitunggal, 11: hijau kampung melayu, 12: hijau sambe lama, 13: Hijau simpang nangka, 14: hijau batu galing, 15: putih perbo, 16: putih PUT, 17: Curup lubuk kembang, 18: kuning kemumu, 19: kuning Lais), 20: kuning pondok kelapa, 21: kuning putri hijau, 22: kuning arga makmur, 23: Hijau ketahun, 24: kuning kabawetan, 25: kuning pekalongan, 26: putih merigi, 27: hijau hujan mas, 28: Hijau tebat monok, 29: dingin jalur 2 kepahiang.

The study revealed monomorphic and polymorphic DNA patterns in the Ambon banana samples. The samples exhibited high genetic similarity, with some variations and distinct genetic relationships observed. Furthermore, the dendrogram demonstrates variations in genetic similarity, forming groups based on coefficient distances. These findings provide insights into the genetic diversity and relationships among local Ambon bananas from Bengkulu.

Conclusions

Based on the results of the study, several conclusions can be drawn:

1-The study investigated the molecular characteristics of 29 samples of local Ambon bananas from Bengkulu using the PCR technique with 12 primers. The amplification results revealed monomorphic (uniform pattern) and polymorphic (varying patterns) DNA patterns in the samples. Based on the described analysis results, the overall patterns of PCR fragment bands using 12 primers are monomorphic—only primer number 8 and number 11 show different fragment band patterns.

2-The amplification results with different primers showed varying fragment band patterns. Most samples exhibited a single monomorphic pattern with a size of around 450 base pairs. However, primer 4 resulted in two monomorphic ways at 500 sample base pairs. Primers 8 and 11 also produced multiple fragment band patterns, indicating polymorphism among the samples.

3-The study utilized a similarity matrix to determine the genetic similarity relationships among the 29 banana samples. The scoring data based on the presence or absence of bands in the amplified results showed similarity levels ranging from 0.73 to 1. This score indicates a high genetic similarity percentage of 73% to 100% among the samples.

4-The coefficient of genetic similarity was calculated to assess the genetic relationships among the samples further. The highest coefficient of gene similarity was 1, indicating high similarity. Samples 2, 4, 7, 8, 12, 13, 14, 15, 17, 18, and 19 exhibited a coefficient of genetic similarity of 0.96, while samples 1, 3, 5, 6, 11, 16, 20, 21, 22, 23, 27, and 29 also showed a coefficient of genetic similarity of 0.96. On the other hand, samples 9 and 10 had a lower coefficient of gene similarity (0.81) than the other models.

The current study suggest Research (conventional and non-conventional) is very open to explaining the phenomenon of local Ambonese banana diversity from Bengkulu. Thus, the results of these studies can be used for future breeding and conservation purposes. The mechanisms of environmental influence and environmental-genetic interactions on morphological characters deserve to be studied in more depth.

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Contribution of authors

R.S, conducted and carried out this research study.

I.S., and W.W., designed and supervised this research study.

A.Z. and S.S., analyzed the data and edited the manuscript.

All the authors read and approved the manuscript to be published.

ORCID

R.S., https://orcid.org/0009-0001-1796-7571
I.S., https://orcid.org/0000-0001-7497-229X
W.W.,https://orcid.org/0000-0003-3354-2335
A.Z., https://orcid.org/0009-0000-8371-2748
S.S., https:https://orcid.org/0000-0002-1969-5442

Conflicts of interest

The author declare that they have no conflict of interests.

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التوصيف الجزيئي لموز بنجكولو أمبون المحلي من خلال علامات تكرار تسلسل البلاستيدات الخضراء البسيطة (SSR)

ريني سورياني[1]، عرفان سوليانسياه[2]، وارنيتا وارنيتا [2]، أبريزال زينل[2]، سوكارتيني سوكارتيني[3]

طالبة دكتوراة، في العلوم الزراعية، جامعة ألاندلس، كامبوس ليماو مانيس بادانج، إندونيسيا [1]

قسم الهندسة الزراعية، كلية الزراعة، جامعة ألاندلس، كامبوس ليماو مانيس بادانج، إندونيسيا [2]

معهد بحوث الفواكه الاستوائية، جامعة ألاندلس، كامبوس ليماو مانيس بادانج، إندونيسيا[3]

المستخلص: تهدف هذه الدراسة إلى استكشاف التنوع الجيني والعلاقات الجزيئية للأنماط الجينية المختلفة لنبات الموز صنف Bengkulu Ambon المزروع محليا. وشمل التحقيق استخدام واسمات SSR البلاستيدات الخضراء وتقنيات تفاعل البوليميراز المتسلسل مع 12 بادئا لفحص 29 عينة. كشفت النتائج عن نطاقات أحادية الشكل (نمط موحد) ومتعددة الأشكال (أنماط مختلفة) داخل العينات. في جميع العينات تقريبا، وجد أن نمط النطاق أحادي الشكل هو السائد عند حوالي 450 زوجا من القواعد. وفي الوقت نفسه ، في 500 زوج أساسي (التمهيدي 4) ، تم العثور على اثنين من أحاديات الأشكال المختلفة. بالإضافة إلى ذلك ، أظهر البادئان 8 و 11 أنماط شريط شظايا متعددة ، مما يشير إلى وجود تعدد الأشكال بين العينات. تظهر نتائج تحليل dendogram أن مستوى التشابه بين جميع العينات مرتفع نسبيا. ومع ذلك، هناك مجموعات تمثل التنوع الجيني. عند مستوى التشابه 0.82 وجد هناك ثلاث مجموعات و وجد أربع مجموعات عند 0.85 ، سبع مجموعات عند 0.9 وهكذا. من بين 29 عينة تمت دراستها ، كما لوحظ أن هناك 11 عينة متشابهة وراثيا تماما. تقدم هذه الدراسة رؤى قيمة حول التنوع الجيني والعلاقات بين الموز صنف بنجكولو أمبون المزروع محليا. تساهم النتائج في الحالية فيما يتعلق بالخصائص الجزيئية لهذا الموز ولها أهمية لمبادرات الحفظ والتربية DNA.

الكلمات المفتاحية: التضخيم، التنوع الوراثي، التحليل الجزيئي، تعدد الأشكال.