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Genetic diversity and molecular analysis using RAPD markers of banana cultivars in the five regions of East Java, Indonesia

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Abstract. *Slameto. 2023. Genetic diversity and molecular analysis using RAPD markers of banana cultivars in the five regions of East Java, Indonesia. Biodiversitas 24: 5035-5043.* The study explored banana plants in East Java, Indonesia, specifically in the districts of Jember, Banyuwangi, Bondowoso, Situbondo, and Lumajang, using Random Amplified Polymorphic Deoxyribonucleic Acid (RAPD) markers. Thirty accessions of *Musa* ssp. were collected from these five districts, revealing a high level of genetic diversity. Primers OPA-04 and OPC-05 were suitable for assessing genetic variation in banana plants. It was found that the OPD-07 primer produced the fewest DNA bands (8 bands and 7 polymorphic bands), while the OPA-04 primer produced the maximum number of DNA bands (14 bands, with 9 polymorphic bands). The OPB-08 primer had the lowest polymorphism percentage (56%), and the OPC-05 primer had the greatest percentage (92%). With genetic similarity scores ranging from 0.04 (Bigih Tanjung Glugur and Lilin Banyuwangi) to 0.94 (Agung Banyuwangi and Let). The phylogenetic tree of 30 banana plants formed two primary clusters: cluster I and II, included 26 and 4 banana varieties respectively. In addition, molecular variance (AMOVA) resulted a significant proportion (93%) of genetic diversity within the population. Further studies using more precise genetic markers are still needed to determine the exact identity of the banana plant genome.

Keywords: Banana, exploration, genetic diversity, molecular phylogeny, RAPD

INTRODUCTION

Bananas (*Musa* spp.) is one of the most important fruit trees in the world, and has been cultivated by humans since the beginning of agriculture (Padam et al. 2014). Various types of bananas are essential and nutritious part of the diets of millions people living in the tropical and subtropical regions. Since 2019, global banana production has continuously exceeded the 100 million ton mark, with total production reaching approximately 20,073 million ton in 2021 (FAO 2022). Indonesia's banana production currently stands at 9.6 million tons, with Java Island accounting for 4.5 million tons. The origin and diversity of cultivated bananas are assumed to be centered in Southeast Asia, particularly in Indonesia. Both wild species and domesticated kinds of bananas are abundant in the country (Simmonds 1959; Nasution 1991; Sulistyaningsih 2016; Harto et al. 2019). There are approximately 30 species of two-seeded bananas included in the Eumusa range; *Musa acuminata* and *Musa balbisiana* contributed to the A and B genomes, respectively, and are the source of most varieties used for food. Edible genetic variations fall into six categories: AA, AAA, AB, AAB, ABB, and ABBB. These are based on their ploidy level and a taxonomic scoring system that takes into account 15 morphological features (Simmonds and Shepherd 1955). The future sustainability of banana cultivation requires better banana plants, but existing breeding efforts for resistant varieties are based on too narrow of genetic variation basis. Breeding needs to incorporate more diversity, notably from resilient local landraces and wild species. Furthermore, climate change is expected to induce structural changes in these wild and domestic genetic resources. According to numerous research, the primitive *M. balbisiana* species is more resilient to biotic and abiotic stress, such as the dry season, than *M. acuminata*. Therefore, research is required to examine the natural and produced banana diversity in Indonesia and to comprehend how this diversity is organized following climatic factors as well as the taste and demands of the local population. The first edible bananas are thought to have arisen through natural hybridization between two subspecies: *Musa acuminata* (contributing to the A genome) and *Musa balbisiana* (contributing to the B genome). Over time, farmers have selectively cultivated and domesticated these bananas from the descendants of one or both of these wild parent species. Therefore, banana varieties can be divided into AA and AB (diploid), AAA, AAB, ABB and BBB (triploid), AAAA, AABB, and ABBB (tetraploid), based on the combination of genomes they carry (Simmonds 1959). Entire genomes can be randomly amplified using the Random Amplified Polymorphic DNA (RAPD) profiling method, which uses single primers of arbitrary nucleotide sequences. RAPD is a reliable method to determine the presence or absence of banana varieties (Premabati et al. 2013; Kiran et al. 2015) and other species (Shu et al. 2003; Tsuda et al. 2004; Khaled et al. 2015; Thangjam and Thangjam 2017; Bi et al. 2021; Wahibah et al. 2023).

Kasiamdari et al. (2019) and Wahibah et al. (2023) used RAPD to analyze genetic variation in a cost-effective manner.

RAPD has also been used to evaluate banana genetic species and assess banana genetic diversity (Mukunthakumar et al. 2013; Poerba and Ahmad 2013; Izquierdo et al. 2014; Lamare and Rao 2015; Hinge et al. 2022). Furthermore, Lamare and Rao (2015) used the RAPD to analyze the genetic diversity, population size, and genetic composition of *Musa* species.

The primary objective of this study is to conduct a comprehensive survey on banana diversity in East Java, with a special focus on understanding habitat characteristics. Currently, there is no database listing different types of bananas in Indonesia. This is mainly due to lack of prior research in this area. Therefore, the aim of this study is to fill this knowledge gap by addressing the genetic diversity and molecular analysis of bananas and list the banana varieties found in the five regions of East Java.

MATERIALS AND METHODS

Exploration of banana plants in five districts in East Java

Exploring banana plants was employed in the East Java Province, Indonesia, which included five districts: Lumajang, Jember, Bondowoso, Situbondo, and Banyuwangi (Figure 1). The exploration of plant sampling used surveys and direct observation in the field. To collect information about the selected locations, interviews were conducted with local community members and banana traders at the local fruit markets, which are considered banana cultivation centers or banana cultivation areas. Data was also collected from local land stakeholders and local government agency personnel.

The target genomes were cultivated AA, BBssp/ variety, AA (unique like banana fruit), and unknown AAB/ ABB bananas. Direct surveys were conducted within the district as a part of exploratory study. Interviews were conducted with local communities, banana traders, and/or local farmers who recognize the area as a banana production area. Information was also collected from national stakeholders in the region and heads of national agricultural authorities. Extensive information about banana plants was inventoried, recorded, and documented.

Identification of banana genomes

The genome identification was conducted using Simmonds and Shepherd's genome-based nomenclature system (Simmonds and Shepherd 1955). Basic characteristics that distinguish cultivars of *Musa acuminata* from *Musa balbisiana* and hybrids include pseudo stem color, curvature of leaf and flower stalks, ovules, back and roll of inflorescence petals, inflorescence shape, tip and color, male flower petals, color, and stigma color. Therefore, in order to observe the similarity index and dendrogram of banana genotype associations, Writer used the SPSS program uses the average linkage (between-groups) hierarchical clustering method to collect evaluation data from characteristic observations. was calculated.

Molecular characterization

Fresh leaves of banana cultivars were selected from the germplasm collection, immersed in liquid nitrogen, and stored at 70°C until the DNA extraction. Total genomic DNA was extracted from preserved leaves using a modified CTAB method and stored at -20°C for molecular analysis.

Random Amplified Polymorphic DNA Analysis (RAPD)

RAPD-PCR was performed using the following 7 primers: OPA-04 AATCGGGCTC, OPA-08 GTGACGTAGG, OPB-12 CCTTGACGCA, OPC-04 CCGCATCTAC, OPC-05 GATGACCGCC, OPC-07 GTCCCGACGA, OPD-07 TTGGCACGGG.

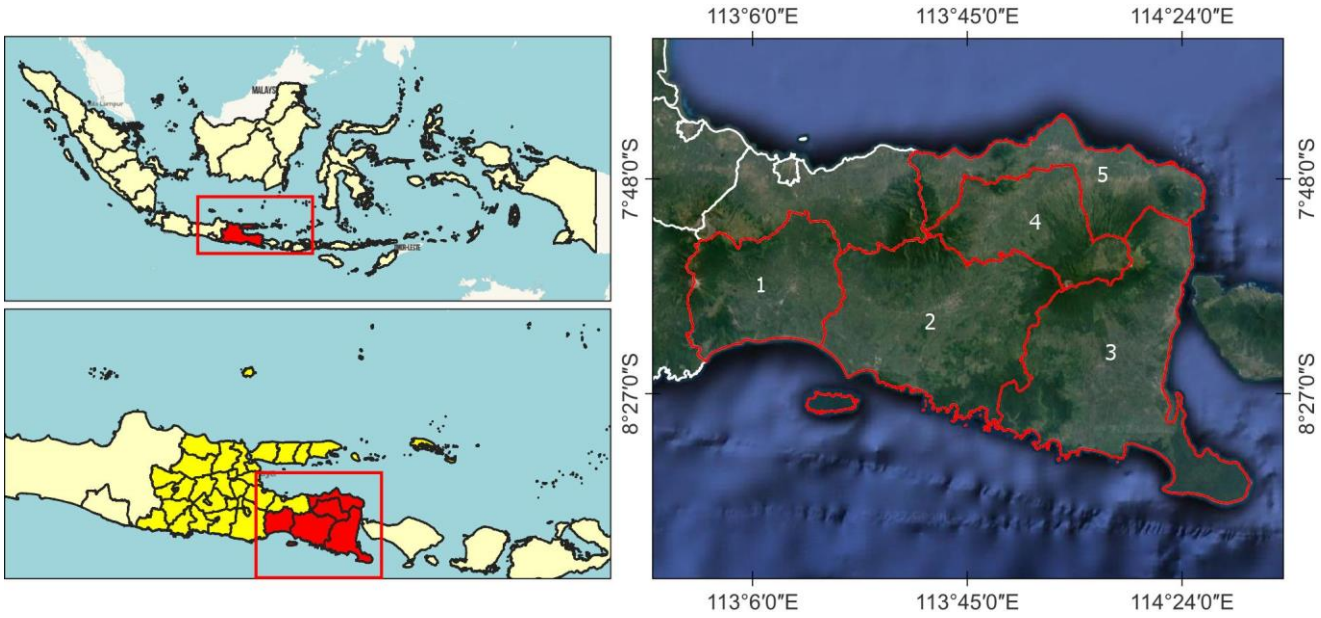

Figure 1. Location map of banana exploration in five districts of East Java Province, Indonesia: 1. Lumajang, 2. Jember, 3. Banyuwangi, 4. Bondowoso, and 5. Situbondo

PCR reactions were carried out per the published Kit's manual, and RAPD data analysis was completed as shown in Table 1. Gel analysis of the electrophoresis results was then performed and each DNA band present or absent in RAPD was assigned a score of 0/1. According to Shiddalingeswara et al. (2018), calculations were made to determine the total number of bands, the number of polymorphic bands, and the amount of polymorphic information. PCR was performed in a total volume 10 µL with Type Volume, 5µL Master MIX My Taq Bioline, 0.5 µL primers, 2 µL template DNA, and 2.5 µL ddH_2O.

The amplification protocol was as follows: Pre-denaturation at 95ºC for 3 minutes, denaturation at 95ºC for 15 seconds, annealing in 30 seconds for the primer OPA-04 at 32ºC, OPA-08 and OPC-04 at 30ºC, OPB-12 at 32ºC, OPC-05 and OPC-07 at 36ºC, and OPD-07 at 38ºC. Then, 2% of DNA amplification fragments were separated and stained with 3 µL of Red Safe. Amplifications were performed in a Techne Prime Thermal Cyclers.

Analysis of Molecular variation (AMOVA) with GenAIEx 6.503 (Peakall and Smouse 2006), which divided the overall genetic variation into "among populations" and "within populations," was used to construct Analysis of Molecular Variance (AMOVA) input files.

Standardized morphological data and similarity matrices were calculated using coefficient simple matching. In this study, a manual scoring process was carried out to assess the presence of distinct and clearly visible bands within DNA fragments. A score of (1) was given if the band was present, while a score of (0) indicated the absence of a band. These evaluation results were collected as binary data and used for cluster analysis using the unweighted pair group method with the arithmetic averaging (UPGMA) method. Consistent and polymorphic bands were used to create a binary matrix for statistical analysis using NTSYS-pc 2.1.1. In addition, the similarity matrix for the RAPD data was calculated using coefficient method of Jaccard (1908).

RESULTS AND DISCUSSION

Banana's inventory from five districts in East Java

In this study, the exploration results found that all banana plants in five regencies were cultivated bananas. Exploration was carried out outside the residential areas but partly on the outskirts of the forest, locally name-moor. From the exploration, 30 bananas were collected (Table 1). They consisted of 7 diploid cultivars and 23 triploid cultivars. Further, according to the consumption type of cultivars by the local community, 16 were consumed as a dessert, 12 for cooking/processing, and 2 banana types were used for both. Following the residents' preferences, the 2 types of bananas were found for dessert and processed in five regencies: Rojo Temen and Nangka.

Table 1. Inventory of bananas collected from five districts in East Java, Indonesia

Local name	Code	Consumed as	Locality	Habitat	Genome (morphology)
Musang	V1	Dessert	Jember	Moor/Yard	AAA
Palembang	V2	Dessert	Banyuwangi	Moor/Yard	AAA
Rayap	V3	Dessert	Jember	Moor/Yard	AA
Candi Matahari	V4	Processed	Jember	Moor/Yard	AAB
Usuk	V5	Processed	Jember	Moor/Yard	AAA
Agung Banyuwangi	V6	Processed	Banyuwangi	Moor/Yard	AAA
Mas Kuring	V7	Dessert	Jember	Yard	AA
Let	V8	Dessert	Lumajang	Moor/Yard	ABB
Selendang	V9	Processed	Jember	Moor/Yard	AAA
Pandhek	V10	Processed	Situbondo	Moor/Yard	AAA
Bigih	V11	Processed	Situbondo	Moor/Yard	BB
Pakak Madu	V12	Processed	Bondowoso	Moor/Yard	AAA
Sabha	V13	Processed	Situbondo	Moor/Yard	ABB
Cavendish	V14	Dessert	Lumajang	Moor/Yard	AAA
Agung Lumajang	V15	Processed	Lumajang	Moor/Yard	AAB
Mas	V16	Dessert	Jember	Yard	AA
Susu	V17	Dessert	Jember	Moor/Yard	AAA
Rojo Temen	V18	Dessert/ Processed	Bondowoso	Moor/Yard	AAB
Kosta	V19	Processed	Situbondo	Moor/Yard	ABB
Makassar	V20	Processed	Jember	Moor/Yard	ABB
Berlin	V21	Dessert	Situbondo	Yard	AA
Lumut	V22	Dessert	Situbondo	Moor/Yard	AAA
Lilin	V23	Dessert	Banyuwangi	Yard	AA
Sebulan	V24	Dessert	Jember	Yard	AA
Ijo	V25	Dessert	Jember	Moor/Yard	AAA
Kayu	V26	Processed	Jember	Moor/Yard	AAA
Mbuk	V27	Dessert	Bondowoso	Moor/Yard	AAA
Puspo	V28	Dessert	Banyuwangi	Yard	ABB
Slangket	V29	Dessert	Banyuwangi	Moor/Yard	AAB
Nangka	V30	Dessert/ Processed	Bondowoso	Moor/Yard	AAB

Quantification of DNA from thirty banana varieties

Table 2 details the RAPD profiles established in banana samples studied using 7 RAPD primers. RAPD markers can detect genetic diversity and grouping based on DNA banding patterns, which can indicate the presence or absence of carriers a gene or allele of interest (Čížková et al. 2015). The 7 RAPD primers generated a total of 77 DNA bands (Figure 2). In previous studies, RAPD primers have been widely used to study the genetic diversity of banana plants from different countries. Each RAPD primer used has a different polymorphism due to some genetic variation differences between different accessions (Kiran et al. 2015). CR-RAPD technology has high efficiency in amplifying DNA fragments. Remarkably, this method requires only a minimal quantity of DNA buffer to replicate specific DNA fragments (Susilo et al. 2018). When 7 RAPD primers were used for amplification, multiple DNA fragments with different bands were generated. Primer OPA-04, in particular, resulted in the formation of 14 bands, 9 bands, of which showed polymorphism. Primer OPB-08 produces 9 bands, 5 bands are polymorphic, and primer OPB-12 has 13 bands, of which 11 are polymorphic. Primer OPC-04 produced 10 bands, of which 9 bands are polymorphic. Primer OPC-05 produced 13 bands, and 12 bands being polymorphic. Primer OPC-07 produced 10 bands, 7 of which were polymorphic. Furthermore, primer OPD-07 produced 8 bands which 7 bands were polymorphic. the OPA-04 primer produced the most DNA bands, 14 bands, including 9 polymorphic bands, while the OPD-07 primer produced the least number of DNA bands, 8 bands and 7 polymorphic bands.

This study various levels of polymorphism induced by 7 RAPD primers. The OPB-08 primer had the lowest percentage of polymorphisms (56%), and the OPC-05 primer had the highest percentage of polymorphism (92%). Several studies demonstrated that the RAPD marker was successful in producing stable and readable bands for genetic evaluation among 5 cultivars of the genus *Musa* spp. Susilo et al. (2018) reported that primer OPB 1-04 achieved the highest polymorphism with 100%, while the primer OPB 4 obtained 25% of polymorphism. The greater the number of polymorphic bands produced by a primer, the higher the percentage of polymorphisms of a primer will be gained. Otherwise, the high polymorphism of a primer is influenced by the complementary arrangement of nucleotide bases found on its primer and the total locus amplified by the RAPD primer (Hinge et al. 2022).

Furthermore, these results indicate that the OPA-04 and OPC-05 primers are suitable for detecting genetic diversity in banana cultivars based on the number of polymorphic bands and polymorphism. Determining the optimal primers in genetic diversity studies is based on the number of DNA bands produced in the PCR products, the number of polymorphic DNA bands, and the percentage of polymorphism. (Probojati et al. 2019). Based on the large number of DNA bands generated by the PCR products and the proportion of polymorphisms, the primers OPA-04 and OPC-05 can thus be recommended as the best primers in investigating genetic diversity in banana plants. Significant polymorphisms are a sign of the significant genetic diversity in a species. Some other RAPD primers suitable for genetic diversity analysis of banana plants based on high polymorphism of 100% include OPC-08, OPA-10, OPA-11, and OPA-12 (Hinge et al. 2022). The RAPD primer mechanism, which involves the amplification of genomic DNA using short oligonucleotide primers with random sequences, played an important role in generating the high levels of polymorphism observed in this study. This high polymorphism facilitated the identification of complementary base pairs between the genomic DNA and the primers, simplifying the DNA amplification process (Verma et al. 2017).

Using RAPD markers, the current study was conducted to assess the level of genetic diversity in banana cultivars. Polymorphism information is necessary for genetic diversity studies and indicates the efficiency of primers efficiency in identifying genetic diversity in banana plants. In Addition, polymorphism indicates the success of the primers in identifying genetic diversity within the sample. The values above 50% are considered highly polymorphic. (Tawfik et al. 2019). However, studies on polymorphism that compare RAPD and ISSR primers have not determined the best primer. This is supported by the results of a study on the genetic diversity of *Prunus salicina* L. plants in which RAPD primers produced 81.59% polymorphism and ISSR primers produced 87.74% polymorphism (Li et al. 2022). In another study on the genetic diversity of five kalanchoe plant (Al-Khairy et al. 2022), RAPD primer showed a higher polymorphism of 60.25% compared to 15% for ISSR primer. Then, the ISSR primer was used to evaluate the molecular diversity of banana cultivars; the ISSR markers showed an average polymorphism of 79.62%; of all the ISSR markers used, the five ISSR primers (ISSR-810, ISSR818, ISSR-826, ISSR-857 and ISSR-858) generated 100% of the polymorphisms (Hinge et al. 2022). Hence, the use of DNA fingerprinting techniques combined with botanical and physiological evaluations provides a solid foundation biological selection and conservation procedures of different banana.

Dendrogram based on RAPD markers

Genetic diversity analysis of banana plants using RAPD primers is shown through a kinship tree/dendrogram to determine the hierarchical relationship between one genotype and another (Figure 3). This made it easier to determine the parents for breeding programs.

Table 2. List of primers and their sequences used for RAPD analysis of 30 accessions of *M. acuminata*

Primer	Sequence (5'-3 ')	Total DNA band	Polymorphic bands	Polymorphism (%)
OPA-04	AAT CGG GCT C	14	9	64%
OPB-08	GTG ACG TAG G	9	5	56%
OPB-12	CCT TGA CGC A	13	11	85%
OPC-04	CCG CAT CTA C	10	9	90%
OPC-05	GAT GAC CGC C	13	12	92%
OPC-07	GTC CCG ACG A	10	7	70%
OPD-07	TTG GCA CGG G	8	7	88%
	Total	77	60	544%
	Mean	11	8,57	78%

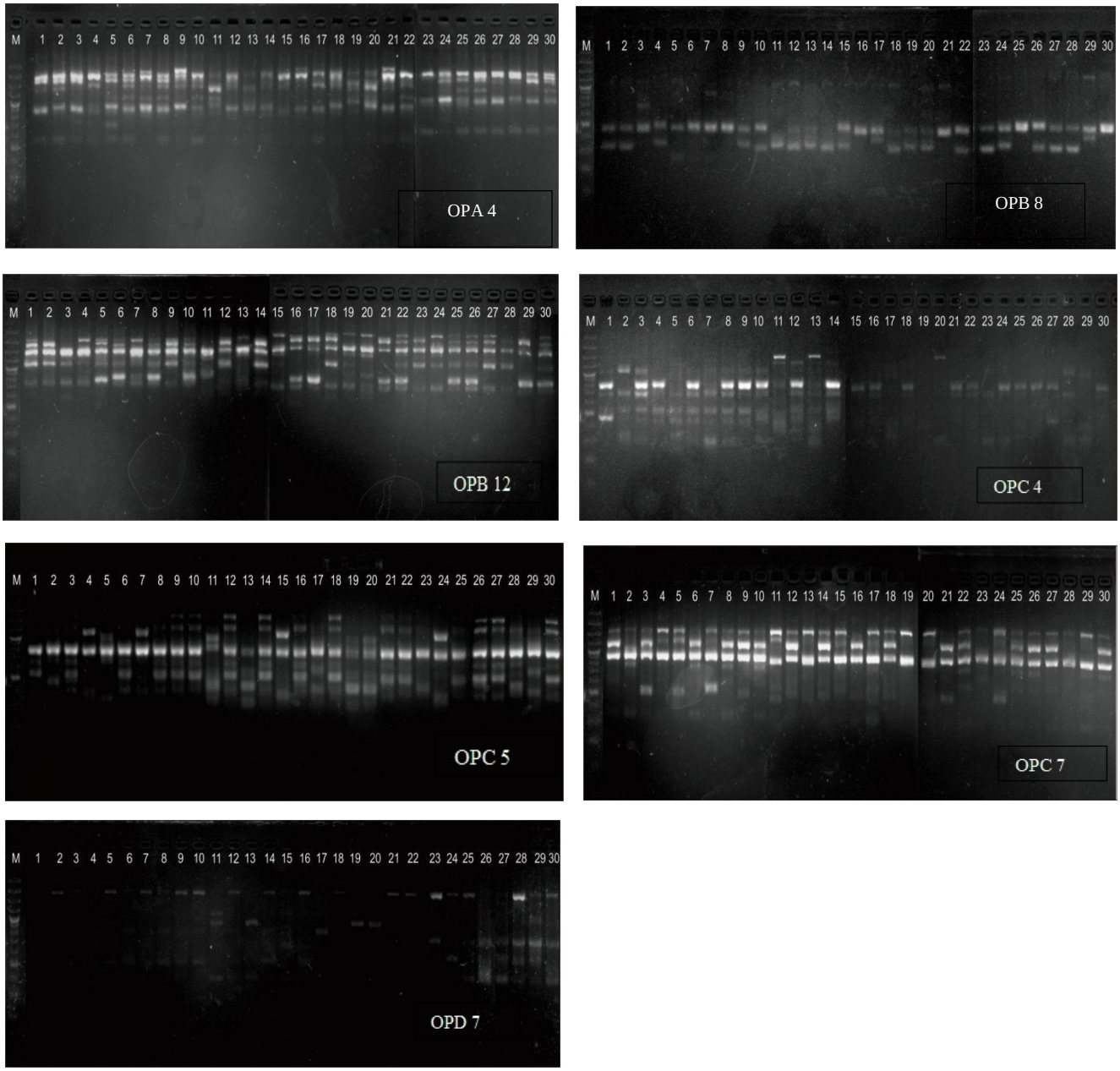

Figure 2. RAPD profile of primers OPA 4, OPB 8, OPB 12, OPC 4, OPC 5, OPC 7, and OPD 7. M: 100 bp molecular ladder. Banana cultivar: 1. Musang, 2. Palembang, 3. Rayap, 4. Candi Matahari, 5. Usuk, 6. Agung Bannyuwangi, 7. Mas Kuning, 8. Let, 9. Selendang, 10. Pandhek, 11. Bigih, 12. Pakak Madu, 13. Sabha, 14. Cavendish, 15. Agung Lumajang, 16. Mas, 17. Susu, 18. Rojo Temen, 19 Kosta, 20. Makasar, 21. Berlin, 22. Lumut, 23. Lilin, 24. Sebulan, 25. Ijo, 26. Kayu, 27. Mbuk, 28. Puspo, 29. Slangket, 30. Nangka

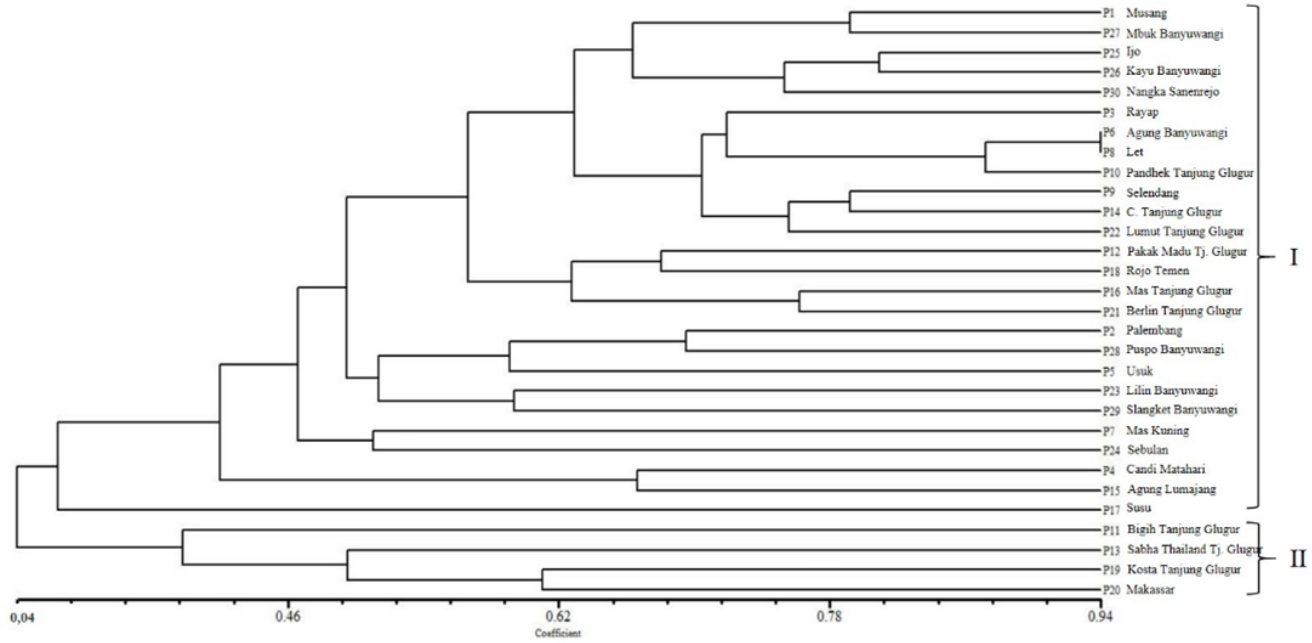

Figure 3. Dendrogram of 30 genotype banana varieties from East Java, Indonesia using UPGMA with Jaccard's index

The analysis of kinship tree/dendrogram and genetic similarity score were performed using UPGMA (Unweight Pair Group Method with Arithmetic Mean) with SIMQUAL (Similarity Quantitative Jaccard's Index) function using NTSYS (Numerical Taxonomy and Multivariate) software version 2.1.1. The resulting dendrogram showed that the 30 samples were divided into two groups. The research results in Figure 3 show that all banana varieties form two major clusters: I and II. Cluster I included 26 banana varieties, and Cluster II of 4. Cluster I comprised of banana varieties Musang, Mbuk Banyuwangi, Ijo, Kayu Banyuwangi, Nangka Sanenrejo, Rayap, Agung Banyuwangi, Let, Pandhek Tanjung Glugur, Selendang, C. Tanjung Glugur, Lumut Tanjung Glugur, Pakak Madu Tanjung Glugur, Rojo Temen, Mas Tanjung Glugur, Berlin Tanjung Glugur, Palembang, Puspo Banyuwangi, Usuk, Lilin Banyuwangi, Slangket Banyuwangi, Mas Kuning, Sebulan, Candi Matahari, Agung Lumajang, and Susu. Cluster II consisted of Bigih Tanjung Glugur, Sabha, Tanjung Glugur, Kosta Tanjung Glugur, and Makassar. Due to the differences in the amplified region, the cluster separation, number of bands, and intensity of the DNA bands for each genome in each sample are different. Poerba and Ahmad (2013) state that the initial amplification results do not consistently yield bands with uniform intensity compared to subsequent amplification results. This discrepancy occurs because the binding site of the primers on the DNA template have a significant influence on the amplification of each RAPD primer.

Genetic similarity and variation of thirty banana varieties

The genetic similarity values in Table 3 ranged from 0.04 (Bigih Tanjung Glugur and Lilin Banyuwangi) to 0.94 (Agung Banyuwangi and Let). Based on the dendrogram results, we recorded Agung Banyuwangi (AAA) and Let (ABB) have the closest similarity, while the furthest one is observed between Bigih Tanjung Glugur (BB) and Lilin Banyuwangi (AA). This suggests a lack of close relationship between these banana varieties, mainly because they belong to two separate chromosomal groups, AA and BB. As shown in the results of a prior study, the highest degree of genetic similarity, assessed as 1.00, was observed among Thai Banana varieties, specifically 'Kluai Khai KamphaengPhet' (belonging to the AA group) and 'Kluai Namwa Tha Yang' and 'Kluai Namwa Nuan Chan' (both from the ABB group) (Boonsrangsom et al. 2023). These findings provide evidence of close relationships between cultivars within specific genetic groups. It is worth noting that bananas carrying AAB genome exhibit distinct banding patterns, highlighting the significant genetic diversity present among bananas belonging to the AAB gene cluster. In this study banana varieties among Agung Lumajang, Slangket, Rojo Temen, Nangka, and Candi Matahari all belong to the AAB gene group. Therefore, further experiments with other AAB banana cultivars are necessary to confirm the unique banding patterns generated from the *Musa* AAB genome in this research.

The higher the genetic similarity value between two varieties indicates that the two varieties are closely related, whereas the lower the value, the less similar the two varieties are (Hanum et al. 2020). Determination of parents in the banana plant breeding program can be done based on the relationship between the obtained varieties. Furthermore, the more distant the relationship between parents allows the opportunity to produce new cultivars with wide genetic variations. In contrast, the closer the relationship between parents will produce new cultivars with fewer genetic

variations (Hasan and Khasim 2018; Jeon et al. 2023). The Analysis of Molecular Variance (AMOVA) also supported this statement. The genetic variation in 30 banana cultivars was estimated using (AMOVA) with GenAlEx 6.503. The results indicated high and low variation among populations, (93%) and (7%). The findings through the Analysis of Molecular Variance (AMOVA) are presented in Table 4.

The population variation of bananas, including allele frequencies (Na and Ne), across five distinct regions (Jember, Bondowoso, Lumajang, Situbondo, and Banyuwani) is presented in Table 5. In this study, the banana population exhibited allele frequencies that were relatively consistent with the research conducted by Lamare and Lao (2015) but lower than the study by Hinge et al. (2022). Notably, the Lumajang and Bondowoso bananas yielded lower allele frequencies. In the comparison of allele frequencies, our study discovered a higher value of 1.74, while Lamare and Lao (2015) reported a slightly lower value of 1.58. Additionally, our study revealed lower values of 0.7, whereas Lamare et al. (2015) reported a higher value of 1.81. In contrast, the research conducted by Hinge et al. (2022) found higher allele frequencies at 2.0 and lower frequencies at 1.5. These variations in allele frequencies highlight the diversity and differences in genetic characteristics across these studies.

In the current investigation, the observed (Na) and expected number of alleles (Ne) values for the number of alleles varied from 0.70 to 1.74 and 1.26 to 1.44, respectively. Genetic diversity in banana cultivars is significantly influenced by genomic groups (Resmi et al. 2011). Banyuwangi, Situbondo, Lumajang, and Bondowoso districts show a lower observed (Na) value compared to the expected number of alleles (Ne) due to the presence of high among banana cultivars. This occurs because the number of samples observed in the districts of Banyuwangi, Situbondo, Lumajang, and Bondowoso is relatively small compared to the total sample size, resulting in a lower observed number of alleles (Ne).

Generally, bananas and plantains usually reproduce by budding, resulting in low genetic diversity. Natural mutations are able to create genetic variations. Identifying genes and gene mutations causing to the phenotypic changes is essential for understanding different biological processes (Sahu et al. 2020). HS-SPME (Headspace Solid-Phase Microextraction) and GC-MS (Gas Chromatography-Mass Spectrometry) techniques to generate profiles of volatile compounds associated with the genotype. Thus, this information provides as a valuable resource for farmers, helping them to develop new banana varieties with delicious taste, pleasing aroma, smooth texture, and appealing color. It also empowers scientists and farmers to safeguard the genetic stability of commercially cultivated banana varieties.

Table 3. Genetic similarity values of 30 banana varieties based on the Jaccard's coefficient

	Banana variety	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	30
1	Musang	1,00																													
2	Palembang	0,62	1,00																												
3	Rayap	0,77	0,58	1,00																											
4	Candi Matahari	0,47	0,45	0,48	1,00																										
5	Usuk	0,48	0,58	0,57	0,44	1,00																									
6	Agung Banyuwangi	0,72	0,48	0,72	0,41	0,56	1,00																								
7	Mas Kuning	0,44	0,46	0,58	0,53	0,54	0,52	1,00																							
8	Let	0,70	0,52	0,74	0,45	0,54	**0,94**	0,59	1,00																						
9	Selendang	0,61	0,50	0,73	0,48	0,65	0,68	0,62	0,70	1,00																					
10	Pandhek Tanjung Glugur	0,73	0,54	0,69	0,40	0,53	0,84	0,58	0,90	0,73	1,00																				
11	Bigih Tanjung Glugur	0,24	0,27	0,19	0,26	0,30	0,24	0,20	0,23	0,30	0,23	1,00																			
12	Pakak Madu Tanjung Glugur	0,66	0,48	0,66	0,45	0,43	0,57	0,51	0,60	0,70	0,66	0,27	1,00																		
13	Sabha Thailand Tanjung Glugur	0,27	0,31	0,30	0,30	0,34	0,36	0,42	0,40	0,34	0,34	0,39	0,40	1,00																	
14	C Tanjung Glugur	0,66	0,55	0,70	0,39	0,54	0,60	0,45	0,63	0,79	0,70	0,29	0,76	0,24	1,00																
15	Agung Lumajang	0,45	0,33	0,41	0,66	0,23	0,29	0,35	0,33	0,32	0,32	0,27	0,42	0,37	0,35	1,00															
16	Mas Tanjung Glugur	0,59	0,34	0,55	0,40	0,29	0,48	0,41	0,47	0,55	0,51	0,12	0,69	0,29	0,51	0,42	1,00														
17	Susu	0,39	0,32	0,31	0,37	0,31	0,23	0,25	0,27	0,27	0,27	0,18	0,32	0,26	0,30	0,28	0,51	1,00													
18	Rojo Temen	0,62	0,46	0,54	0,39	0,33	0,43	0,36	0,46	0,58	0,54	0,25	0,68	0,23	0,68	0,41	0,65	0,50	1,00												
19	Kosta Tanjung Glugur	0,36	0,25	0,34	0,41	0,19	0,25	0,27	0,30	0,34	0,29	0,34	0,30	0,45	0,32	0,37	0,33	0,36	0,37	1,00											
20	Makassar	0,31	0,26	0,29	0,40	0,17	0,26	0,32	0,34	0,34	0,46	0,34	0,53	0,32	0,47	0,33	0,64	0,20	0,37	0,61	1,00										
21	Berlin Tanjung Glugur	0,59	0,39	0,59	0,30	0,46	0,57	0,37	0,56	0,63	0,63	0,17	0,60	0,24	0,65	0,42	0,76	0,35	0,55	0,27	0,23	1,00									
22	Lumut Tanjung Glugur	0,75	0,64	0,70	0,40	0,58	0,69	0,46	0,72	0,74	0,82	0,27	0,60	0,26	0,76	0,38	0,52	0,37	0,68	0,35	0,34	0,65	1,00								
23	Lilin Banyuwangi	0,61	0,53	0,52	0,45	0,42	0,50	0,52	0,53	0,38	0,57	**0,04**	0,39	0,33	0,36	0,36	0,43	0,29	0,36	0,25	0,27	0,37	0,48	1,00							
24	Sebulan	0,45	0,47	0,51	0,50	0,46	0,40	0,51	0,47	0,55	0,46	0,25	0,39	0,39	0,41	0,47	0,33	0,20	0,41	0,44	0,57	0,28	0,52	0,54	1,00						
25	Ijo	0,62	0,46	0,62	0,48	0,45	0,65	0,45	0,68	0,54	0,66	0,20	0,51	0,33	0,45	0,56	0,46	0,35	0,50	0,32	0,37	0,51	0,72	0,63	0,60	1,00					
26	Kayu Banyuwangi	0,62	0,44	0,62	0,54	0,47	0,61	0,29	0,64	0,54	0,58	0,31	0,52	0,31	0,51	0,66	0,43	0,32	0,51	0,35	0,34	0,52	0,68	0,43	0,52	0,80	1,00				
27	Mbuk Banyuwangi	0,79	0,52	0,62	0,45	0,47	0,61	0,29	0,60	0,50	0,62	0,31	0,60	0,31	0,59	0,61	0,43	0,27	0,59	0,30	0,34	0,56	0,68	0,53	0,47	0,72	0,76	1,00			
28	Puspo Banyuwangi	0,59	0,69	0,59	0,40	0,59	0,53	0,51	0,60	0,55	0,63	0,29	0,47	0,34	0,65	0,31	0,38	0,30	0,51	0,27	0,33	0,42	0,65	0,64	0,47	0,46	0,43	0,56	1,00		
29	Slangket Banyuwangi	0,45	0,47	0,55	0,35	0,42	0,44	0,41	0,52	0,42	0,55	0,17	0,43	0,39	0,41	0,36	0,38	0,30	0,32	0,27	0,33	0,42	0,47	0,59	0,47	0,55	0,47	0,52	0,57	1,00	
30	Nangka Sanenrejo	0,60	0,46	0,64	0,43	0,45	0,62	0,40	0,65	0,60	0,64	0,22	0,61	0,25	0,48	0,40	0,50	0,40	0,53	0,28	0,29	0,50	0,61	0,51	0,50	0,77	0,73	0,65	0,45	0,58	1,00

Table 4. Analysis of molecular variance (AMOVA) on 30 banana cultivars

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Estimated variance	Percentage of variance%
Among pops.	4	63.717	15.929	0.878	7%
Within pops.	25	277.917	11.117	11.117	93%
Total	29	341.633		11.994	100%

Table 5. Genetic variation in 30 banana cultivars in five districts of East Java, Indonesia

Population	N	Na	Ne	I	h
Jember	12	1.74±0.08	1.44±0.04	0.41±0.03	0.27±0.02
Banyuwangi	6	1.35±0.11	1.44±0.04	0.38±0.03	0.26±0.02
Situbondo	7	1.29±0.11	1.43±0.05	0.36±0.03	0.24±0.02
Lumajang	3	0.88±0.11	1.30±0.04	0.24±0.04	0.17±0.02
Bondowoso	2	0.70±0.10	1.26±0.05	0.18±0.03	0.13±0.03

Note: N: Sample size, Na: Observed no. of alleles, Ne: Effective no. of alleles, h: Nei's genetic diversity, I: Shannon's information index, SD: Standard deviation

In conclusion, the investigation of 30 banana plants from five districts in East Java Province revealed great genetic diversity. Using 7 RAPD primers, each revealed high polymorphism, according to the findings. The primers OPA-04 and OPC-05 are suitable for detecting genetic diversity in banana plants based on the number of DNA bands formed and the high polymorphism. The phylogenetic tree of 30 banana plants formed 2 main clusters where cluster I consisted of 26 varieties of bananas and cluster II consisted of 4 varieties of bananas with genetic similarity values ranging from 0.04 (Bigih Tanjung Glugur and Lilin Banyuwangi) to 0.94 (Agung Banyuwangi and Let). Molecular studies on banana plants using RAPD primers were able to describe genetic diversity among 30 banana varieties from five districts in East Java. AMOVA distributed 93% of the variance within the subpopulations and 7% variation among the populations, indicating a high degree of genetic differentiation. However, further research is needed to use more specific genetic markers to confirm the exact identity of the banana genomes.

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