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Genome identification and diversity analysis of banana (*Musa spp.*) from Jember based on morphological characters

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ABSTRACT

Introduction: Banana plants are easy to find and grow in Indonesia, including in Jember. Banana production in Jember in 2020 increased by 50% and has the potential to be inventoried and developed. Information regarding the characteristics of various banana cultivars is needed for future banana breeding. This research was conducted to identify and classify the genome of bananas growing in Jember and determine the level of banana diversity. **Methods**: The banana exploration using a purposive sampling method. The observation of 15 morphological characters followed the method of Simmonds and Shepherd. Determination of the genome followed the method of Silayoi & Chamchalow and Singh & Uma. The kinship analysis uses cluster analysis using the average linkage method and Gower distance, by R Studio software. **Results**: There were 23 banana cultivars obtained in this research, consisting of 35% dessert bananas and 65% plantain types. Genome identification results in four genome groups, namely AA/AAA, AAB, ABB, and BB. Cluster analysis produces three main groups, where its branch is generally consistent with the genome grouping. The closest relationship is between Rayap and Seribu bananas (0.96), while the furthest is between Gaje and Agung bananas (0.38). The dissimilarity value for 23 banana cultivars is 0.36, or the similarity is 0.64. **Conclusion**: The 23 banana cultivars are closely related or have low diversity based on the 15 morphological characters observed. Further identification can be carried out with a greater number of morphological characters, accompanied by molecular identification.

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INTRODUCTION

Indonesia is known as one of the centers of biodiversity of bananas (*Musa* spp.) (Sari & Badruzsaufari, 2013). Banana plants grow well throughout Indonesia. There are two types of bananas, i.e., bananas which fruit can be eaten directly (dessert bananas), and those which need to be cooked first (plantain). Other parts of the banana plant, from the roots to the leaves, can also be used. In some countries in Southeast Asia, including Indonesia, banana crops are used in cultural traditions.

Banana domestication was initially determined by the economic value of the banana for food and industrial needs. Bananas cultivated today are generally derived from seedless triploid bananas, by hybridization or genetic improvement from wild bananas such as *Musa acuminata* (genome AA) and *Musa balbisiana* (genom BB) (Espiano *et al.*, 1992). According to Pillay *et al.* (2002), the formation of the current banana involves its ancestors namely *M. acuminata* for the genome A and *M. balbisiana* for the genome B. Genome A is characterized by sweet and soft fruit characteristics, while the B genome is characterized by hard and starchy fruit. In addition, there are specific morphological differences between the two types of bananas. Simmonds and Shepherds (1955) classified bananas based on their morphological characteristics into several genome groups, namely triploid hybrids for dessert bananas including the AAB group and processed bananas (plantains) into the ABB group (Sunaryo *et al.*, 2019).

The identification and classification of bananas' genomes using morphological characteristics has been extensively studied, using 15 morphology characters, to sort and classify the genome based on the Scoring methods by Simmonds and Shepherd (1955). The scoring method is still considered reliable and the easiest to do for banana crops. Genome classification and analysis of banana diversity in Indonesia have been carried out (Fitriyah *et al.*, 2017; Sunaryo *et al.*, 2019). However, not all cultivars of bananas have been observed in their study.

According to BPS (2021) data, banana production in Jember in 2020 reached 183715.1 tons, an increase of more than 50% compared to 2019 production of 74978.1 tons. The morphological characterization activities of germplasms can be guidelines in genetic development, conservation, collection, and utilization of them (Norman & Beah, 2014; Hariyono *et al.*, 2022). Further, Hariyono *et al.* (2022) revealed that the level of diversity will affect the effectiveness of the selection process in the crop breeding program. According to Fitriyah *et al.* (2017), environmental factors such as climate, soil type, and height of place can influence the growth and yield of fruit crops, causing different banana characteristics between regions in Indonesia. This research was conducted to identify and classify the genome of bananas grown in Jember, East Java Province, as well as to determine the kinship and level of diversity.

METHODS

This research was carried out by exploring banana plants in several areas of Jember, using a purposive sampling method. The purposive sampling method is a non-probability sampling method in which the researcher uses his own judgment, research knowledge, or specific criteria to select samples that are considered appropriate and relevant to the research objectives (Thomas, 2022).There are 15 morphological characters observed, and scored, following the method by Simmonds and Shepherd (1982), namely: the Pseudostem color, Petiole canal, Peduncle, Pedicel, Ovules, Bract shoulder, Bract curling, Bract shape, Bract apex, Bract color, Colour fading, Bract scars, Free tepal shape on male flower, Male flower color, and Stigma color. The score is 1 for the character that is similar to *M. acuminata*, 5 for the character that is similar to *M. balbisiana*, and 2-4 for the character between both of them. The grouping of the genome was determined by the total scores, referring to the Silayoi & Chamchalow (1987), and Singh & Uma (1996) methods, as shown in Table 1. Kinship analysis using cluster analysis, by average linkage method and Gower distance, using R studio software.

Table 1. Banana genomes determination

Genome	Total Score Silayoi & Chamchalow (1987)	Total Score Singh & Uma (1996)
AA/AAA	15-25	15-25
AAB	26-46	26-45
AB	-	46-49
ABB	59-63	59-65
ABBB	-	66-69
BB/BBB	70-75	70-75

Source: Singh *et al.* (2014).

The table 1 shows the results of determining the banana genome based on the total score obtained from two assessment methods, namely the Silayoi & Chamchalow (1987) and Singh & Uma (1996) methods. The total score for each scoring method is divided into ranges, each of which represents a different banana genome.

RESULTS AND DISCUSSION

There are 23 banana cultivars explored in Jember, as presented in Table 2, obtained from 16 villages in 11 subdistricts. The types of bananas obtained include desserts and plantains. The dessert types were only 35% and the plantain types were more than 65% of the total sample of banana crops. This shows that plantains are more cultivated due to several considerations, including higher economic value, more resistance to pests and diseases, and can be processed into various processed products.

The scoring results based on two methods showed that the 23 banana cultivars were divided into four genomes, namely AA/AAA, AAB, ABB, and BB (Table 3). It is in line with the statement of Wahyuningtyas (2009), that bananas generally cultivated have AA, AAA, AAB, and ABB genomes. In this study, bananas with the AAB genome were the most frequent. According to Valmayor *et al.* (2000), AAB genomes are more common than others (ABB, AB, AABB, and ABBB). There are 11 cultivars, or around 50% of the total explored bananas, that have the AAB genome, namely Raja, Keripik, Keripik Bali, Gaje, Candi, Susu, Raja Nangka, Agung, Musang, Seribu, and Rayap. There are differences in the estimation of the genome composition of Susu banana. According to Hapsari and Masrum (2012), Susu bananas have the AA/AAA genome, whereas in this study they were grouped as AAB. Differences in genome estimates can occur due to differences while scoring. The determined score range, namely between scores 1-5, can differ between observation results and literature due to trait variations among the 15 genome-determining characters (Nisa *et al.*, 2010; Fitriyah *et al.*, 2017).

The dendrogram resulting from the cluster analysis (Figure 1) shows similarities between bananas based on 15 morphological characters. The dissimilarity value on the x-axis explains that the smaller the value of the dissimilarity, the closer the kinship. The dissimilarity value of 23 banana cultivars is 0.36, or a similarity of 0.64, indicating a close relationship. The study of Rai *et al.* (2023) that grouped 6 local bananas of Bali showed that they have close kinship, with a similarity value of 0.74. The similarity value when less than 0.60 or 60% can be said to be distant (Trimanto, 2012; Szabo *et al.*, 2021).

Table 2. Bananas explored in several subdistricts in Jember

Village	Subdistricts	Coordinates	Cultivar Local Name	Type*
Kreyongan	Patrang	-8.158814, 113.700903	Kluthuk	Plantain
Antirogo	Sumbersari	-8.151805, 113.731956	Raja	Dessert
Jumerto	Patrang	-8.884422, 113.4139213	Keripik	Plantain
Jumerto	Patrang	-8.8102619,113.4139881	Keripik Bali	Plantain
Jumerto	Patrang	-8.866334, 113.4119702	Ambon	Dessert
Karangrejo	Sumbersari	-8.163945, 113.730142	Kepok Kuning	Plantain
Tegal Gede	Sumbersari	-8.162235, 113.724222	Kepok Putih	Plantain
Tendean	Kranjingan	-8.194249, 113.725393	Kayu	Plantain
Pontang	Ambulu	-8.364187, 113.637042	Berlin	Dessert
Jumerto	Patrang	-8.136084, 113.685900	Mas	Dessert
Balung Kidul	Balung	-8.297285, 113.538554	Gaje	Plantain
Andongsari	Ambulu	-8.380973, 113.66099	Candi	Plantain
Balung Lor	Balung	-8.284226, 113.537314	Susu	Dessert
Balung Lor	Balung	-8.284226, 113.537314	Raja Nangka	Dessert
Pasrujambe	Pasrujambe	-8.6582768, 113.353352	Agung	Dessert
Pasrujambe	Jambearum	-8.117946, 113.070559	Cavendish	Dessert
Sari Kemuning	Senduro	-8.122916, 113.129335	Musang	Plantain
Glundengan	Wuluhan	-8.288709, 113.582549	Nangka	Plantain
Sempolan	Silo	-8.185587, 113.865996	Seribu	Plantain
Sempolan	Silo	-8.185658, 113.865868	Rayap	Plantain
Gebang	Kaliwates	-8.165383, 113.678362	Kepok Makasar	Plantain
Tegal Gede	Sumbersari	-8.150000, 113.729079	Awak	Plantain & dessert
Tegal Gede	Sumbersari	-8.149994, 113.729086	Morosebo	Plantain

Note: plantain=cooking banana; *=based on local resident's interview

Table 3. Genome identification of explored banana in Jember

Cultivar Local Name	Binomial Nomenclature	Total Scoring	Genome
Kluthuk	*M. balbisiana* Colla. cv Kluthuk	72	BB
Raja	*M. acuminata* x *M. balbisiana* cv Raja	43	AAB
Keripik	*M. acuminata* x *M. balbisiana* cv Keripik	44	AAB
Keripik Bali	*M. acuminata* x *M. balbisiana* cv Keripik Bali	41	AAB
Ambon	*M. acuminata* Colla. cv Ambon	23	AA/AAA
Kepok Kuning	*M. acuminata* x *M. balbisiana* cv Kepok Kuning	59	ABB
Kepok Putih	*M. acuminata* x *M. balbisiana* cv kepok Putih	60	ABB
Kayu	*M. acuminata* Colla. cv Kayu	23	AA/AAA
Berlin	*M. acuminata* Colla. cv Berlin	20	AA/AAA
Mas	*M. acuminata* Colla. cv Mas	20	AA/AAA
Gaje	*M. acuminata* x *M. balbisiana* cv Gaje	43	AAB
Candi	*M. acuminata* x *M. balbisiana* cv Candi	46	AAB
Susu	*M. acuminata* x *M. balbisiana* cv Susu	45	AAB
Raja Nangka	*M. acuminata* x *M. balbisiana* cv Raja Nangka	32	AAB
Agung	*M. acuminata* x *M. balbisiana* cv Agung	39	AAB
Cavendish	*M. acuminata* Colla. cv Cavendish	25	AA/AAA
Musang	*M. acuminata* x *M. balbisiana* cv Musang	31	AAB
Nangka	*M. acuminata* Colla. cv Nangka	25	AA/AAA
Seribu	*M. acuminata* x *M. balbisiana* cv Seribu	33	AAB
Rayap	*M. acuminata* x *M. balbisiana* cv Rayap	35	AAB
Kepok Makasar	*M. acuminata* x *M. balbisiana* cv Kepok Makasar	59	ABB
Awak	*M. acuminata* x *M. balbisiana* cv Awak	59	ABB
Morosebo	*M. acuminata* Colla. cv Morosebo	25	AA/AAA

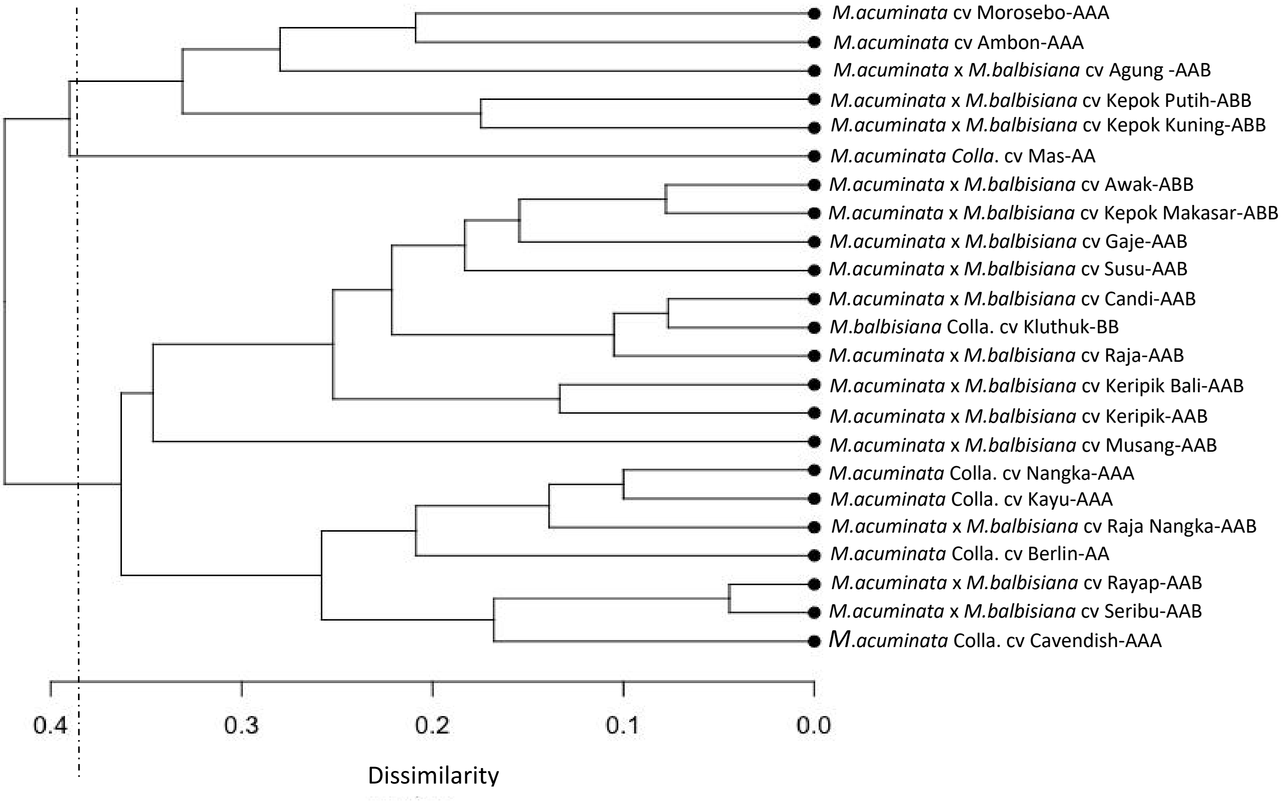
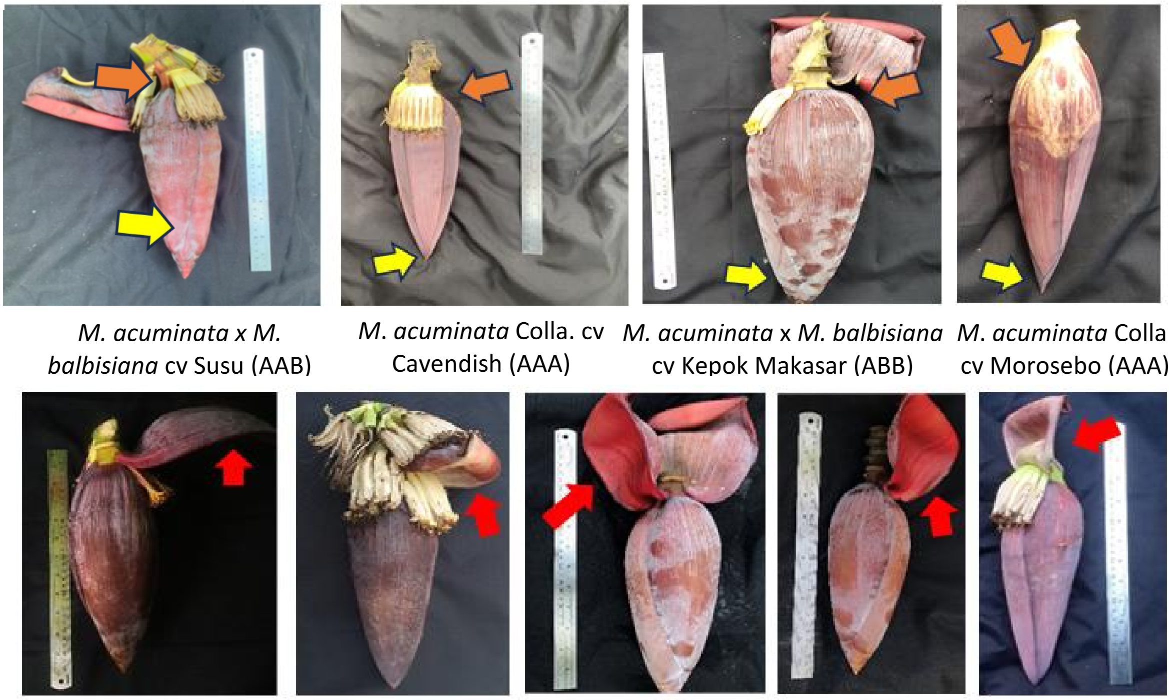


Figure 1. Grouping of bananas based on average linkage and Gower distance methods



M. acuminata x M. balbisiana cv Susu (AAB)

M. acuminata Colla. cv Cavendish (AAA)

M. acuminata x *M. balbisiana* cv Kepok Makasar (ABB)

M. acuminata Colla. cv Morosebo (AAA)

M. balbisiana Colla. cv Kluthuk (BB)

M. acuminata x *M. balbisiana* cv Kepok Putih (ABB)

M. acuminata x *M. balbisiana* cv Kepok Kuning (ABB)

M. acuminata Colla. cv Kayu (AAA)

M. acuminata Colla. cv Berlin (AA)

Figure 2. Bract character variation. Bract shoulder (orange arrow), bract apex (yellow arrow), and Bract curling (red arrow)

Figure 1 also shows that at the level of dissimilarity of 0.36, the banana cultivars were divided into three main groups. The first group consisted of Canvendish, Seribu, Rayap, Berlin, Raja Nangka, Kayu, and Nangka banana. The second group consists of Musang, Kepok, Kepok Bali, Raja, Kluthuk, Candi, Susu, Gaje, Kapok Makassar, and Awak banana. And the rests, belong to Group 3, namely Emas, Kepok Kuning, Kepok Putih, Agung, Ambon, and Morosebo banana. Banana cultivars in group 1 have similarities in the Color characteristics of male flowers: cream; Bract scars: very prominent; Bract sediment: very pronounced; Color fading: pigmentation is uniform and continues until the base; and Ovules characters: have two regular rows in each locule. The cultivar of bananas in group 2 has similarities in the character of their long peduncle, whereas group 3 has similarities in the Fading color, where their color discontinues towards the base (loss of pigmentation at the base).

The dendrogram also shows that several bananas with the same genome are in the same group/cluster. Morosebo and Ambon bananas have the AAA genome; Kepok Putih and Kepok Kuning have the ABB genomes; Awak and Kepok Makassar have ABB genomes; Keripik Bali and Keripik have AAB genomes, Nangka and Kayu have the AAA genomes; Rayap and Seribu have AAB genome. Character resemblance can reflect the genome in it. The greater the morphological resemblance, the more genome-like it is (Rinaldi *et al.*, 2014). Similarly, according to Abdullah *et al.* (2012), bananas with the same genome show higher similarities compared to different bananas, where other cultivars are on different clusters of the genome. The research of Simmond (1955) showed that although a genotype has the same AAA genome constitution, they are in different clusters. This can happen because banana pollination is open-pollinated. According to Rinaldi *et al.* (2014), free pollination causes genome variations that can vary in the composition of the genome on a cultivar.

Figure 2 shows the variation of bract characters in some banana cultivars. Bananas with the genome AA, AAA, and AAB have rolling, curling, or rollback braktea, whereas ABB genome groups have lifting but not rolling braktea (Wahyudi *et al.*, 2020).

Table 4. Similarity Value (1- dissimilarity) of 23 banana cultivars in Jember

Local Cultivar Name	Kluthuk	Raja	Keripik	Keripik Bali	Ambon	Kepok Kuning	Kepok Putih	Kayu	Berlin	Emas	Gaje	Raja Nangka	Agung	Musang	Nangka	Seribu	Rayap	Morosebo	Cavendish	Kepok Makassar	Awak	Candi	Susu
Kluthuk	1,00																						
Raja	0,90	1,00																					
Keripik	0,78	0,79	1,00																				
Keripik Bali	0,65	0,68	0,87	1,00																			
Ambon	0,52	0,51	0,46	0,39	1,00																		
Kepok Kuning	0,68	0,58	0,68	0,68	0,59	1,00																	
Kepok Putih	0,56	0,48	0,59	0,65	0,71	0,82	1,00																
Kayu	0,69	0,66	0,68	0,54	0,64	0,57	0,62	1,00															
Berlin	0,68	0,72	0,71	0,60	0,59	0,49	0,56	0,81	1,00														
Emas	0,67	0,63	0,45	0,43	0,58	0,54	0,56	0,61	0,68	1,00													
Gaje	0,73	0,70	0,70	0,70	0,51	0,70	0,64	0,66	0,65	0,45	1,00												
Raja Nangka	0,66	0,69	0,74	0,61	0,62	0,54	0,60	0,89	0,74	0,55	0,60	1,00											
Agung	0,62	0,58	0,40	0,40	0,74	0,56	0,65	0,57	0,53	0,71	**0,38**	0,54	1,00										
Musang	0,64	0,61	0,66	0,77	0,42	0,67	0,62	0,58	0,46	0,46	0,74	0,62	0,41	1,00									
Nangka	0,66	0,69	0,69	0,64	0,70	0,60	0,68	0,90	0,84	0,59	0,70	0,83	0,58	0,57	1,00								
Seribu	0,73	0,77	0,69	0,63	0,58	0,59	0,60	0,78	0,68	0,69	0,53	0,82	0,64	0,59	0,79	1,00							
Rayap	0,69	0,72	0,69	0,63	0,58	0,59	0,65	0,74	0,64	0,64	0,49	0,82	0,64	0,55	0,75	**0,96**	1,00						
Morosebo	0,60	0,56	0,51	0,45	0,79	0,70	0,79	0,75	0,65	0,69	0,62	0,72	0,71	0,63	0,76	0,69	0,69	1,00					
Cavendish	0,72	0,66	0,61	0,54	0,51	0,63	0,58	0,75	0,61	0,61	0,64	0,75	0,56	0,60	0,76	0,85	0,80	0,66	1,00				
Kepok Makassar	0,83	0,75	0,81	0,79	0,53	0,68	0,69	0,69	0,70	0,54	0,86	0,62	0,48	0,71	0,68	0,61	0,56	0,59	0,55	1,00			
Awak	0,82	0,78	0,87	0,80	0,52	0,71	0,65	0,68	0,73	0,53	0,83	0,61	0,47	0,63	0,71	0,59	0,59	0,58	0,54	0,92	1,00		
Candi	0,92	0,89	0,75	0,64	0,50	0,63	0,56	0,65	0,71	0,59	0,81	0,58	0,57	0,62	0,68	0,66	0,61	0,61	0,70	0,81	0,84	1,00	
Susu	0,76	0,73	0,79	0,72	0,60	0,57	0,51	0,69	0,74	0,47	0,77	0,62	0,41	0,58	0,72	0,56	0,52	0,52	0,55	0,82	0,86	0,78	1,00

The similarities of 23 banana cultivars based on 15 morphological characteristics are shown in Table 4. The greatest degree of similarity is between Rayap and Seribu bananas, with a value of 96%. This indicates that the two bananas have a close relationship. The lowest degree of similarity is between Gaje and Agung bananas, with a value of 38%. Both are easily distinguished by the morphological appearance of the fruit. However, the two bananas, based on genetic identification, have genetic similarity of the AAB. This can happen, because in the identification of genetic determination of the group of the genome based on a certain range of scores.

A grouping of 23 banana cultivars provides information that bananas in Jember have the potential for further development. According to Rinaldi *et al.* (2014), the greater the genetic distance, the greater the chance of genetic variation to be inherited, and the potential to be used as parents to be crossed in plant breeding programs. However, identification based on morphological characteristics needs to be strengthened by molecular marker technology.

According to Wijaya *et al.* (2020), the effectiveness of using morphological characters for determining the relationship of genotypes should be considered. The use of morphological markers has a limitation in dealing with cultivars with close genetic relationships and environmental factors that have a major influence on morphological variation (Poerba *et al.*, 2010; Rusdiana *et al.*, 2023).

CONCLUSION

The exploration in Jember obtained 23 banana cultivars, consisting of 35% dessert bananas and 65% plantains. A grouping based on 15 characters used for genome determination results in four groups of genome AA/AAA, AAB, ABB, and BB. Cluster analysis results in three main groups, and branches of groups generally consistent with genome grouping. The closest relationship is between Rayap and Seribu banana, both have the AAB genomes. Whereas the most distant are Gaje and Agung bananas. Overall, the dissimilarity of the 23 banana cultivars is 0.36 or the similarity is 0.64, which means that those banana cultivars have close kinship based on the 15 observed morphological characters.

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