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PHENOTYPIC VARIATION AND RAPD POLYMORPHISM OF PISANG KEPOK LOCAL CULTIVARS (Musa acuminata x Musa balbisiana, ABB, SABA SUBGROUP)

VARIASI FENOTIPIK DAN POLIMORFISME RAPD KULTIVAR LOKAL PISANG KEPOK (Musa acuminata x Musa balbisiana, ABB, SUBGRUP SABA)

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Pisang Kepok is a major local banana cultivar in Indonesia with high economy, social and cultural value. Particularly on the island of Java, there are several variations of Pisang Kepok recognised with their own local names, which makes difficulties in taxonomic identification and grouping. Morphological features are used in conventional banana cultivar classification, but they are deemed less precise due to their subjectivity, thus, it is supposed to be complemented with molecular approach. This study aims to identify the phenotypic variation of Pisang Kepok local cultivars also their genetic polymorphism using Random Amplified Polymorphic DNA marker. Phenotypic variation was observed using 35 morphological characters. Six RAPD primers were used, i.e. OPA2, OPA3, OPA4, OPA11, OPA12 and OPA18. Clustering analysis, both phenotypic and genetic were performed using PAST v4.02. The morphological characterisation identified four variants of Pisang Kepok i.e. Kepok Abang, Kepok Putih, Kepok Manurun, and Kepok Australi; which all confirmed as ABB genome group. Phenotypic clustering showed that Pisang Kepok cultivars were separated into 3 clusters based on their local name, with a high similarity value of >90%. PCA biplot showed that the fruit flesh colour was the most important character contributed to the cultivar variation. RAPD marker also showed that each specimen was grouped according to its local name and source, with a similarity value of >80%. Both morphology and molecular (RAPD) markers resulted in the branching of Pisang Kepok which was closer to Pisang Klutuk than Pisang Barlin.

ABSTRACT

Keywords: characterisation, molecular, morphology, Pisang Kepok, RAPD

ABSTRAK

Pisang Kepok merupakan kultivar pisang lokal utama di Indonesia dengan nilai ekonomi, sosial dan kultural yang tinggi. Khususnya di Pulau Jawa, terdapat beberapa variasi Pisang Kepok yang dikenal berdasarkan penamaan lokal, sehingga menimbulkan permasalahan dalam identifikasi dan pengelompokan taksonomi. Karakter morfologi digunakan dalam klasifikasi kultivar pisang konvensional, namun hal tersebut dirasa kurang tepat karena subjektivitasnya, sehingga perlu dilengkapi dengan pendekatan molekuler. Penelitian ini bertujuan untuk mengidentifikasi variasi fenotipik kultivar lokal Pisang Kepok, serta polimorfisme genetiknya menggunakan penanda Random Amplified Polymorphic DNA. Variasi fenotipik diamati menggunakan 35 karakter morfologi. Enam primer RAPD yang digunakan yaitu OPA2, OPA3, OPA4, OPA11, OPA12 dan OPA18. Analisis klastering baik fenotipik maupun genetik dilakukan menggunakan program PAST v4.02. Karakterisasi morfologi mengidentifikasi terdapat 4 varian Pisang Kepok yaitu Kepok Abang, Kepok Putih, Kepok Manurun, dan Kepok Australi; yang semuanya terkonfirmasi bergenom ABB. Klastering fenotipik menunjukkan kultivar Pisang Kepok terbagi menjadi 3 klaster berdasarkan nama lokalnya, dengan nilai kemiripan tinggi >90%. Hasil PCA biplot menunjukkan bahwa warna daging buah merupakan karakter terpenting yang berkontribusi terhadap variasi kultivar. Penanda RAPD juga menunjukkan bahwa setiap spesimen mengelompok sesuai dengan nama lokal dan asalnya, dengan nilai kemiripan >80%. Kedua penanda baik morfologi maupun molekuler (RAPD), menghasilkan percabangan Pisang Kepok yang lebih dekat dengan Pisang Klutuk dibandingkan dengan Pisang Barlin.

Kata kunci: karakterisasi, molekuler, morfologi, Pisang Kepok, RAPD

INTRODUCTION

Banana (*Musa* spp., Musaceae) is a major tropical fruits and horticultural commodity in Southeast Asia, particularly in Indonesia [1]. Indonesia is regarded to be the origin and main diversity center of banana species, including genetic resources for both wild and cultivated bananas [2, 3]. Indonesia appears to have at least 325 banana cultivars [4]. Bananas serve as crucial roles in economy, social and cultural life,

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particularly in the rural community. They earn income for farmers, are utilised as an alternative food supply, provide nutritional value for consumptions either fresh or cooked, and play as a fundamental aspect in social and religious ceremonies [5].

Numerous local banana cultivars have been identified in Indonesia, which is considered to be home to a diverse range of bananas. It has a wide variety of morphological traits and is further confounded by a large number of cultivar names and synonyms in several languages, generating categorisation and nomenclature difficulty [6]. To deal with this scenario, a new banana-specific taxonomy is required. As a result, a new taxonomical scheme for banana cultivars was proposed and agreed upon in 1999 [4]. The taxonomy classification established was based on Chessman's suggestion that current banana cultivars were derived from two major ancestors i.e. Musa acuminata and Musa balbisiana as A and B genome donors, respectively. The genomicbased nomenclature systems have three tiers consisting of species, genome group, and cultivar name [4, 7].

Specifically, Pisang Kepok is a triploid hybrid cultivar (Musa acuminata x Musa balbisiana), identified to have an ABB genome group and included in the Saba subgroup [6]. It is one of the popular banana cultivars today in Southeast Asia particularly in Indonesia, Malaysia, Thailand, Vietnam and the Philippines with many names and synonims in each country. It is known as Pisang Abu Keling or Nipah in Malaysia, Kluai Hak Muk or Hin in Thailand, and Saba or Cardaba in The Phillipines [8]. In East Java, Pisang Kepok also has synonyms such as Pisang Gajih, Pisang Bung, and Pisang Saba [5, 9]. It is primarily a cooking banana, the fruits are prefer cooked before consumption, such as steamed, boiled, fried, etc. It has higher carbohydrates as a source of nutrition than dessert bananas [10]. Furthermore, from the perspective of molecular breeding, Pisang Kepok is a valuable genetic resource for developing drought-tolerant variety [1, 11], and resistant to bunchy top disease [12].

In Java island, there are many variants of Pisang Kepok, which are recognised by their regional or local names. The banana variant naming by local people mostly resemble to its differentiating morphology or perceptual characteristics, utilization and the origin area. However, some of the names do not have resemble to morphology or anything. At least there are 15 names of Pisang Kepok local cultivars recorded in East Java [5] including Kepok Bung, Kepok Merah (Abang), Kepok Putih, Kepok Bali, Kepok Australi, etc. Hence, in this study, we conduct morphological characterisation to some variants of Pisang Kepok from Java, to identify the distinguishing and similar characters among them. Furthermore, an identification technique based on morphological characters is required to collect some visible data about the principal components that affect the variation of bananas [13].

However, morphological identification might be subjective, resulting in discrepancies amongst researchers when defining genome groups and variation within and among banana cultivars [7, 14]. As a result, a molecular approach is required to provide more reliable results. One of the molecular markers often used in the identification of banana cultivars is Random Amplified Polymorphic DNA (RAPD). The advantages of using RAPD are an easy technique, fast process, and efficient method [15, 16, 17]. Therefore, in addition to phenotypic variation, the objective of this study also to characterise the genetic polymorphism of Pisang Kepok using RAPD marker. The findings of this study are necessary in the taxonomy context as well as to provide a basis for further breeding and conservation strategy for genetically valuable Pisang Kepok local cultivars in Java. Moreover, the analysis of prospective RAPD primers from this study can be utilised as a reference for other and further banana studies.

METHODS

Plant materials. The plant materials examined in this study comprising of 16 specimens of Pisang Kepok local cultivars originated from several regions in Malang, East Java. Two outgroups were used, i.e. Pisang Klutuk representing BB group and Pisang Barlin representing AA group.

Morphological characterisation. The morphological characters were identified according to 15 main diagnostic characters to differentiate the two ancestors i.e. *M. acuminata* with *M. balbisiana* and their hybrids to confirm the genomic group [4]. All samples were also morphologically characterised using a minimum Descriptor of Banana by IPGRI [18], with a total of 35 characters observed (Table 1).

Extraction and evaluation of DNA. The plant samples used were fresh young banana leaves. The DNA extraction kit (Promega Wizard®) was employed to extract the total genomic DNA. It provides step-by-step instructions for DNA isolation of plants. The DNA extracted was then evaluated both qualitatively and quantitatively. Electrophoresis on a 1% agarose gel was used to determine DNA quality, while AE-Nanodrop 200 Nucleic Acid was used to quantify DNA amount.

| No. | Morphological characters | Code | No. | Morphological characters | Code |
|-----|---------------------------------|------|-----|--------------------------------|------|
| 1 | Leaf habit | LH | 19 | Free tepal colour* | FTC |
| 2 | Pseudostem height [m] | PH | 20 | Free tepal shape* | FTS |
| 3 | Pseudostem colour* | PC | 21 | Free tepal apex development | FTA |
| 4 | Sap colour | SC | 22 | Style basic colour | SBC |
| 5 | Wax on leaf sheaths | WLS | 23 | Style shape | SS |
| 6 | Blotches at petiole base | BPB | 24 | Stigma colour* | STC |
| 7 | Petiole canal leaf III* | PCL | 25 | Ovary shape* | OS |
| 8 | Shape of leaf blade base | SLB | 26 | Ovary basic colour | OBS |
| 9 | Colour of midrib dorsal surface | CMD | 27 | Dominant colour of male flower | DCM |
| 10 | Rachis position* | RP | 28 | Arrangement of ovules* | AO |
| 11 | Male bud shape* | MBS | 29 | Fruit position* | FP |
| 12 | Bract base shape* | BBS | 30 | Transverse section of fruit | TSF |
| 13 | Bract apex shape* | BAS | 31 | Fruit apex | FA |
| 14 | Colour on the bract apex* | CBA | 32 | Immature fruit peel colour | IFP |
| 15 | Bract behaviour before falling* | BBF | 33 | Wax on the fruit peel | WFP |
| 16 | Wax on the bract | WB | 34 | Pulp colour at maturity | PCM |
| 17 | Compound tepal basic colour* | CTB | 35 | Presence of seed | PS |
| 18 | Lobe colour of compound tepal | LCC | | | |

 Table 1. Morphological characters observed in this study

Annotation: *= 15 main diagnostic characters

RAPD PCR amplification & visualisation. DNA amplifications were carried out using six selected RAPD primers (Operon Technologies) out of twenty, including OPA2, OPA3, OPA4, OPA11, OPA12 and OPA18. Those six primers were selected based on previous studies as effective RAPD primers for bananas [7, 17]. The process was performed in a PCR Thermocycler with 10 µl total volume of PCR reaction comprised of 25 ng DNA sample 1 µl, primer (10 pmol) 1 µl, nucleasefree water 3 µl, and PCR Master Mix (Genaxon and Hs Master Mix) 5 µl. The cycle of PCR amplification consisted of pre-denaturation (4 minutes at 94°C), 45-cycle of denaturation (30 seconds at 94°C), annealing (30 seconds at 37-45°C, depends on melting temperature of each primer), and elongation (90 seconds at 72°C). The final step was post-elongation (5 minutes at 72°C). The RAPD amplification result was then confirmed by electrophoresis separation on 1.5% gel agarose and visualised by Gel Documentation (Bluegel) with a 100 bp marker DNA ladder.

Data analysis. Clustering analysis. The morphological characters observed were scored, a score of '1' was assigned for a character closer to *M. acuminata* and '5' for a character with strong expression of *M. balbisiana*. Whilst, a score of '2' or '3' or '4' is assigned for an intermediate character. Further, the data score was analysed for clustering and similarity using Paleontological Statistics/PAST program version 4.02 with the UPGMA method and Bray-Curtis coefficient. Meanwhile, the RAPD DNA visualisation was scored as binary data i.e. score '1' for presence band and '0' for absence band. The data score was then analysed for clustering and similarity using

PAST v4.02 with UPGMA method and Jaccard coefficient.

PCA analysis. Principal Component Analysis (PCA) was performed to determine the contribution of the most influential phenotypic characters to clustering in PAST v4.02 [19].

Polymorphism and primer discriminatory power analysis. Polymorphism of RAPD products was analysed visually based on the band pattern. Analysis of the primer discriminatory power uses several parameters including Polymorphism Information Content PIC), Effective Multiplex Ratio (EMR), Marker Index (MI), and Resolution Power (RP) [20].

RESULTS AND DISCUSSION

Phenotypic variation of Pisang Kepok. The morphological characterisation of 16 specimens of Pisang Kepok was comprised of 4 main cultivars, including Kepok Abang, Kepok Putih, Kepok Manurun, and Kepok Australi. All 15 diagnostic characters observed in Pisang Kepok local cultivars showed a high resemblance to M. *balbisiana* as the putative ancestor. The scoring value results ranged from 59 to 63, therefore all of them were confirmed to be identified as ABB genome group (Table 2). The highest score was raised by Pisang Kepok Manurun, differentiated from other cultivars due to its male flower character, especially the compound tepal. It resembles strong M. balbisiana's colour (reddish purple), so it gains 5 points (Figure 1). Meanwhile, for other cultivars have cream-coloured male flowers which much more resemble M. acuminata [4]. The anthocyanin content is possibly the cause of a reddish colour pigmentation (pinkish to purplish) in male banana flowers [21].

The spots on petiole base are main distinguishing features between ancestors *M. balbisiana* and *M. acuminata* [4]. *M. acuminata* has large spots on its petiole base, whilst *M. balbisiana* has fewer spots. In this study, both species were represented by Pisang Barlin (AAcv) and Pisang Klutuk (BBw). Meanwhile, Pisang Kepok cultivars showed intermediate characteristics inherited from both ancestors but closely related to *M. balbisiana* ranging from very small to small spots at the petiole base (Figure 2).

All specimens of Pisang Kepok examined showed four rows of ovules arrangement and closed margin of petiole canal, as in Pisang Klutuk. In the contrary, Pisang Barlin has two rows of ovules arrangement and opened margin of petiole canal (Figure 3). Likewise, Pisang Kepok cultivars have a homogenous red bractea colour, as well as Pisang Klutuk (Figure 4 A-E). This homogenous red colour is caused by a glycol-conjugated anthocyanin which is responsible for the primary pigments of the bracts [22]. Whilst Pisang Barlin has a yellowish red colour (Figure 4 F).

Phenotypic clustering of Pisang Kepok. In clustering analysis based total. on 35 morphological characters was conducted on 18 specimens comprising 16 in-groups and two outgroups. The clustering resulted in five main clusters, in which each specimen was clustered based on its local name and source. The outgroups were separated in Cluster I and II, i.e. Pisang Barlin dan Pisang Klutuk, respectively. Further, the phenotypic dendrogram (phenogram) shows that Pisang Kepok cultivars were clustered more closely related to Pisang Klutuk than Pisang Barlin. It was separated into three clusters, with a high similarity value of >90% (Figure 5).

Each cluster has synapomorphy and autapomorphy characters distinguishing as characters. Synapomorphy character is inherited trait possessed by two or more groups of taxa, while autapomorphy character is a unique trait which derived from and belongs to only one group of taxa [23]. Cluster III consists of 5 specimens of Pisang Kepok Manurun with autapomorphy characters, *i.e.* male flower colour (pinkish purple) and leaf midrib colour (red-purple) (Figures 1 & 2). Cluster IV consists of 8 specimens of Pisang Kepok Abang, Kepok Australi, and Kepok Putih with synapomorphic characters of male flower colour (cream without pink pigmentation) and leaf midrib colour (green) (Figures 1 & 2). Cluster V consists of 3 specimens of Pisang Kepok Putih from Gedangan and Jabung because of the pseudostem height (>3 m), fruit colour (white evenly), and base shape of bractea (wide) (Figures 4 & 6).

Furthermore, the morphological characters such as fruit flesh are commonly used as a reference for cultivar naming by the local community in Indonesia, particularly in Java [5]. Pisang Kepok Putih was named after its white fruit flesh, while Pisang Kepok Abang was named after its yellow fruit flesh (Table 2, Figure 6). Kepok "abang" is in Javanese basically meaning "red", however it is commonly referred to Pisang Kepok Abang with "vellow" fruit flesh. Meanwhile, Pisang Klutuk was named after its seeded fruit (Table 2). In addition, the names are also given by the origin area. Pisang Kepok Australi was suspected first introduced to Java from Australia. Nevertheless, some of the cultivar names do not resemble to morphology or anything, such as Pisang Kepok Manurun (manurun = down) and Pisang Barlin (Table 2).

| Sample | Locality source | Cultivar name | Perceptual meaning | Morphology | Genome |
|--------|-----------------|--------------------|----------------------------|------------|--------|
| code | | | | Score | group |
| K1 | Singosari | P. Kepok Abang 1 | Abang $(Jvn) = red$ | 59 | ABB |
| K2 | Singosari | P. Kepok Abang 2 | Abang $(Jvn) = red$ | 59 | ABB |
| K3 | Jabung | P. Kepok Putih 1 | Putih $(Jvn, Idn) = white$ | 59 | ABB |
| K4 | Jabung | P. Kepok Putih 2 | Putih $(Jvn, Idn) = white$ | 59 | ABB |
| K5 | Wajak | P. Kepok Abang 1 | Abang $(Jvn) = red$ | 59 | ABB |
| K6 | Wajak | P. Kepok Abang 2 | Abang $(Jvn) = red$ | 59 | ABB |
| K7 | Dampit | P. Kepok Manurun 1 | Manurun (Sdn) = down | 63 | ABB |
| K8 | Dampit | P. Kepok Manurun 2 | Manurun (Sdn) = down | 63 | ABB |
| K9 | Sumbermanjing | P. Kepok Manurun 1 | Manurun (Sdn) = down | 63 | ABB |
| K10 | Sumbermanjing | P. Kepok Manurun 2 | Manurun (Sdn) = down | 63 | ABB |
| K11 | Gedangan | P. Kepok Australi | Originated from Australia | 59 | ABB |
| K12 | Gedangan | P. Kepok Putih | Putih $(Jvn, Idn) = white$ | 59 | ABB |
| K13 | Donomulyo | P. Kepok Abang | Abang $(Jvn) = red$ | 59 | ABB |
| K14 | Donomulyo | P. Kepok Manurun | Manurun (Sdn) = down | 63 | ABB |

Table 2. Morphological scoring and identification results based on 15 diagnostic characters

| Sample code | Locality source | Cultivar name | Perceptual meaning | Morphology Score | Genome group |
|----------------|-----------------|----------------|----------------------------|---------------------|-----------------|
| K15 | Kepanjen | P. Kepok Putih | Putih $(Jvn, Idn) =$ white | 59 | ABB |
| K16 | Kepanjen | P. Kepok Abang | Abang $(Jvn) = red$ | 59 | ABB |
| 01 | Jabung | P. Klutuk | Klutuk (Jvn) = seed | 75 | BB |
| O2 | Gedangan | P. Barlin | No meaning | 15 | AA |

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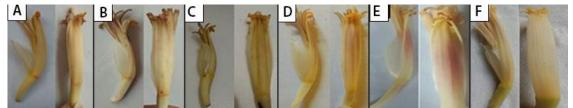


Figure 1. Phenotypic variation in compound tepal: A) Kepok Abang, B) Kepok Putih, C) Kepok Australi, D) Kepok Manurun, E) Klutuk, and F) Barlin

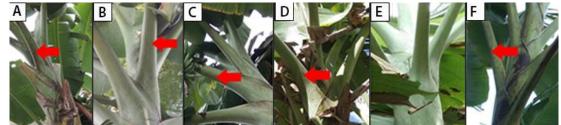


Figure 2. Phenotypic variation in petiole base: A) Kepok Abang, B) Kepok Putih, C) Kepok Australi, D) Kepok Manurun, E) Klutuk, and F) Barlin

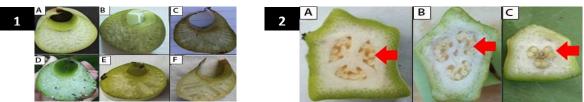


Figure 3. 1. Phenotypic variation in petiole canal margin. A) Kepok Abang, B) Kepok Putih, C) Kepok Australi; D) Kepok Manurun, E) Klutuk, and F) Barlin. **2.** Ovules arrangement: A) Kepok Abang, B) Kepok Putih, and C) Barlin

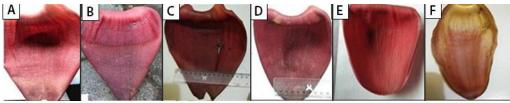


Figure 4. Phenotypic variation in bractea. A) Kepok abang; B) Kepok putih; C) Kepok australi; D) Kepok manurun; E) Klutuk; and F) Braktea Barlin

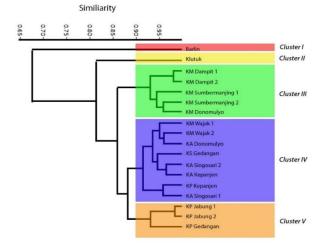


Figure 5. Dendrogram of clustering analysis based on phenotype. KM= Kepok Manurun, KA= Kepok Abang, KP= Kepok Putih, and KS= Kepok Australi

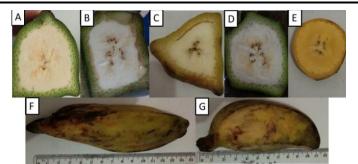


Figure 6. Phenotypic variation in fruit flesh: A) Kepok Abang, B) Kepok Putih, C) Kepok Australi, D) Kepok Manurun, and E) Barlin; and fruit shape: F) Kepok Australi, and G) Kepok Putih

PCA biplot analysis of Pisang Kepok based on morphology. A biplot is a way of simplifying the visualization of results from PCA, as they combine principal component scores of specimens (dots) and loading vectors in a single biplot view. The farther away the vectors are from the origin PC (long lines): the more influence they have on that PC [19]. The biplot analysis of 18 specimens of Pisang Kepok local cultivars and outgroups based on 35 morphological characters showed several characters with short and long vector lines from the centre point (PC origin). The longest vector line is represented by fruit flesh colour (PCM = pulp colour at maturity) with a score of 0.75 on PC1 and pseudostem colour with a score of 0.64 on PC2. Hence, the fruit flesh colour was found as the most important character that contributed to the variation of Pisang Kepok cultivars. It was supported by the fact that the fruit flesh colour, which is a mix of white and yellow, is the distinguishing feature of Pisang Kepok Manurun. Likewise, the fruit flesh colour of Pisang Kepok Putih is white, Kepok Abang is yellow, and Kepok Australi is white flesh (Figure 7).

RAPD polymorphisms of Pisang Kepok. The amplification using RAPD primers established both strong and weak bands. For the purposes of further analysis, only the strong bands were evaluated. Based on the six primers used (OPA2, OPA3, OPA4, OPA11, OPA12 and OPA18), there

were 13 bands which successfully amplified (Table 3).

DNA visualisation shows that there are two types of DNA bands produced monomorphic and polymorphic bands (Figure 8). The monomorphic band is produced on primer OPA2 (500 bp). The monomorphic band can be used as a marker for all banana cultivars examined. However, in this study, RAPD was unable to explain the coded trait of the band [24].

There were 12 polymorphic bands produced, with lengths ranging from 150–1500 bp. The resulting polymorphic bands show variation among the cultivars. Polymorphic bands indicated high genetic diversity in an organism [25]. Five out of six primers used are successfully producing polymorphic bands, i.e. OPA3, OPA4, OPA11, OPA12 and OPA18. Furthermore, based on the primer discriminatory power analysis, OPA3 was considered as the best primer for use in this study since it gives the best results of PIC, EMR, and MI value (Table 3).

Interestingly, the visualisation result shows the appearance of a unique band that was only owned by Pisang Kepok cultivars, not in the outgroups. The band was produced by OPA2 primer at a length of 300 bp (Figure 8 A). This band can be proposed as a distinguishing marker for Pisang Kepok. Further study is required to perform sequencing of this band in order to determine what specific gene is represented.

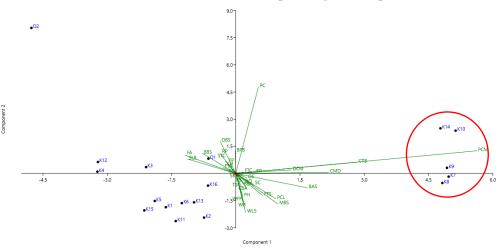


Figure 7. PCA scatter biplot of Pisang Kepok based on morphological characters

| Primers | TNB | NPB | PB (%) | PIC | EMR | MI | RP |
|---------|------|-----|---------------|------|------|------|-------|
| OPA2 | 3 | 2 | 66.6 | 0.06 | 1.3 | 0.08 | 5.63 |
| OPA3 | 4 | 4 | 100 | 0.17 | 4 | 0.68 | 3.38 |
| OPA4 | 2 | 2 | 100 | 0.11 | 2 | 0.22 | 2.00 |
| OPA11 | 2 | 2 | 100 | 0.17 | 2 | 0.34 | 0.88 |
| OPA12 | 1 | 1 | 100 | 0.19 | 1 | 0.19 | 0.50 |
| OPA18 | 1 | 1 | 100 | 0.23 | 1 | 0.23 | 0.75 |
| Total | 13 | 12 | 566.67 | 0.93 | 11 | 1.74 | 13.13 |
| Mean | 2,17 | 2 | 94.44 | 0.16 | 1.89 | 0.29 | 2.19 |

Table 3. RAPD polymorphism analysis results

Annotation: TNB= Total Number of Band, NPB= Number of Polymorphic Band, PB= Percentage of Polymorphic Band, PIC= Polymorphism Information Content, EMR= Effective Multiplex Ratio, MI= Marker Index, RP= Resolving Power.

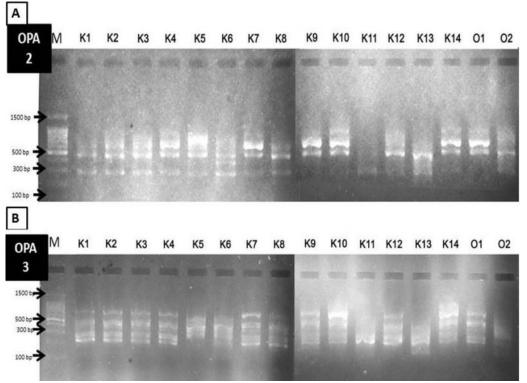


Figure 8. RAPD polymorphism visualisation: A) OPA2 and B) OPA3

RAPD clustering of Pisang Kepok. The results of clustering analysis using the RAPD marker were performed on 16 specimens, resulting in 14 ingroups and two outgroups. The clustering produced seven major groups based on a similarity value of >80%. Hence, Pisang Kepok local cultivars were found to be molecularly more diverse than phenotypically. However, the topology of dendrogram based on RAPD was quite similar to that of morphology, where each specimen was grouped according to its local name and source (Figure 9). Similar to morphological characters, these outgroups were divided into Clusters I and II, correspondingly, Pisang Barlin and Pisang Klutuk.

Furthermore, Cluster III consists of 1 specimen (KA Donomulyo) and Cluster IV consists of 3 specimens (KA Wajak 1, KA Wajak 2 and KA Singosari). Kepok Abang was clustered based on a similarity value of >70%. Cluster V consists of Kepok Manurun (KM Dampit 1 and 2, KM Sumbermanjing, and KM Donomulyo). Kepok Manurun clustered based on similarity values >80%. Kepok Manurun has similar bands in OPA 2 (600 bp, 500 bp, and 300 bp) (Figure 8A). Cluster VI consists of 2 specimens (KS Gedangan and KA Kepanjen). KA Kepanjen was not clustered with the other Kepok Abang due to several bands that did not appear in the same base pair length. Cluster VII consists of four specimens from Kepok Putih (KP Gedangan, KP Jabung 1, KP Jabung 2 and KP Kepanjen). Kepok Putih was clustered based on a similarity value of >80% (Figure 9). Based on band visualisation, Pisang Kepok cultivars were clustered closer to Pisang Klutuk due to their dominant characters. With a similarity value of more than 30%, Pisang Kepok has closer branches to Pisang Klutuk than Pisang Barlin.

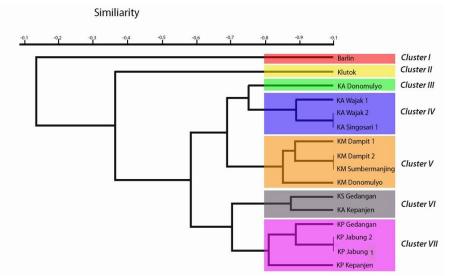


Figure 9. Dendrogram of clustering analysis based on RAPD. KM= Kepok Manurun, KA= Kepok Abang, KP= Kepok Putih, and KS= Kepok Australi.

CONCLUSION

Morphological characterisation of 16 specimens of Pisang Kepok local cultivars originating from several regions in Malang, East Java, was identified into four variants, which were confirmed as ABB genome group. The fruit flesh colour was found as the most important phenotypic character that contributed to the cultivar variation. Result of the molecular (RAPD) was a complement to the phenotypic approach, both dendrogram clusterings show a quite similar pattern following their local name and source. Pisang Kepok local cultivars were found molecularly more diverse than phenotypically, with high similarity values, i.e. >80% and >90%, respectively. This study provides basic information on the morphological and molecular characterisation of Pisang Kepok local cultivars that are useful as a basis for further development.

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