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INTRASPECIFIC VARIATION OF THIRTEEN PISANG AMBON CULTIVARS (*Musa Acuminata* CV. AAA) FROM EAST JAVA AND CENTRAL JAVA (INDONESIA) BASED ON RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKER

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Abstract

Pisang Ambon (M. acuminata cv. AAA) is popular dessert banana in Indonesia, included as Gros Michel. Particularly at Java, there are some variation of Pisang Ambon local cultivars which need to be clearly classified. This study was aims to reveal the intraspecific genetic variation and subgrouping within thirteen of Pisang Ambon cultivars from East Java and Central Java based on RAPD markers. Twenty RAPD markers were performed i.e. OPA1-OPA20. Morphologically, Pisang Ambon cultivars are mostly distinguished by their fruit size, peel colours, pulp colors and pulp taste. Indeed, the fruits are reflecting their distinct morphological or perceptual characteristics. The amplification bands produced in each sample have various lengths, ranging from 140 bp to 1500 bp. In total, there are 101 amplified. In total, 101 characters (RAPD bands) were subjected to dissimilarity distance and hierarchical clustering analysis. The tree resulted from RAPD profiles was clearly illustrated the genetic intraspecific relationship within Pisang Ambon cultivars in Java bands identified. It comprised of 86.82% polymorphic and 13.18% monomorphic bands. The derived cultivars of Pisang Ambon were separated into four sub-clusters, with low dissimilarity distances ranged 0.24 to 0.51. The conclusion of this study revealed that RAPD analysis was effective to study the genetic relationship in intraspecific level of Pisang Ambon cultivars. Furthermore, the finding of this study was showed that within Pisang Ambon cultivars were genetically variable.

Keywords*: Genetic variation; Gros Michel; Musa acuminata; Pisang Ambon; RAPD; Triploid*

Introduction

Bananas (*Musa* L.) are important crop commodity; high nutritious, and have social, economic, and cultural values worldwide, especially in tropical countries [1-7]. In Southeast Asia, as the center origin and diversity of bananas, numerous banana cultivars were recognized. It has high variability of morphological characteristics and is complicated by many cultivar names and synonyms in different languages, causing confusion in classification and nomenclature. To cope with this situation, a new taxonomical system special for banana is needed [8]. Therefore, *N.W. Simmonds and K. Shepherd* [9] proposed a new taxonomical system for bananas [10, 11] and has been determined by consensus in the 1999 [8].

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The taxonomical system proposed by *N.W. Simmonds and K. Shepherd* [9] was adopted from Chessman recommendation, that the common banana cultivars were derived from their wild ancestor *Musa acuminata* (A genome donor) and *Musa balbisiana* (B genome donor). It has three system levels, namely species name, genome group and cultivar known as genome-based nomenclature systems [8]. For example, Pisang Ambon has the scientific name *M. acuminata* (AAA) cv. Ambon

To differentiate the genome group of *M. acuminata* cultivars from *M. balbisiana* and their hybrids, *N.W. Simmond and K. Shepherd* [9] designed scorecard by using 15 diagnostics based on morphological both vegetative and phenological characters. However, identification by using the morphological approach sometimes is subjective, causing differences between several researchers in determining genomes groups in the same individual banana [12-14]. Hence, a molecular approach is needed to get more valid results. Some molecular approaches that provided useful information and new insight into the classification also to study the diversity of bananas include AFLP [15], PCR-RFLP [16-18], RAPD [19-22] barcoding DNA [23, 24] and microsatellite [25].

In particular, of triploid *M. acuminata* cultivars (AAA) are considered as globally important dessert bananas [1]. They have vigorous performance, bear heavy symmetrical bunches of large fruit and markedly curved, creamy white flesh, soft and fine textured, sweet taste with aromatic flavours so that become very prominent over the diploids [11, 26]. Triploid *M. acuminata* cultivars were arose from diploids, perhaps following crosses between edible diploids and wild *M. acuminata* subspecies, giving rise to a wide range of AAA genotypes [27, 28]. There are some subgroups of triploid M. acuminata cultivars recognized, such as Cavendish, Gros Michel/ Ambon, Red, Susu, Green Red, Lakatan, etc. [8, 10].

Specifically, the Gros Michel subgroup is locally known in Indonesia and Malaysia as Pisang Ambon [8, 10, 11]. Previously in the early 20th century, Pisang Ambon was dominated the international trade and later was replaced by Cavendish due to its susceptibility to Fusarium wilt [1, 29]. The distinguishing characters are the green-yellow peel and the green or pale pink underlying pseudo stem sheath compared to the bright yellow peel and bright red pseudo stem of Cavendish cultivars [11, 27].

There are many morphological variations of Pisang Ambon, particularly in Java [4, 12, 26]. Further, they were recognized it with their local names of each region such as Ambon Hijau (green), Ambon Kuning (yellow), Ambon Merah (red), Ambon Kecil (small), Ambon Emprit (small), Ambon Sepet (astringent taste) etc. Banana cultivar names given by local communities mostly reflect distinct morphological or perceptual characteristics, as well as uses, although some of the names do not refer to appearance or anything at all [4]. The high diversity of those Pisang Ambon local cultivars is a valuable material for banana breeding and commercial development efforts as alternative cultivars to complement the readily popular dessert bananas in the market. Hence, further studies need to be conducted.

This study was aims to reveal the intraspecific genetic variation and subgrouping within thirteen of Pisang Ambon cultivars from East Java and Central Java based on RAPD markers. RAPD markers were proven to be moderately effective to identify the genomic and study the genetic diversity of bananas, including intraspecific variation within *Musa acuminata* group [30, 31]. This method also has several advantages in the simplicity of technique, fast process, inexpensive, random primers are commercially available, only requires a small amount of DNA samples (0.5-50ng), no need initial genome information, high genomic abundance and are randomly distributed throughout the genome [32]. A proper of identification of Pisang Ambon local cultivars is important as a basic information for further selection of character for banana breeding, development, and conservation effort

Experimental

Materials

About thirteen Pisang Ambon cultivars were analyzed as ingrup, without outgroup (un-rooted). All sample of the specimens examined were collected from Banana Germplasm Garden, Yogyakarta Department of Agriculture, Indonesia (Table 1).

Each of Pisang Ambon cultivar was previously identified morphologically based on minimal descriptor for bananas [33], and cross-checked with information from the curators, also from some banana morphological references [12, 18]. The plant material used, young leaves (furled) which dried with silica gel prior to analysis, was one sample per cultivar.

Table 1. Identity of Pisang Ambon cultivars examined in this study

Code	Local name	English meaning (perceptual character)	Origin of collection
A1	Ambon Byok	Byok = mbunch (many hands in a bunch)	Bantul, Central Java
A2	Ambon Merah	Merah = red (pulp color)	Tlekung, Batu, Malang, East Java
A3	Ambon Warangan	Warangan = material to clean the dagger (not related to plant characters)	Kulon Progo, Central Java
A4	Ambon Putih	Putih = white (pulp color)	Gunung Kidul, Central Java
A5	Ambon Sepet	Sepet = astringet (pulp taste)	Gunung Kidul, Central Java
A6	Ambon Kuning	Kuning = yellow (pulp color)	Tlekung, Batu, Malang, East Java
A7	Ambon Lumut	Lumut = Green-moss (peel color)	Tembarak, Temanggung, Central Java
A8	Ambon Emprit	Emprit = small (fruit size)	Purworejo, Central Java
A9	Ambon Kecil	Emprit = small (fruit size)	Malang, East Java
A10	Ambon Jaran	Jaran = horse (not related to plant characters)	Bantul, Central Java
A11	Ambon Hong	-	Purworejo, Central Java
A12	Ambon Hijau	Hijau = green (peel color)	Tlekung, Batu, Malang, East Java
A13	Ambon	-	Dongkelan, Yogyakarta

Methods

DNA extraction

The whole genome DNA of each sample was extracted using The Wizard® Genomic DNA Purification Kit Promega. DNA extraction step was following its manufacturer's procedure for plants. DNA yield were then qualitatively evaluated by electrophoresis separation on 1% gel agarose and using DNA ladder 1-Kb (Thermo Scientific) as marker. Quantitative evaluation was conducted using AE-Nano200 Nucleic Acid Analyzer 2.0. Good DNA purity level indicated by the value of the optical density 260/280nm ratio approximately 1.8-2.0 [34].

PCR-RAPD

DNA amplification was performed with PCR Thermocycler (BIORAD) using 20 RAPD primers from Operon Technology Ltd (Table 2). The PCR mixture was conducted with a total volume of 10µL consisting of 3µL ddH2O, 1µL OPA Primer (10pmol), 5µL DreamTaq Green PCR Master Mix (2x) from Thermo Scientific and 1µL DNA template (5-20ng/µL). PCR cycling step consists of initial denaturation at 94ºC for 4 minutes, followed by 45 cycles of 30 seconds at 94ºC, 30 seconds of annealing at different temperatures based on the melting temperature of each primer (Table 2), 5 minutes at 72ºC of extension and final extension at 72ºC for 7 minutes. The PCR products were then evaluated by electrophoresis separation on 1.5% agarose gel, with DNA ladder 100bp (Thermo Scientific) was used to determine the size of DNA amplification bands and photographed under UV transiluminator (BioRAD).

Table 2. DNA sequences of OPA primes used in this study

Primer	Sequence	Tm (ºC)	TA (ºC)	GC (%)
OPA-01	5' - CAG GCC CTT C – 3'	36.4	41	70
OPA-02	5' - TGC CGA GCT G – 3'	40.7	45	70
OPA-03	5' - AGT CAG CCA C – 3'	34.3	39	60
OPA-04	5' - AAT CGG GCT G – 3'	35.1	40	60
OPA-05	5' - AGG GGT CTT G– 3'	32.6	37	60
OPA-06	5' - GGT CCC TGA C– 3'	35.2	40	60
OPA-07	5' - GAA ACG GGT G – 3'	33.2	38	60
OPA-08	5' - GTG ACG TAG G – 3'	31.1	36	60
OPA-09	5' - GGG TAA CGC C – 3'	37.4	42	70
OPA-10	5' - GTG ATC GCA G – 3'	33.1	38	60
OPA-11	5' - CAA TCG CCG T – 3'	36.7	41	60
OPA-12	5' - TCG GCG ATA G – 3'	34.0	39	60
OPA-13	5' - CAG CAC CCA C – 3'	37.7	42	70
OPA-14	5' - TCT GTG CTG G – 3'	34.3	39	60
OPA-15	5' - TTC CGA ACC C – 3'	34.2	39	60
OPA-16	5' - AGC CAG CGA A – 3'	38.3	43	60
OPA-17	5' - GAC CGC TTG T – 3'	35.7	40	60
OPA-18	5' - AGG TGA CCG T – 3'	36.2	41	60
OPA-19	5' - CAA ACG TCG G – 3'	34.2	39	60
OPA-20	5' - GTT GCG ATC C – 3'	33.5	38	60

Data analysis

The RAPD gel profiles were gathered based on the presence and absence of the amplified bands. It was scored as "1" for the present band and "0" for the absent band of each primer. Only bands which reproducible, well resolved and non-ambiguous were considered. The binary data matrix was tabulated for further analysis.

Discriminatory power of each RAPD primers was evaluated by means of four parameters, viz. Polymorphism Information Content (PIC), Effective Multiplex Ratio (EMR), Marker Index (MI) and Resolution Power (Rp) [35]. PIC was calculated using formula proposed by *I. Roldan-Ruiz et al.* [37]. EMR was measured referred to *H. Laurentin and P. Karlovsky* [38]. MI was calculated referred to *R.K. Varshney et al.* [39]. Resolution power (Rp) was calculated referred to *A. Prevost and M.J. Wilkinson* [40].

Basic genetic population statistics were calculated using the software GENALEX 6.1 [40] to determine the percentage of polymorphic, Shannon's Information Index and expected diversity of Pisang Ambon cultivars in Java. Further, to construct the intraspecific and subgrouping tree within Pisang Ambon cultivars, hierarchical clustering was conducted to both morphology and RAPD data using Unweighted Pair Group Method with Arithmetic Mean/UPGMA method option and Jaccard dissimilarity index on DARwin6 software package Version 6.0.21 [41, 42].

Results and discussion

Morphological appearances of Pisang Ambon cultivars

According to local farmers (Javanese people), within Pisang Ambon cultivars are just slightly morphologically different. They are mostly distinguished by their fruit size, peel colors,

pulp colors, and pulp tastes (Table 1). Unfortunately, during the field observation only three cultivars which were fruiting i.e. Ambon Emprit, Ambon Byok and Ambon Hong. Indeed, the fruits are reflecting their distinct morphological or perceptual characteristics (Fig. 1). As typical to Musa AAA group, the fruits of Pisang Ambon cultivars are in curved shape i.e. slight to sharp curved, with fruit transverse section slightly ridged to rounded [26]

The minimum morphological characterization has conducted only to the vegetative organs. In general, they have leaf habit intermediate to drooping, due to the large size of leaves. The pigmentation of underlying colour of pseudo stem of the Ambon cultivars are green-yellow, green-red or pale pink as mentioned by *N.W. Simmonds* [10], *E.W.M. Verheij* [11] and *J. Daniells et al.* [27]. Most of the cultivars have small to large blotches at petiole base in brown to brown-black colors. Petiole canal mostly open with margins spreading and winged. Those two characteristics are typical to *M. acuminata* cultivars [10]. In particular, Pisang Ambon Merah (red Ambon) has very distinctive red-purple coloration on its vegetative appearances from pseudo stem, petiole, and midrib (Fig. 1).

Fig. 1. Some morphological characteristics of Pisang Ambon cultivars. Remarks: A1 (Ambon Byok), A2 (Ambon Merah), A8 (Ambon Emprit), A10 (Ambon Jaran), and A11 (Ambon Hong)

Hierarchical clustering and dissimilarity distance analyses based on 35 vegetative morphological characteristics resulted a phenetic tree which separated in two main groups (Figure 3). Pisang Ambon Hijau, Ambon Jaran and Ambon Byok were clustered in Cluster I, whilst the rest cultivars were clustered in Cluster II. Further Cluster II was separated into four sub-clusters. Dissimilarity distance within Pisang Ambon cultivars based on vegetative morphology were low to moderate ranged from 0.38 to 0.69.

Sub-cluster 1 was comprised of Pisang Ambon Emprit, Ambon Warangan and Ambon Sepet (Dis.: 0.60 – 0.67). Sub-cluster 2 was comprised of Pisang Ambon and Ambon Merah (Dis.: 0.50). Pisang Ambon Lumut and Ambon Hong was the most similar pair (Dis.: 0.38) and clustered in sub-cluster 3. Sub-cluster 4 was comprised of Pisang Ambon Putih, Ambon Kuning and Ambon Kecil (Dis.: 0.46). This phenetic tree is considered not completely reflected the relationship within Pisang Ambon cultivars due to the incomplete characterization (vegetative

only) and very subjective. Thus, molecular approach is needed, because it is considered fast method, no need to wait until its fruiting, and need only small amount of plant tissues [32, 43, 44]. In addition, a complete morphological characterization particularly the fruit characteristic is necessary as it is related to consumer's preference and commercial interests [3, 6, 7].

RAPD profiles of Pisang Ambon cultivars

It was selected about 11 out of 20 RAPD primers with high reproducibility of polymorphic bands in Pisang Ambon, specifically OPA 1, OPA 2, OPA 3, OPA 4, OPA 5, OPA 11, OPA 16, OPA 17, OPA 18, OPA 19, and OPA 20. This selected OPA primers result was in accordance to previous RAPD study by *R.T. Probojati et al.* [23] to Pisang Raja (*M. acuminata* x *M. balbisiana* cv. ABB), except OPA 8 was not selected for Pisang Ambon due to low reproducibility. However, some samples were indicated not successfully amplified by certain primers i.e. Pisang Ambon Lumut (A7) by OPA 3, OPA 4, OPA 5, and OPA 17; Ambon Kecil (A9) by OPA 3; Ambon (A13) by OPA5, etc. (Fig. 2). It possibly due to its genetic material, the samples do not have the sequences which match to the OPA primer sequences.

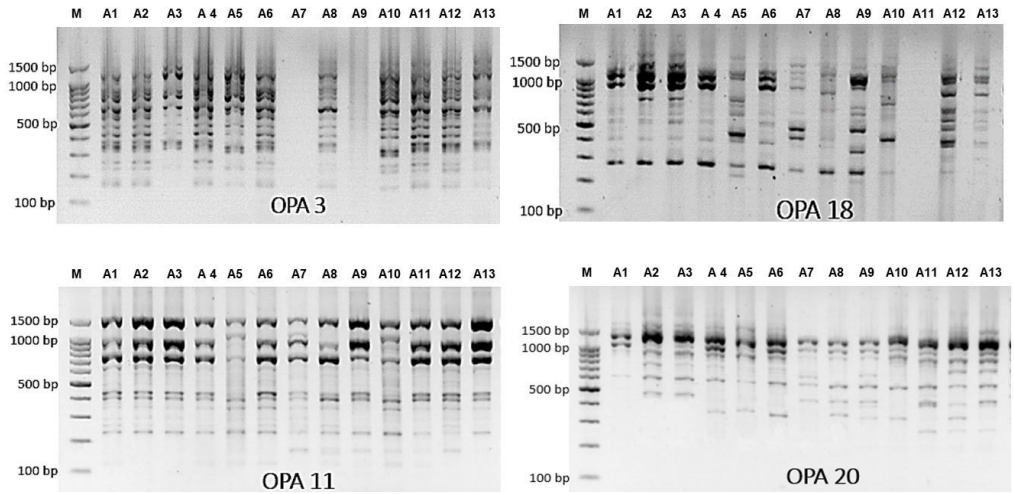

Fig. 2. Electrophoregram PCR RAPD of some selected primers: OPA 3 and OPA 18 (high polymorphisms); OPA 11 and OPA 20 (low polymorphisms). Remarks: M (marker 100 bp), A1 (Ambon Byok), A2 (Ambon Merah), A3 (Ambon Warangan), A4 (Ambon Putih), A5 (Ambon Sepet), A6 (Ambon Kuning), A7 (Ambon Lumut), A8 (Ambon Emprit), A9 (Ambon Kecil), A10 (Ambon Jaran), A11 (Ambon Hong), A12 (Ambon Hijau), and A13 (Ambon)

The amplification bands produced in each sample have various lengths, ranging from 140 bp to 1500 bp. In total, there are 101 amplified bands identified. It comprised of 86.82% polymorphic and 13.18% monomorphic bands (Table 2). The maximum number of polymorphic bands (12 bands) were obtained by primers OPA 3 and OPA 18. Whilst minimum number was obtained by OPA 20 (3 bands) (Table 2). The presence of bands on each and all bands size of samples indicate monomorphism while the presence of bands in some and not all the samples indicate polymorphisms. These polymorphisms are considered to be primarily due to variation in the primer annealing sites, but they can also be generated by length differences in the amplified sequence between primer annealing sites [32]. Each polymorphic band is an informative character to describe the genetic diversity and construct the genetic relationships among samples [21, 32].

Further, monomorphic bands were indicated no genetic variation in all samples. The monomorphic bands identified in this study produced by primers OPA 11 (220, 400, 450, 750 and 1500bp); OPA 16 (450, 520 and 700bp) and OPA 20 (600, 800, 1000, 1100 and 1300bp)

(Fig. 2). Further, RAPD markers are not locus-specific, band profiles cannot be interpreted in terms of loci and alleles (dominance of markers), and similar sized bands (monomorphic bands) may not be homologous [32, 37]. RAPD are dominant markers and hence have limitations in their use as markers for mapping, however it can be overcome to some extent by selecting those markers that are linked in coupling [45].

Discriminatory power of RAPD primers for Pisang Ambon cultivars

PIC analysis can be used to evaluate the most informative markers for genetic mapping and phylogenetic analysis [35]. The range of PIC values in dominant markers such as RAPD is ranging from 0 - 0.5. The OPA 3 and OPA 1 were considered as the most informative primers to determine genetic variation in Pisang Ambon cultivars with high PIC value (0.36), meanwhile the lowest PIC value was indicated by OPA 11 (0.17) (Table 3).

Table 3. Discriminatory power analysis of OPA1-20 RAPD primers in Pisang Ambon cultivars

No	Primer	TNB	NPB	PB(%)	PIC	EMR	MI	RP
1	OPA 1	9	9	100	0.36	81	28.97	6.62
2	OPA 2	8	8	100	0.31	64	20.07	5.69
3	OPA 3	12	12	100	0.36	144	51.98	12.15
4	OPA 4	11	11	100	0.23	121	28.12	5.54
5	OPA 5	8	8	100	0.33	64	21.40	5.38
6	OPA 11	10	5	50	0.17	50	8.28	15.38
7	OPA 16	9	6	67	0.18	54	9.80	12.92
8	OPA 17	7	7	100	0.33	49	16.07	10.53
9	OPA 18	12	12	100	0.26	144	38.06	7.54
10	OPA 19	7	7	100	0.34	49	16,90	5.23
11	OPA 20	8	3	38	0.18	32	5.68	13.69
Total		101	88	955	3.05	852	245.33	100.67
Mean		9.20	8.0	86.82	0.28	77.45	22.30	9.15

Remarks: TNB: total number of bands; NPB: number of polymorphic bands; PB (%): polymorphic band percentage; PIC: polymorphism information content; EMR: effective multiplex ratio; MI: marker index; Rp: resolution power

The EMR value is used to determine the effective primers in producing the number of polymorphic bands in the observed sample [37]. The EMR value of each primer in this study was ranged from 32 – 144, with an average of 77.45 per primer (Table 3). The most effective primer in producing polymorphic bands in Pisang Ambon cultivars were OPA 3 and OPA 18; whilst OPA 20 was the most ineffective primer.

The high MI value is reflected the efficiency of marker to simultaneously analyze a large number of bands, rather than the level of polymorphism detected [35, 38]. The MI value of each primer in this study was ranged from 5.68 - 51.98 with an average of 22.30 per primer (Table 3). The most efficient primer in producing polymorphic bands in Pisang Ambon cultivars is OPA 3, while OPA 20 was the most inefficient.

The value of Rp is the ability of each primer to detect level of variation between individuals [35]. Further, it can be used to determine the strength of a primer in producing clear bands. The Rp value of each primer in this study was range between 5.23 – 15.38 with an average of 9.15 per primer. The OPA 11 was considered as the best primer to produce clear bands and detect variation in Pisang Ambon cultivars, while the lowest Rp value is generated by OPA 19. Hence, according to four polymorphisms discriminatory power parameters, those selected primers are suitable and recommended to determine genetic variability in Pisang Ambon cultivars, except OPA 11 and OPA 20 which considered less suitable due to its low discriminatory power of polymorphisms.

Genetic variation within Pisang Ambon cultivars

Genetic variation in plants are valuable information in plant conservation and management, for further genetic improvement and development. The level of genetic diversity observed corroborates with the wide range of morphological variability observed in banana populations [30]. Result of the genetic population statistic of this study with assumption the

local cultivars samples as part of a population of Pisang Ambon in Java showed that it has high of polymorphism percentage about 83.17%. Further, the Shannon's information index was considered moderate (I = 0.39); and also, moderate expected genetic diversity (He = 0.27). Hence, this study confirmed that the morphological variation within Pisang Ambon cultivars is not due to somatic mutations but genetically variable in moderate level.

M. acuminata species was exhibited higher genetic diversity compared to those reported in *M. balbisiana* [24, 30, 31]. The high level of polymorphisms detected was pressumable due to banana plant adaptation to climate and environments which lead to mutations. Further, it was maintained by vegetative propagation (clonal), which then elaborated by natural and artificial selection, also dispersal by human, etc. Banana domestication has been a relatively continuous process, is extremely complex, occurred over thousands of years and involved multiple stages, and often separated in time and place [28, 46, 47]. Large chromosomal translocations mechanism in *M. acuminata* subspecies is proposed to have played a role in the emergence of triploid cultivars [48].

Intraspecific relationship of Pisang Ambon cultivars based on RAPD profiles

In total, 101 characters (RAPD bands) were subjected to dissimilarity distance and hierarchical clustering analysis. The tree resulted from RAPD profiles was clearly illustrated the genetic intraspecific relationship within Pisang Ambon cultivars in Java. The tree topology (RAPD) was very different to morphology. Pisang Ambon Lumut was placed as a basic cultivar of Pisang Ambon in Java with moderate distance 0.61 (Figure 3). A basic cultivar (clone) is a selected clone from a cultivated or wild population, which became popular in and beyond its original area of existence as a result of diffusion, migration and so on [46]. Previous phylogenetic study by *K. Hapsari et al.* [25] using ITS region also confirmed that Pisang Lumut was the basic cultivar of AAA group in Java.

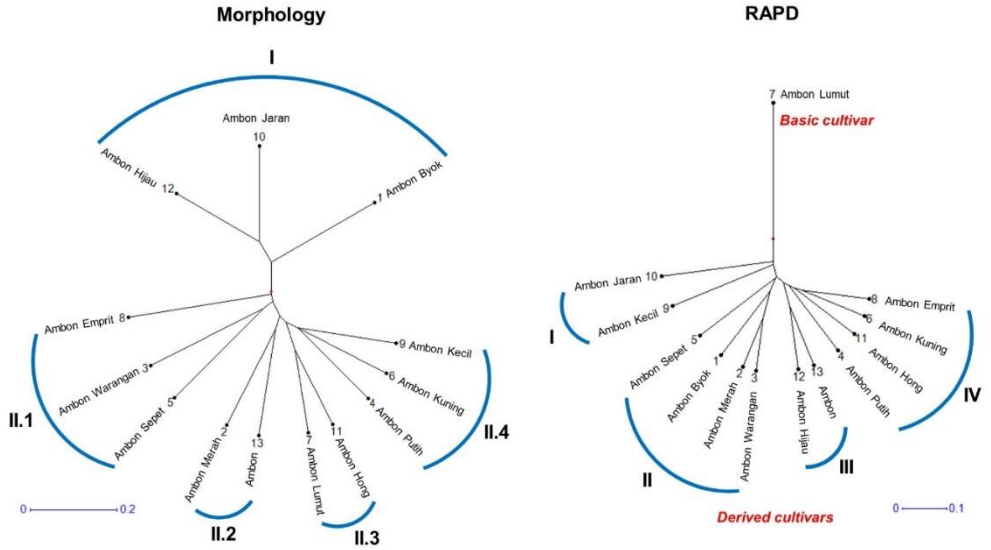

Fig. 3. Phenetic tree topology based on morphology (left) and genetic tree topology based on RAPD profiles (right)

The more vigorous and nearly sterile triploids basic cultivars of AAA were considered come first (after intersubspecific AA diploids), then AAB and later on ABB [46]. *R.C. Perrier et al.* [28] have proposed that the development of numerous other triploid subgroups was contributed by the continual dispersal of AAcvs by people and followed through hybridization with local diploids. The globally distributed commercial AAAs, Gross Michel and Cavendish

were presumably deriving genetically from a 2N gamete of the AAcv Mlali subgroup and an N gamete of the AAcv Khai (The northern origin of these AAA—around the Gulf of Thailand or the South China Sea). Later, the basic cultivar was further selected and dispersed lead to somatic mutants produced subsequently through clonal propagation over a timespan of centuries or perhaps even millennia and separated into some derived cultivars [46].

Further, the rest of Pisang Ambon cultivars were considered as derived cultivars (Figure 3). It is a clone with a slightly different morphology and presumed to stem vegetative from somatic mutation of the basic cultivar [46]. However according to this study, the derived cultivars (twelve Pisang Ambon cultivars) were confirmed genetically variable (moderate level) from its basic cultivar (Pisang Ambon Lumut). Clonal plants would appear to be at a particular disadvantage due to their limited capacity for adaptation. However, epigenetic mechanisms in clonal propagated plants (including bananas) also involved to the adaptation and evolution process. It facilitates and optimize phenotype variation in response to environmental change may permit them to be well suited to projected conditions [49].

The derived cultivars of Pisang Ambon were separated into four sub-clusters, with low dissimilarity distances ranged 0.24 to 0.51. Cluster I was comprised of Pisang Ambon Kecil and Ambon Jaran (Dis.: 0.51). Cluster II comprised of Pisang Ambon Sepet, Ambon Byok, Ambon Merah, and Ambon Warangan (Dis.: 0.24 – 0.44). Further, Pisang Ambon Merah and Ambon Warangan was the closest related pair (Dis.: 0.24). Pisang Ambon and Ambon Hijau was also revealed as close related with low dissimilarity (Dis.: 0.29), and clustered in Cluster III. Whilst Cluster IV comprised of Pisang Ambon Putih, Ambon Hong, Ambon Kuning and Ambon Emprit (Dis.: 0.31 – 0.37) (Fig. 3).

Conclusions

RAPD analysis was effective to study the genetic relationship in intraspecific level of Pisang Ambon cultivars. The finding of this study was revealed that within Pisang Ambon cultivars were genetically variable. Pisang Ambon cultivars in Java has high of polymorphism percentage about 83.17% and moderate genetic diversity. The tree topology (RAPD) was very different to morphology. Pisang Ambon Lumut was placed as a basic cultivar (clone) of Pisang Ambon in Java with moderate dissimilarity distance 0.61, and the rest of Pisang Ambon cultivars were considered as derived cultivars with low dissimilarity distances ranged 0.24 to 0.51. The derived cultivars were separated into four clusters. This basic genetic information of Pisang Ambon cultivars from Java in study was important to support further breeding and development program of bananas. A complete morphological characterization followed by nutrient testing and other pest and diseases resistance testing were required to find superior characteristics in order to improve the existing commercial Gros Michel bananas.

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