CHARACTERIZATION AND EVALUATION OF MUSA ACUMINATA CULTIVARS IN INDONESIA BASED ON MICROSATELLITE MARKERS

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Amin Retnoningsih, Rita Megia & Alex Hartana. 2010. Karakterisasi dan Evaluasi Kultivar *Musa acuminata* di Indonesia Berdasarkan Penanda Mikrosatelit. *Floribunda* 4(1): 21–32. — Kesulitan mengidentifikasi kultivar *Musa acuminata* disebabkan karena tingginya variasi, banyaknya nama dan sinonim. Karakter morfologi menyediakan informasi yang sangat terbatas, karena itu identifikasi kultivar sangat sulit dilakukan. Tujuan dari studi ini adalah untuk menjelaskan status taksonomi, mengidentifikasi dan menghasilkan informasi hubungan genetika aksesi pisang berdasarkan penanda mikrosatelit. DNA dari 59 aksesi pisang yang secara morfologi diklasifikasikan ke dalam kelompok genetik AA dan AAA dianalisa dengan menggunakan 12 primer. Hasil analisa memperlihatkan bahwa 8 primer menghasilkan diskrit dan pengulangan alel. Jumlah alel per primer berkisar dari 5 untuk MaCIR327b sampai 15 untuk MaCIR108 dan Ma-1-139. Tiga alel terbesar untuk MaCIR108 termasuk alel genomik B, sementara 12 alel lainnya termasuk dalam kisaran panjang alel genomik A. Oleh karena itu, 11 aksesi yang mempunyai alel genomik A dan B telah diklasifikasikan ke dalam kultivar *M. acuminata* secara salah. Sebelas aksesi tersebut harus ditempatkan pada jenis hibrid *M* x *paradisiaca*. Empat puluh delapan aksesi dalam studi ini tidak memperlihatkan perubahan status atau tidak memiliki alel spesifik untuk membedakan kelompok genomik AA dan AAA. Kesulitan membedakan AA dari AAA juga kemungkinan disebabkan oleh pengaruh alel mikrosatelit. Sebagai akibatnya, aksesi yang memiliki maksimum 2 alel pada masing-masing primer seharusnya dikelompokkan sebagai kelompok genomik AA atau AAA. Karena adanya 6 genotip yang identik yang terdiri dari 12 aksesi, terdapat 42 genotip yang dideteksi dari 48 aksesi yang digunakan dalam studi ini dengan 8 primer. Hubungan kekerabatan memperlihatkan bahwa kelompok genomik kultivar *acuminata* secara jelas tidak dapat dibedakan, sehingga kelompok tersebut cenderung dikelompokkan berdasarkan tingkat ploidinya.

Kata kunci: Pisang, kelompok genomik, mikrosatelit, *M. acuminata,* status taksonomi

Amin Retnoningsih, Rita Megia & Alex Hartana. 2010. Characterization and Evaluation of *Musa acuminata* Cultivars in Indonesia Based on Microsatellite Markers. *Floribunda* 4(1): 21–32. — Difficulties in identifying *Musa acuminata* cultivars are caused by their large variations, numerous names and synonyms. The morphological characters provide limited information, therefore identifying cultivars could be difficult. In line with the difficulty to identify the cultivars, thus this study was aimed to verify taxonomic status, to identify and to reveal genetic relationships among banana accessions based on microsatellite markers. The DNA of 59 banana accessions which have morphologically been classified into AA and AAA genomic groups was analyzed using 12 primers. The results showed that only 8 primers producing discrete and repeatable alleles. The number of alleles per primer ranged from 5 for the MaCIR327b to 15 for the MaCIR108 and the Ma-1-139. The largest three of the MaCIR108 alleles included in length range of the B genomic alleles, whereas the other 12 alleles fell into length range of the A genomic alleles. Hence, the 11 accessions having alleles of the A and B genomes have been incorrectly classified into *M. acuminata* cultivars. They should be placed in hybrids species *M.* x *paradisiaca*. Forty eight accessions studied neither exhibited status change, nor possessed specific alleles to distinguish between AA and AAA genomic groups. Difficulties in separating AA from AAA may also as a result of the dosage effects of a microsatellite allele. Therefore, the accessions having maximum 2 alleles in each primer should be designated as AA or AAA genomic group. Due to the presence of 6 identical genotypes consisting of 12 accessions, there were detected only 42 genotypes resulted from analysis of the 48 accessions using the 8 primers. Genetic relationship analysis showed that genomic groups of those pure *acuminata* cultivars could not be differentiated significantly, though most of them tend to be clustered according to ploidy level.

Keywords: Banana, genomic group, microsatellite, *M. acuminata,* taxonomic status.

Large variations, numerous names and synonyms in *Musa acuminata* cultivars led to difficulties in cultivar identification (Valmayor et al. 2000). The differences in the A genome size as reported by Lysak et al. (1999), Kamate et al. (2001) and Dolezel et al. (2004) indicated that they contribute large variations within *M. acuminata* cultivars. Distinguishing cultivars based on morphological characters could be difficult because the information provided by morphological characters are limited especially in determining morphologically overlapped cultivars (Sefe et al. 2001). Therefore, International Network for the Improvement of Banana and Plantain (INIBAP) (2006a) proposed to investigate synonymy and variation among cultivars through molecular studies.

Molecular markers have played essential role in plants identification (Schulman 2006; Heslop-Harrison & Schwarzacher 2007). These techniques were more capable in distinguishing one individual or group of individuals from the others than morphological method (Ford-Lloyd et al. 1997). Several molecular techniques have been employed for analyzing genetic diversity of *Musa*, such as AFLP (Ude et al. 2002; Wong et al. 2002), cpDNA PCR-RFLP (Ge et al. 2005), ISSR (Racharak and Eiadhong 2007), AP-PCR (Agoreyo et al. 2008), and SSR or microsatellite (Creste et al. 2003, 2004).

Microsatellite marker analysis is one of the most informative methods for exposing diversity of banana cultivars (Kaemmer et al. 1997; Crouch et al. 1998). The markers are abundant and widely dispersed throughout *Musaceae* genomics (Lagoda et al. 1997). Microsatellite are highly polymorphic since their length may vary among individuals (Creste et al. 2005). In additions, the markers were codominant, reproducible, and easy to be interpreted (Crouch et al. 1999). Microsatellites were easier to be used than RFLP (Holton 2001) because they can be amplified using PCR methods. The methods have some advantages compared to hybridization-based techniques, particularly if large numbers have to be investigated (Kaemmer et al. 1997). Microsatellites are also indicated as a good method for marker-assisted selection in *Musa* (Crouch et al. 1998). Kaemmer et al. (1997) suggested that microsatellite should be used as an anchor marker for a core map of banana genetic.

The purposes of this study were to clarify taxonomic status, to determine the importance of microsatellite markers in identifying *M. acuminata* cultivars previously identified based on morphological characters, and to reveal their genetic relationships using microsatellite markers.

MATERIALS AND METHODS

Plant materials and microsatellite primers

Fifty nine accessions of *M. acuminata* cultivars from various regions in Indonesia were used in this study (Table 1). These accessions have been previously identified based on morphological characters, and classified into AA and AAA genomic groups (Jumari 2000; Jumari and Pudjoarinto 2000; Edison et al. 2004; INIBAP 2002a; Siddiqah 2002).

Characterization and evaluation of AA and AAA genomic groups was conducted using 12 microsatellite primers synthesized by Invitrogen life technologies Japan® (Table 2). The eight primers were designed by Kaemmer et al. (1997) while the remaining 4 primers were designed by Crouch et al. (1998). The MaCIR108 designed by Kaemmer et al. (1997) was known to provide diagnostic characters for banana accessions containing B genome (Kaemmers et al. 1997; Creste et al. 2003). The B genomic characters were derived from the other ancestral parent of most cultivated bananas namely *M. balbisiana* Colla (Stover & Simmonds 1987).

DNA extraction and PCR condition

Total DNA was extracted from the youngest fresh leaves for each accession by modification of the SDS method described by Dixit (1998). Samples were treated without PEG solution, quantified using spectrophotometer, and diluted to an estimated concentration of 10 ng µl-1. Amplification of microsatellite region was per-formed following modified Kaemmer et al. (1997) procedure. PCR reactions were mixture of 10 µl volume containing 1.5 µl DNA template, 1 µl 10 x PCR buffer with 20 mM MgCl2, 0.2 µl 10 mM dNTPs, 0.2 µl 10 µM of each primer, and 0.06 µl 5 µl-1 Taq DNA Polymerase Native (GenScript Corporation®). The amplifications were performed in a Perkin Elmer 2400 thermocycler®, programmed with an initial denaturing step at 94ºC for 5 min, followed by 35 amplification cycles consisting of 30 s denaturation at 94ºC, 30 s annealing at specific melting temperature of each primer (Table 2), and 30 s extension at 72ºC, and ending with a final extension at 72ºC for 10 min.

Table 1. List of 59 accessions used in the study of *M. acuminata* cultivars

Accession	Subgroup[a]	Genomic group	Region	Source/ collection[b]
Berlin	Pisang Mas	AA	Banyuwangi	Diperta
Cici Gading	Pisang Becici	AA	Tegalsari	Diperta
Cici Kuning	-	AA	Tegalsari	Diperta
Cici Merah	-	AA	Sukoharjo	Diperta
Emas Batangmerah	-	AA	Bukit tinggi	RIF
Fungkah Mos	-	AA	Amban Manokwari	RIF
Keja	-	AA	Sentani Jayapura	RIF
Koja Pretel	Pisang Mas	AA	Gunung Kidul	Diperta
Koumus	-	AA	Amban Manokwari	RIF
Lilin	Pisang Becici	AA	Palembang	Diperta
Mas	-	AA	Jasinga Bogor	Bogor
Mas Besar	Pisang Mas	AA	Uknown	Diperta
Mas 40 Hari	Pisang Mas	AA	Uknown	Diperta
Monyet	Pisang Becici	AA	East Java	Diperta
Mulih Hijau	-	AA	Jasinga Bogor	Bogor
Mulıh Putıh	-	AA	Jasınga Bogor	Bogor
Neij Sehi	-	AA	Ransiki Jayapura	RIF
Penjalin	Pisang Mas	AA	Sleman	Diperta
Pinang	Pisang Mas	AA	Banyuwangi	Diperta
Raja Wligi	Pisang Mas	AA	Purworejo	Diperta
Rejang	Pisang Mas	AA	Sleman	Diperta
Rejang	-	AA	Unknown	RIF
Tegetmolo	-	AA	Bokondini Jayawijaya	RIF
Tolu	-	AA	Unknown	RIF
Uli	-	AA	Jasinga Bogor	Bogor
Abu	-	AAA	Jasinga Bogor	Bogor
Agung Pasuruan	Mauli	AAA	Pasuruan	Diperta
Ambon	Ambon	AAA	Yogyakarta	Diperta
Ambon	-	AAA	Jasinga Bogor	Bogor
Ambonaae	Ambon	AAA	Sentani Jayapura	RIF
Ambon Hijau	Ambon	AAA	Unknown	RIF
Ambon Hong	Ambon	AAA	Purworejo	Diperta
Ambon Jaran	Ambon	AAA	Bantul	Diperta
Ambon Lumut	-	AAA	Jasinga Bogor	Bogor
Ambon Merah	-	AAA	Malang	Diperta
Ambon Putih	Ambon	AAA	Jambi	RIF
Ambon Sepet	-	AAA	Gunung Kidul	Diperta
Ambon Warangan	-	AAA	Kulon Progo	Diperta
Angleng Kuning	Potho	AAA	Bogor	Diperta
Anjasmara	Ambon	AAA	Sleman	Diperta
Barangan	-	AAA	West Kalimantan	Diperta
Bole	-	AAA	Bokondini Jayawijaya	RIF
Cebol	Ambon	AAA	Yogyakarta	Diperta
Cebol Morosebo	Ambon	AAA	Kota Gede	Diperta
Comot Abang	-	AAA	Malang	Diperta
Gorohito	Ambon	AAA	Bantul	Diperta
Kidang Ijo	Potho	AAA	Sleman	Diperta
Koumusona	-	AAA	Amban Manokwari	RIF
Lase	Ambon	AAA	Bogor	Diperta
Mauli	Mauli	AAA	Samarinda	Diperta
Nona	-	AAA	Girimulyo	Diperta
Papan	-	AAA	Jasinga Bogor	Bogor
Potho Ijo	Potho	AAA	Bantul	Diperta
Potho Merah	-	AAA	Bantul	Diperta
Potho Wangi	Potho	AAA	Kulon Progo	Diperta
Sri Nyonya	Mauli	AAA	Banyuwangi	Diperta
Raja Delima	Potho	AAA	Malang	Diperta
Raja Kriyak	Ambon	AAA	Temanggung	Diperta
Usuk	-	AAA	Banyumas	Diperta

a. following the subgroup determination key constructed by Jumari (2000) and Jumari and Pudjoarinto (2000)

b - Diperta, Dinas Pertanian dan Kehewanan, Yogyakarta

- RIF, Research Institute of Fruits, Solok West Sumatera

Table 2. Primers used for detecting genetic variations among the 59 banana accessions of *M. acuminata* cultivars

Primers	Forward primer sequence Reverse primer sequence 5'-3'	Optimized annealing temperature (ºC)
MaCIR108[a]	F: TTTGATGTCACAATGGTGTTCC R: TAAAGGYGGGTTAGCATTAGG	55
MaCIR327a [a]	F: TCCCATAAGTGTAATCCTCAGTT R: CTCCATCCCCCAAGTCATAAAG	53
MaCIR327b [a]	F: AAGTTAGTCAAGATAGTGGGATTT R: CTTTTGCACCAGTTGTTAGGG	50
MaCIR332a [a]	F: TCCCAACCCCTGCAACCACT R: ATGACCTGTCGAACATCCTTT	53
Ma-1-5 [a]	F: GGTTGGAACGGAGGTATACTAA R: TCCAAGCTTATCGATCTACG	56
Ma-1-17 [a]	F: AGGCGGGGAATCGGTAGA R: GGCGGGAGACAGATGGAGT	56
Ma-1-27 [a]	F: TGAATCCCAAGTTTGGTCAAG R: CAAAACACTGTCCCCATCTC	56
Ma-1-132 [a]	F: GGAAAACGCGAATGTGTG R: AGCCATATACCGAGCACTTG	53
Ma-3-48[b]	F: CCCGTCCCATTTCTCA R: TTCGTTGTTCATGGAATCA	52
Ma-3-104 [b]	F: AGAACGTTTGCTGTTGGAG R: GCTTCTGTCATCGTTTTGTC	53
Ma-3-90 [b]	F: GCACGAAGAGGCATCAC R: GGCCAAATTTGATGGACT	56
Ma-3-139 [b]	F: ACTGCTGCTCTCCACCTCAAC R: GTCCCCCAAGAACCATATGATT	56

[a] Primers designed by Kaemmer et al. (1997).
[b] Primers designed by Crouch et al. (1998).

Electrophoresis and polymorphism detection

Electrophoresis was performed in 6% denaturized polyacrylamide gels 7 M Urea (Sigma-Aldrich Chemie Germany®), and its product was visualized by silver staining according to modified *Creste* et al. (2001) method. Solutions were prepared using distilled water, all chemicals used for staining were analytical grade.

Data analysis

Characterization and evaluation were carried out based on the presence of microsatellite bands from each accession. Each band variant was treated as an allele. Different sizes of these alleles were estimated by a 100-bp ladder molecular size standard (Invitrogen life technologies Japan®). Alleles were scored (1) for presence or (0) for absence. Heterozygous genotype exhibited 2 alleles for diploid or triploid and 3 alleles for triploid, while homozygous genotype exhibited only 1 allele. Similarity estimation was conducted based on Jaccard coefficient using Similarity of Qualitative Data (SYMQUAL) procedure. Sequential, Agglomerative, Hierarchical and Nested (SAHN) clustering was performed using procedure of Unweighted Pair-Grouping Method with Arithmatic Average (UPGMA) and plotted in a dendrogram using the Tree Plot function of the NTSYSpc version 2.02 (Rohlf 1998).

RESULTS AND DISCUSSION

Eight of the 12 primers used for analyzing the accessions generated discrete and repeatable alleles and showed polymorphic banding patterns. The size of alleles approximately 110–436 bp with polymorphism degree per primer ranged 5–15 alleles. The highest degree of polymorphism was observed in the Ma–3–139 and the MaCIR108, and the lowest was detected in the MaCIR327b. The allele polymorphism obtained from primer MaCIR327b is shown in Figure 1.

The largest three of 15 alleles of the MaCIR108 were indicated as diagnostic characters

for banana containing the B genome. The length of those alleles was within the size range of *M. balbisiana* alleles, whereas the size of other 12 alleles fell into the length range of *M. acuminata* alleles. Similar allele sizes were first discovered by Kaemmer et al. (1997), and these have been used for characterizing banana germplasm in Brazil (Creste et al. 2003). According to Kaemmer et al. (1997), the flanking region size of the B genomic allele is 259 bp which is 49 bp longer than that of the A genomic allele (Figure 2). The number of perfect repeat varied between 4 and 30 units, depending on the microsatellite consensus. Besides that, the perfect sequence repeats of the MaCIR108 are dinucleotide types. Thus, expectation of minimum size of the B genomic alleles was 259 plus 8 bp and maximum size of the A genomic alleles was 210 plus 60 bp.

Accessions containing the number of perfect repeat of less than 5 tended to produce a monomorphic banding pattern (Kaemmer et al. 1997). For that reason, alleles of the MaCIR108 that longer than 270 bp were assumed as alleles of the B genome. This result supported by the study of Creste et al. (2003) which also obtained alleles for the B genome from the same primer. The MaCIR108 microsatellite marker could be used for evaluating taxonomic status of banana cultivars, especially for the accessions derived from inter and intraspecific hybridization of *M. acuminata* (AA) and *M. balbisiana* (BB). The presence of alleles of the MaCIR108 with the size of more than 270 bp in the 11 accessions indicated that they should not be classified into *M. acuminata* cultivar. In addition to the B genomic allele, they also possessed the A genomic alleles derived from interspecific crosses between *M. acuminata* and *M. balbisiana*. As reported by Kaemmer et al. (1997), the present study also exhibited that the A genomic alleles of the MaCIR108 tend to be shorter than alleles of the B genome.

Ten of the 11 accessions consisting of 'Keja', 'Abu', 'Agung Pasuruan', 'Ambon', 'Ambon Jaran' 'Ambon Sepet', 'Koumusona', Photo Wangi', 'Raja Delima', and 'Usuk' showed 1 allele from the alleles size range of the B genome, while the other accessions namely 'Comot Abang' showed 2 alleles from the same

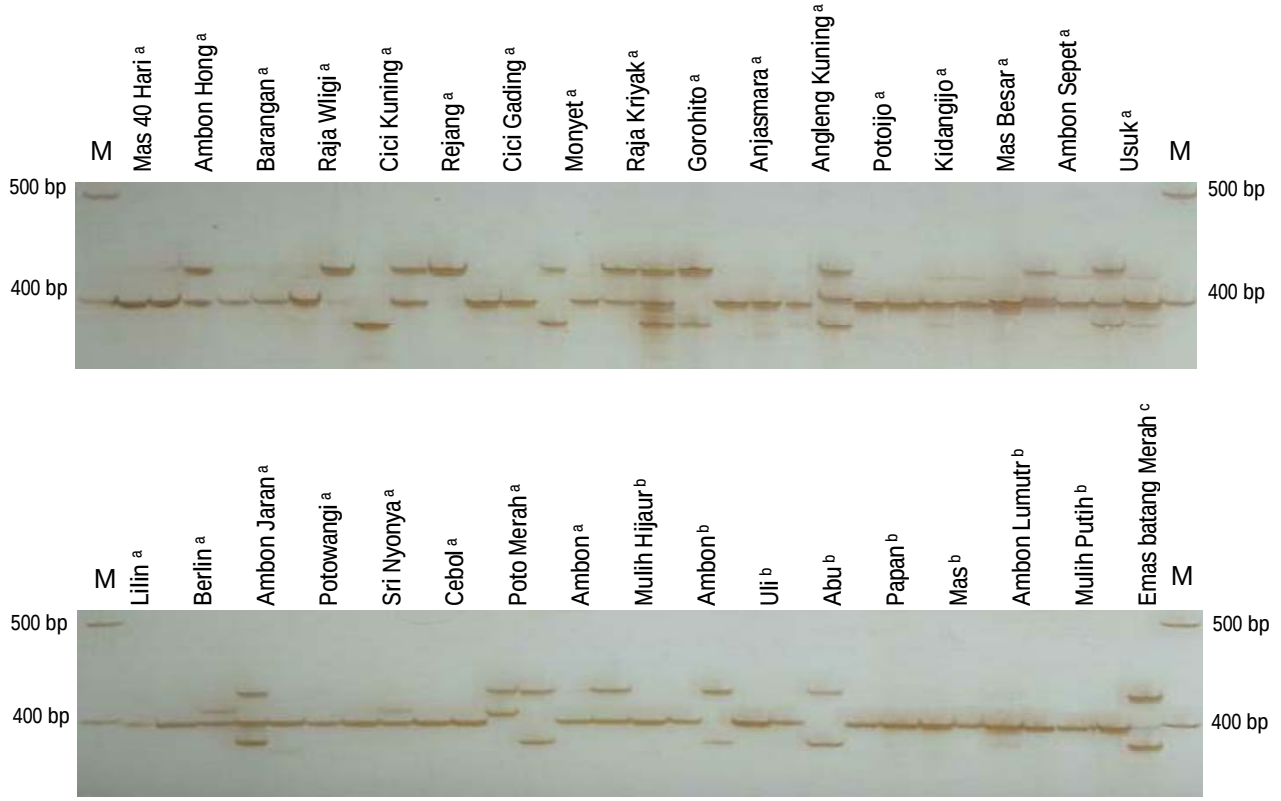

Figure 1. Allele pattern of primer MaCIR327b. Lane M is 100-bp DNA ladder, accession$_a$ obtained from collection of Diperta, Yogyakarta, accession$_b$ obtained from fields of cultivated bananas in Jasinga Bogor, West Java, and accession $_c$ obtained from collection of RIF Solok, West Sumatera.

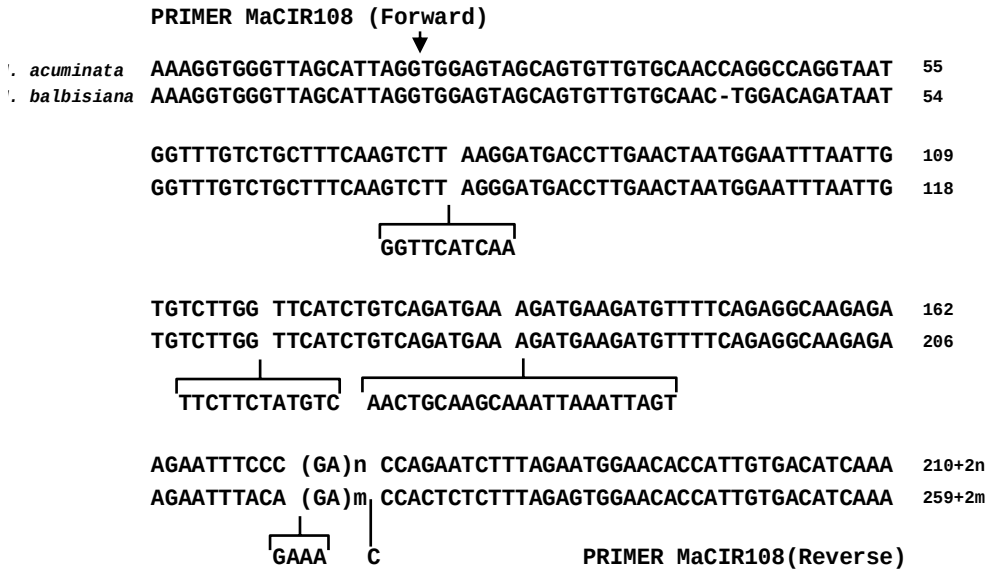

Figure 2. Sequences comparison of *acuminata* and *balbisiana* alleles of locus MaCIR108. n, m= number of repeats (Kaemmer et al. 1997).

size range. The Ambon group may be mislabeled due to the names used were well-known to designate *M. acuminata* cultivars. Nomenclature of banana cultivars was determined by their genomic composition (Pillay et al. 2004) and how cultivars arising in the cultivation (Brickell et al. 2004). According to International Code of Botanical Nomenclature (ICBN) appendix I and International Code of Nomenclature for Cultivated Plants (ICNCP) article 16, the correct name for the 11 banana accessions is *M.* x *paradisiaca* L.

The study observed 2 accessions containing the B genome, 'Keja' and 'Koumusona' have been documented in *Musa* Germplasm Information System (MGIS) (Guinard et al. 2004), as AA and AAA genomic groups, respectively. This incorrect identification may due to subjectivity of scoring systems of the morphological characters (Silayoi & Chomchalow 1987). A cultivar having score between the ranges of 2 genomic groups was difficult to be classified (Karamura 1998). In this case, a genomic group is usually determined based on general appearance of the plant; such as leaf habit, canal of leaf petiole and leaf blade base. As an example, 'Potho Wangi' which possessed score 25 (between AA, AAA, and AAB genomic groups), was classified into the AAA genomic group because the canal of leaf petiole is opened with margins spreading and the both side of leaf blade base is rounded. Both characters are usually found in the AAA genomic group (Jumari 2000). The present analysis of 'Potho Wangi' using microsatellite markers detected 1 allele of the B genome and 2 alleles of the A genome. Therefore, the accession should be placed into the AAB genomic group and should be designated under the name *M.* x *paradisiaca*.

Classification and nomenclature of cultivated banana remain to be complicated issue (Valmayor et al. 2000; INIBAP 2006b). There is no publication or consensus that could be used as a reference by the banana community. Therefore, Taxonomy Advisory Group (TAG) for *Musa* attempts to develop an accepted nomenclature and glossary of terms by which the *Musa* research community can communicate and understand each other (INIBAP 2006b). It is hope that molecular analysis in this study could provide important contribution for supporting the TAG attempts.

Taxonomic status of the 48 accessions studied did not change. These accessions should be included in *M. acuminata* cultivars. Using the 8 primers (Table 2) minus the primers Ma–1–5; MaCIR327a; Ma–3–48; and Ma–3–104, a total of 82 alleles were produced, with the mean value of 10.3 alleles per primer. The number of alleles per primer from the 48 accessions of pure *acuminata* ranged 4–14. The highest degree of polymorphism was observed in the Ma–3–139 and the lowest in the MaCIR327b, with the mean observed heterozygosity of 0.769. The range of allele MaCIR108 size of pure *acuminata* groups was from approximately 220–268 bp (Table 3). Expectation of maximum size of the A genomic alleles is 270 bp,

therefore the length range from 220 to 268 bp should be used as a diagnostic character for the A genome. This result illustrated a variation in the A genome supported by the cytometric analysis (Lysak et al. 1999, Kamate et al. 2001, Dolezel et al. 2004).

The number of alleles obtained in the study was larger than in previous study. Using the primers MaCIR108; Ma–1–17; Ma–3–90; and Ma–3–139, Creste et al. (2003) obtained 29 alleles from 35 accessions containing the A and B genomes. By the same primers, the present study observed 50 alleles from the 48 accessions of the A genome alone. Accessions investigated also exhibited a greater value of observed heterozygosity than those of banana germplasm in Brazil (Creste et al. 2003, 2004). The high genetic diversity supported the hypothesis that Indonesia is one of the main centre of diversity and most likely the centre of origin of *M. acuminata* cultivars (Daniells et al. 2001), although according to Heslop-Harisson and Schwarzacher (2007) the diversity can be found throughout Southeast Asia.

The number of alleles per accession per primer ranged 1–3 either for diploid or triploid accessions. Some accessions exhibited greater alleles than expected from their level of ploidy. The primers Ma–1–17; Ma–3–90; Ma–3–139; and MaCIR332a detected 3 alleles in the diploid 'Koja Pretel'; primer Ma–1–17 detected 3 alleles in 'Tolu' and primer Ma–3–90 detected 3 alleles in 'Tegetmolo'. This result may explain the occurrence of duplicated alleles or duplicated chromosomal regions as reported in previous study in banana (Kaemmer et al. 1997; Creste et al. 2003, 2004). The level of ploidy probably has also been incorrectly determined due to the subjectivity of morphological characters. Therefore, based on microsatellite markers, the accessions which have 3 alleles at least in one of the primers used should be grouped into the triploid cultivars. However, the homozygous and heterozygous accessions having only 1 and 2 alleles respectively could not be determined their ploidy level. It may due to the dosage effects of a microsatellite allele (Provan et al. 1996). Those accessions should be designated as AA or AAA genomic group.

The genomic group of AA was difficult to be differentiated from the AAA. It is due to the polyploidy nature of banana. The dosage effects of a microsatellite allele, either simplex or duplex can not be distinguished because each allele is interpreted as unique character without considering the effect of its gene dosage (Provan et al. 1996; Cerenak et al. 2004). For example, the diploid and triploid cultivars having the same 2 alleles in a certain locus, the first cultivar has simplex dosage for A1A2 and the second, duplex for A1A1A2, both seem identical by microsatellite markers. According to Creste et al. (2003) the 2 cultivars were only possible distinguished using methods that allow the estimation of allele dosage such as qualitative PCR. Although the genomic groups could not be differentiated by microsatellite markers, the taxonomic status of these cultivars did not change. Both cultivars should be designated under the name of *M. acuminata*. Using only primer MaCIR108, the pure *acuminata* accessions can be significantly distinguished from accessions containing the B genome.

The study recorded that some alleles were present only in AA and the others existing only in AAA accessions. Among the identified alleles, 24 alleles were located only in AA accessions, while 45 were found only in AAA accessions. Most alleles were discovered in accessions of both

Table 3. Discrete and repeatable alleles produced by the 8 microsatellite primers in pure *acuminata* cultivars

Primer	Alleles size range (bp)	Number of alleles	Observed heterozygosity
MaCIR327b	388–436	4	0.125
Ma-1-132	330–378	10	0.980
MaCIR332a	260–296	10	0.792
MaCIR108	220–268	12	0.813
Ma-3-139	132–177	14	0.917
Ma-3-90	132–172	12	0.875
Ma-1-27	122–142	8	0.729
Ma-1-17	110–154	12	0.917
Total		82	
Mean		10.3	0.769

banana genomic groups. None of these alleles were consistent detected only in AA or AAA. Therefore, none of alleles were could be considered as a marker for separating AA from AAA genomic groups. Microsatellite markers used in this study could not distinguish AA from AAA genomic groups because a specific microsatellite allele either for the diploid or the triploid accessions was not found. Similar result was also reported by Creste et al. (2004). The AA genomic groups most probably closely related to AAA because they were derived from the same subspecies of *M. acuminata* having continuous variation (Simmonds 1962). According to Valmayor et al. (2000), the addition of one set of chromosomes through autopolyploidy also did not change the cultivar genetic constitutions.

The dendrogram generated from UPGMA clustering revealed the genetic relationship of the 48 accessions of *M. acuminata* cultivars (Figure 3). The genetic similarity coefficients ranged 0.15–1.00. Coefficient 0.15 to 0.95 indicated different cultivars, and 1.00 indicated identical cultivars. The most resemble similarity in all primers was found in 6 pairs of banana accessions.

The eight primers combination (Table 2) minus the primers Ma–1–5; MaCIR327a; Ma–3–48; and Ma–3–104 detected only 42 genotypes that allowed the discrimination of all cultivars except 6 pairs of identical accessions. Accession 'Mas 40 Hari' had identical genotype with 'Berlin', 'Mulih Hijau' with 'Mulih Putih', 'Rejang' from Diperta with 'Rejang' from RIF collections, 'Cici Kuning' with Cici Merah', 'Ambon Hijau' with 'Ambonaae', and 'Cebol Morosebo' with 'Cebol' (Figure 3). Justification of the 6 identical genotypes will clear using greater number of locus. Courtois (2002) suggested that at the beginning, characterization using 8 to 9 microsatellites loci amplifying 6 to 10 alleles was adequate, but at least 15 loci were required to get an accurate identity of a cultivar.

Clustering analysis based on the 82 alleles could not completely separate AA or AAA; from AAA genomic groups because several accessions of AA or AAA were clustered together with AAA or vice versa. Nevertheless, most accessions tend to be clustered according to ploidy level. Ploidy levels of *acuminata* accessions were difficult to be determined because accessions only having 1 or 2 alleles could be considered as diploid or triploid. Valmayor et al. (2000) illustrated the development of the triploid cultivar that always involved cultivated diploid, with or without its wild type of *M. acuminata*. In the taxonomic scorecard suggested by Silayoi and Chomchalow (1987), AA and AAA genomic groups have the same of score range (15–25). This explained that the diploid AA also has morphological characteristics of triploid AAA (Simmonds 1962; Stover and Simmonds 1987).

The dendrogram grouped the 48 accessions into 2 clusters at similarity coefficient of 0.15. The accessions of diploid 'Monyet' and 'Uli' and triploid 'Gorohito' grouped in one cluster (I) presented the lowest similarity values than those of the main cluster (II). The main cluster consisted of two subclusters, IIa and IIb. The subcluster IIa was dominated by the accessions of AA or AAA genomic group, whereas the subcluster IIb was dominated by AAA genomic group.

Based on morphological characters, 'Monyet' was the most closely related to wild type of *M. acuminata* (Jumari 2000). Taxonomically, this accession was classified into *M. acuminata* subsp. *zebrina* (Simmonds 1962). The cultivated bananas were commonly derived from subsp. *banksii* or subsp. *errans* (Carreel et al. 2002). The development of 'Gorohito' and 'Uli' perhaps associate with a wild type of *M. acuminata* subsp. *zebrina*.

Most accessions especially for AA or AAA genomic groups collected from Diperta, based on microsatellite markers tend to be clustered according to their subgroups constructed based on fruit morphology (Jumari 2000; Jumari & Pudjoarinto 2000). The subcluster IIa most probably consisted of diploid AA. The diploid AA was divided into 2 subgroups: Pisang Mas and Pisang Becici, while the triploid AAA was separated into 3 subgroups: Ambon, Mauli and Potho as presented in Table 1. Subgroups of the diploid AA were differentiated from each other by the number of seeds and taste of fruits, while subgroups of the triploid AAA were separated by the length, apex and peel color of fruits. Generally, the subcluster IIa contained accessions of subgroup Pisang Mas bearing seedless and sweet fruit, but in the subcluster IIb, there are not found specific alleles which could differentiate subgroups of the triploid AAA.

This study also detected the presence of variability of accessions from the same subgroup. On the other hands, Creste et al. (2003) reported high level genetic similarity between cultivars in Brazil from the same subgroup. The results showed that banana accessions in Indonesia most

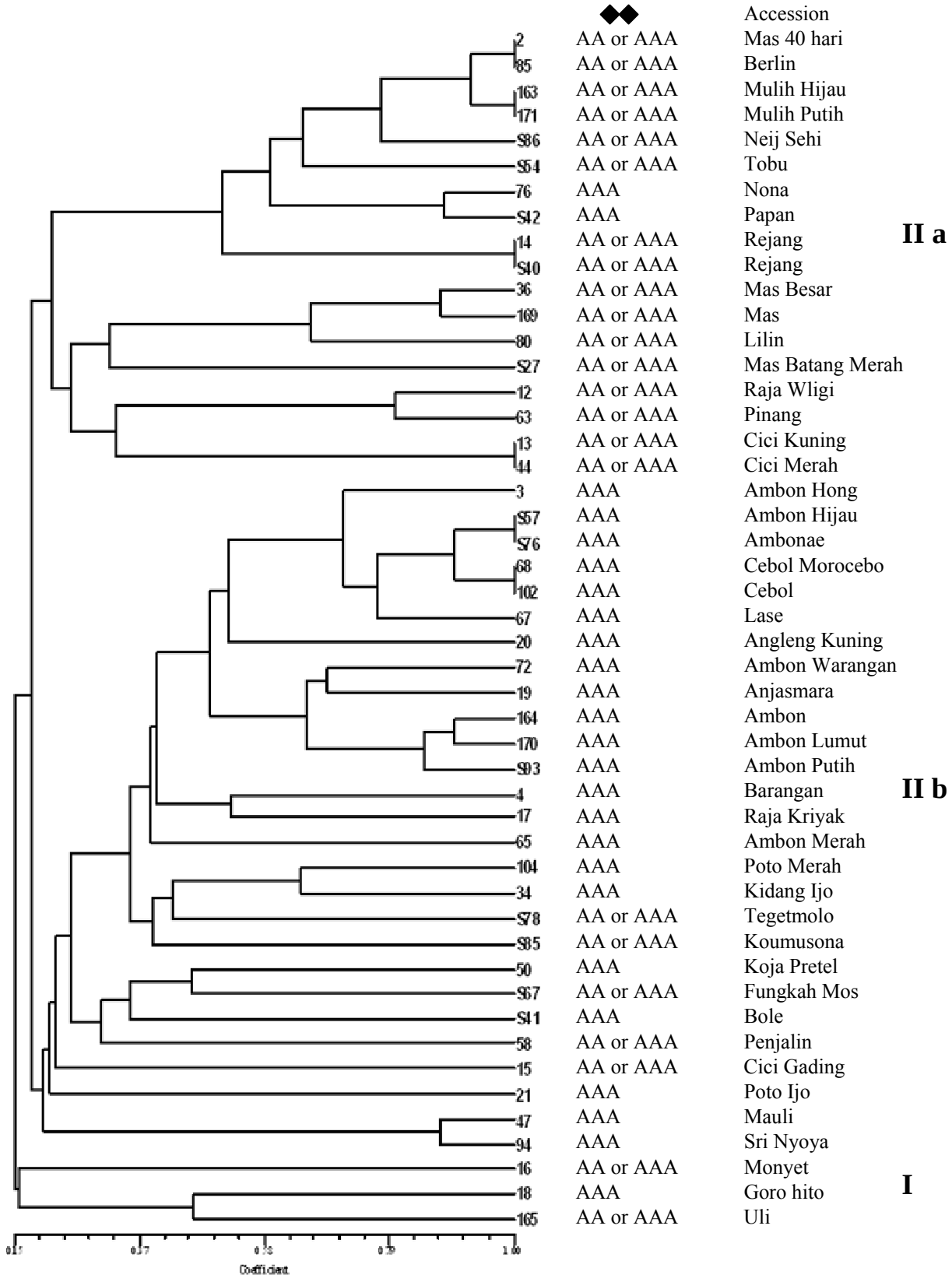

Figure 3. Dendrogram generated from analysis of the 48 accessions of pure *acuminata* cultivars using the 8 microsatellite primers. f& genomic group based on morphological characters and f&f& genomic group based on primer MaCIR108.

probably have a larger diversity than those in Brazil. A high degree polymorphism within the subgroups of the A genome alone was possible to indicate a high degree of variability which present within the *M. acuminata* complex (Carreel et al. 2002) discovered throughout Indonesia (Daniells et al. 2001; Pollefeys et al. 2004).

CONCLUSION

Taxonomic status of cultivated bananas in Indonesia could be determined using only one microsatellite primer namely the MaCIR108. The eleven accessions previously classified using morphological characters as *M. acuminata* should be changed into *M.* x *paradisiaca*. The key character for this alteration is 3 alleles of the MaCIR108 which longer than 270 bp. These alleles included in the length range of the B genomic alleles, whereas the other alleles which less than 270 bp fell into the length range of the A genomic alleles. Due to possess the A and the B genomic alleles, the 11 accessions should be placed in hybrids species *M.* x *paradisiaca.* Taxonomic status of the 48 banana accessions studied did not change. The analysis result of the 48 accessions based on the 8 primers indicated that no specific alleles which could differentiate AA from AAA genomic groups. Difficulties in separating these genomic groups may also due to the dosage effects of a microsatellite allele. Therefore, the accessions having maximum 2 alleles in each primer were designated as AA or AAA genomic group. Analysis of pure *acuminata* accessions using the 8 primers detected only 42 genotypes because 12 of the 48 accessions clustered within 6 identical genotypes.

Genetic relationship analysis showed that genomic groups of those pure *acuminata* accessions could not be separated significantly, though most of them tend to be clustered according to ploidy level.

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