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Genome identification of bananas (*Musa* L.) from East Java Indonesia assessed with PCR-RFLP of the internal transcribed spacers nuclear ribosomal DNA

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Abstract

East Java Province has high diversity of local banana cultivars with various local names and diverse morphological characteristics. The genome identification of those banana cultivars are needed to provide valid identity of the banana accessions to be used as basic data for *in-situ* and/or *ex-situ* banana conservation management and for further breeding banana material. Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) of the Internal Transcribed Spacer (ITS) region nuclear ribosomal Deoxyribo Nucleic Acid (nrDNA) using *Rsa*I restriction endonuclease enzyme was conducted to 68 banana accessions collected from 17 Regencies in East Java Province. The *Rsa*I digested fragments showed consistent polymorphic banding DNA patterns; in *M. acuminata* the wild species and cultivars produced two fragments of 530 bp and 120 bp whereas *M. balbisiana* species and its hybrid cultivars produced three fragments of 350 bp, 180 bp and 120 bp. From this study it was found that about 45 accessions were identified as *M. acuminata* species (AA/AAA genomes). It comprises of 3 wild species *M. acuminata* (AAw), 17 *M. acuminata* diploid cultivars (AA) and 25 *M. acuminata* triploid cultivars (AAA). Wild species *M. balbisiana* identified only one accession (BBw), and the hybrid cultivars comprises of 14 *M acuminata* × *balbisiana* (ABB) cultivars and 8 *M. acuminata* × *balbisiana* (AAB) cultivars. Clustering analysis result based on restriction fragment ITS region showed that BBw, ABB and AAB genome groups were close related and clustered as sister group whilst AA/AA genome group became the out-group with similarity coefficient between 0,635 to 1.00.

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Introduction

Banana (*Musa* L.) is important food crop that playing crucial roles in social, economic and cultural life worldwide (FAO, 2003; Megia, 2005). Bananas are widely cultivated in more than 130 countries in the tropics and subtropics (Pachuau *et al.*, 2014). It is estimated that there are not less than 1000 banana cultivars were exist and known with various local names and have wide ranges of morpohological appearance. The presence of many local names and any possible synonimies among and within banana cultivars are become problems for taxonomists and horticulturist (Valmayor *et al.*, 2000).

Nomenclature names for edible banana cultivars were first described by Linnaeus in 17th century that were *Musa paradisiaca* L. and *M. sapientum* L. but later it was found restrictive and causing problems for classifying the great diversity of bananas in Asia, especially in Southeast Asia as the center of origin and diversity of banana (Valmayor *et al.,* 2000; Singhs *et al.*, 2001). Simmond and Shepherd (1955) presented the theory of origin of edible bananas. Wild seeded fertile banana species *M. acuminata* Colla (donor of A genome) and *M. balbisiana* Colla (donor of B genome) are believed to be the ancestral parents of main modern banana cultivars. This hypothesis was later confirmed by molecular studies and in addition to A and B genomes it was also indicated the presence of S genome contributed by *M. schizocarpa* and T genome contributed by *M. textilis* (Singh *et al.*, 2001; De Langhe *et al.*, 2009; Li *et al.*, 2013).

Simmonds and Shepherd (1955) suggested the replacement of binomial nomenclature on banana cultivars with genome nomenclature, which consists of generic name, followed by a letter combination indicating the ploidy and the genome sets contributed by their parents, followed by the name of cultivar group and/or the cultivar (Espino *et al.*, 1992; Valmayor *et al.*, 2000; Simmonds, 1959). This genome nomenclature was then approved by a consensus in 1999 as valid name for banana cultivars (Valmayor *et al.*, 2000).

Edible bananas were arisen in the first instance through the development of parthenocarpy and sterility. Those events were then followed by chromosome restitution and further occurrence of out-crossing of the ancestral parents both intra and inter-species to give rise to high bananas diversity with various ploidy levels and genomic combinations like AA, AAA, AB, AAB, ABB, BB, ABB and ABBB (including AxT, AxS, BxT). Selection and vegetative propagation played an important role in the evolution of the edible bananas (Simmonds, 1959; Espino *et al.*, 1992; Danniels *et al.*, 2001; Singhs *et al.*, 2001; Valmayor *et al.*, 2000).

Genome composition in banana cultivars can be estimated by a scoring method based on 15 differentiator characters of morphological traits resulted from phenotype expressions of *M. acuminata* and *M. balbisiana* (Simmonds and Shepherd, 1955; Simmonds, 1959) but the process is time consuming and results were very subjective (Rao, 2004). Latest approach to identify the level of ploidy and genome composition with accurate precision (more than 95%) is by flow cytometry method (Doležel *et al.*, 1994). The molecular approach DNA based may also be used to estimate the genome composition, genetic diversity and relationships through various techniques using PCR methods including PCR-RFLP, Random Amplified Polymorphism DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) or microsatellite (de Jesus *et al.*, 2013). Ploidy level itself can be approached by manual chromosome analysis using staining method (Sharma and Sharma, 1980) or using ploidy analyzer machines (Nwakanma *et al.*, 2003).

East Java Province covers an area in the eastern part of Java Island and Madura Island separated by the Madura Strait and several surrounding small islands. East Java Province is one among the 16 provinces of banana production centres in Indonesia (Tarigan, 2007). Diversity of local banana cultivars in East Java Province is quite high. According to Purwodadi Botanic Garden's records and from banana

exploration data, it was known that not less than 90 banana cultivars with various local names and diverse morphological characteristics available in East Java. In 2010, about 65 banana accessions in field gene bank collection of Purwodadi Botanic Garden were originated from East Java Province (Hapsari, 2011). About 39 banana cultivars were recognized in Pasuruan and Probolinggo Regencies (Hapsari *et al.*, 2012 (unpublished)), whereas in Lumajang, Jember and Banyuwangi were found about 49 cultivars (Hapsari *et al.*, 2013 (unpublished)). Banana exploration in Madura Island recognized 37 banana cultivars with Madurese names (Hapsari *et al.*, 2015). The genome identification of those banana cultivars from East Java are needed to conduct to provide valid identity of the banana accessions to be used as material for further banana breeding program and basic data for *in-situ* and/or *ex-situ* banana collection management.

This study was aimed to identify the genome composition and genetic relationship of bananas from East Java using PCR-RFLP of the Internal Transcribed Spacer (ITS) region of the ribosomal DNA. The ITS region is including ITS1 and ITS2 are part of the nrDNA transcript but not incorporated into ribosomes (Baldwin *et al.*, 1995). ITS region were proved as most widely used molecular markers to reconstruct the phylogenetic of the family Musaceae with more informative results than markers from chloroplast and mitochondrial genomes (Nwakanma *et al.*, 2003; Irish *et al.*, 2009; Li *et al.*, 2010; Liu *et al.*, 2010; Ravishankar *et al.*, 2011; Hřibová *et al.*, 2011; Ekasari *et al.*, 2012; De Jesus *et al.*, 2013; Jingyi *et al.*, 2013; Sulistyaningsih *et al.*, 2014). The *Rsa*I digestion of ITS region in bananas showed consistent marker bands; a single unique 530 bp fragment was diagnostic marker band for the presence of the 'A' genome while 350 and 180 bp fragments were diagnostic for the presence of the B'genome (Nwakanma *et al.*, 2003).

Material and methods

Plant materials

Sixty eight banana accessions (Table 1) collection of Purwodadi Botanic Garden, Indonesian Institute of Sciences (Pasuruan, East Java) were analyzed in this study. The accessions were collected from areas covering 17 Regencies in East Java Province includes in Eastern Java Main-land, Bawean Island and Madura Island (Fig. 1) representing *M. acuminata* and *M. balbisiana* wild banana species and mostly banana cultivars.

Fig. 1. Map of location where the banana accessions collected from East Java Province (Google Earth version 2003).

DNA isolation

Whole genome DNA was isolated from fresh young unfurled leaf tissues using Promega Wizard® Genomic DNA Purification Kit, Wisconsin, USA. The isolation protocol was conducted following its manufacturer's instructions for plants. DNA yields were confirmed qualitatively using electrophoresis separation on 1% agarose gels, stained with 1 µg/ml of *Ethidium bromide* (*Etbr*) in TBE buffer, then visuliased under UV light. Generuller 1-Kb DNA ladder (Thermo Scientific, California, USA) was used to estimate the sizes of whole genome DNA. DNA concentrations and purities were quantified by measuring absorbencies at 260 nm and 280 nm wavelengths using Nanophotometer IMPLEN vers. 7122 V2.3.1. An aliquot of the isolated DNA for each sample was diluted to a final concentration of 25 ng/µl in TE buffer to use in subsequent assays.

PCR-amplification

The ITS region was amplified using primer pairs ITSL (5'-TCG TAA CAA GGT AGG CGT TTC TG-3 ') as a forward primer and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') as a reverse primer according to White *et al.*, 1990; Hsiao *et al.*, 1994; Nwakanma *et al.*, 2003. The ITS pimers flanking the entire ITS region (ITS1 and ITS2) including the intervening 5.8S sub-unit. Amplifications of the ITS region were performed at a volume of 15 µl consisting of 1,5 µl 25 ng DNA sample, 1,5 µl of 10 pmol forward and reverse primers, 1,5 µl of nuclease-free water and 7,5 µl DreamTaq Green PCR Master Mix (2x) from Thermo Scientific, California, USA containing of DreamTaq DNA polymerase, 2x DreamTaq Green buffer, 0,4 mM each of dNTPs and 4 mM MgCl2.

Thermal cycling protocol consisted of one cycle initial denaturation step for 3 minutes at 95°C, followed by 25 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 53°C, and extension for 30 seconds at 72°C. Final extension carried out for 7 minutes at 72 °C. Confirmation of successful amplification were conducted by electrophoresis separation on 1,5% agarose gels, stained with 1 µg/ml of Ethidium bromide in TBE buffer, then visualised under UV light. Generuller 100bp DNA ladder (Thermo Scientific, California, USA) were used to estimate the sizes of amplified DNA fragments.

Restriction fragment-length polymorphism

Each sample's PCR product was digested using *Rsa*I restriction endonuclease enzyme (Thermo Scientific, California, USA). The digestion protocol consists of 10 µl PCR product, 18 µl nuclease-free water, 2 µl 10x Buffer Tango and 2 µl *Rsa*I. Incubation was performed at 37°C water bath for overnight (± 12 to 16 hours). The digested DNA fragments were confirmed using electrophoresis separation on 2% agarose gels, stained with 1 µg/ml of *Etbr* in TBE buffer, then visualised under UV light. Generuller 50bp DNA ladder (Thermo Scientific, California, USA) were used to estimate the sizes of digested DNA fragments.

Data analysis

The *Rsa*I digested DNA fragments per banana accession was analyzed its length polymorphism patterns visually to identify its genomic group. The results of genomic group identification using PCR-RFLP were then compared to results using morphology method which gathered from previous studies and references (Hapsari and Masrum, 2011; Hapsari, 2013; Hapsari, 2014; Hapsari *et al.*, 2015).

The DNA fragment length datas were quantified into numeric scoring datas according to its band intensities. The data matrices were then subjected to cluster analysis with Bray-Curtis similarity measure using Paleontological Statistics (PAST) software version 1.94b.

Result and discussion

DNA isolation and ITS PCR results

Whole genome DNA of all banana accessions were identified at the sized of >10.000 bp (Fig. 2A). Amplification of ITS region were successfully carried out to all banana accessions produced a specific DNA band at a length of ±700 bp (Fig. 2B). ITS region length in Angiosperms varies between 400 to 800 bp, like in families Asteraceae 469-487 bp, Fabaceae 428-448 bp, Malvaceae 517-538 bp, Poaceae 430-445 and

Rosaceae 419-445 bp (Baldwin *et al.*, 1995). The ITS region of 18S-26S nuclear ribosomal DNA has proven to be a useful source of characters for phylogenetic studies in many Angiosperm families including Musaceae.

ITS region is biparental inherited so that more informative in revealing the evolutionary history of organisms that naturally involves crossing both inter and intra species, also occurrence of polyploidy and hybrid speciation events (Álvarez and Wendel, 2003). The two spacers of region ITS1 and ITS2 (each <300 bp) can be readily amplified by PCR and sequenced using universal primer. Primer pairs that are often used in identification, diversity and relationship studies on bananas includes ITSL and ITS4 (Nwakanma *et al.*, 2003; Irish *et al.*, 2009; Ravishankar *et al.*, 2011; Hřibová *et al.*, 2011; Ekasari *et al.*, 2012; Jingyi *et al.*, 2013), ITS4 and ITS5 (Liu *et al.*, 2010; Sulistyaningsih *et al.*, 2014) and ITS1 and ITS4 (de Jesus *et al.*, 2013). ITSL forward primer showed amplification to ITS region better than ITS5 in grasses (Hsiao *et al.*, 1994) and also banana cultivars (Nwakanma *et al.*, 2003).

ITS PCR-RFLP results

Digestion of ITS region fragment in banana accessions using *Rsa*I endonuclease restriction enzyme resulted consistent polymorphic banding DNA patterns in accordance to the research revealed by Nwakanma *et al.* (2003). Banana accessions which produced two DNA fragments of 530 bp and 120 bp identified as *M. acuminata* species and cultivars, whereas accessions which produced three DNA fragments of 350 bp, 180 bp and 120 bp were identified as *M. balbisiana* species and its hybrid cultivars (Fig. 2C). In this study no low molecular weight of DNA fragments (< 50bp) identified which was different from the finding in previous study by Nwakanma *et al.* (2003) and Ekasari *et al.* (2012). It could be an excess primers or excess DNA template or contained primers dimmers in the PCR products.

Table 1. Genome identification of bananas from East Java assessed by PCR-RFLP of ITS region.

No.	Species / Cultivar name	Locality/origin	Diagnostic fragments (bp)				ITS/*Rsa*I Genome		
			530 'A'	350 'B'	180 'B'	120 'A/B'	PCR-RFLP	Morpho-logy	Final
1	*M. acuminata* ssp. rutilifes / Cici	Krawak, Tuban	++	-	-	+	AAw	AAw	AAw
2	*M. acuminata* ssp. flava / Jantung Kuning	Krawak, Tuban	++	-	-	+	AAw	AAw	AAw
3	*M. acuminata* ssp. nakaii / Monyet	Pasuruan	++	-	-	+	AAw	AAw	AAw
4	*M. balbisiana* /Klutuk Ijo	Purwosari, Pasuruan	-	++	++	-	BBw	BBw	BBw
5	Sri	Pasuruan	++	++	++	+	ABB	ABB	ABB
6	Kates	Nongkojajar, Pasuruan	++	++	++	+	ABB	ABB	ABB
7	Raja Prentel	Nongkojajar, Pasuruan	++	++	++	+	ABB	ABB	ABB
8	Sobo Londo	Purwodadi, Pasuruan	++	++	++	+	ABB	ABB	ABB
9	Sobo Awu	Purwodadi, Pasuruan	++	++	++	+	ABB	ABB	ABB
10	Tlekung	Batu, Malang	++	++	++	+	ABB	ABB	ABB
11	Raja Wesi	Ngawi	++	++	++	+	ABB	AAB	ABB
12	Ebung	Siman, Ponorogo	++	++	++	+	ABB	ABB	ABB
13	Gajih Bali	Kedungjajang, Jember	++	++	++	+	ABB	ABB	ABB
14	Awak	Genteng, Banyuwangi	++	++	++	+	ABB	ABB	ABB
15	Tajinan	Glagah, Banyuwangi	++	++	++	+	ABB	ABB	ABB
16	Kusta Putih	Sumenep, Madura Island	++	++	++	+	ABB	ABB	ABB
17	Sabeh Biru	Sampang, Madura Island	++	++	++	+	ABB	ABB	ABB
18	Susu Gabug	Songkopuro, Bawean Island	++	++	++	+	ABB	AAB	ABB
19	Belindang	Batu Putih, Sumenep	++	+	+	+	AAB	AAB	AAB
20	Ongkap	Karang Penang, Sampang	++	+	+	+	AAB	AAB	AAB
21	Kisto	Nongkojajar, Pasuruan	++	+	+	+	AAB	AAB	AAB

Notes: ++ indicates full band intensity, + indicates faint band intensity, - indicates absences of marker band. Rows highlighted with grey showed different genome identification result between PCR-RFLP and morphology method.

The band expression is quantitative based on its genomes, accessions which possessing two sets of 'B' genomes showed stronger band intensity at 350 bp and 120 bp than those with single 'B' genome (Fig. 2C and 2D). For example, pisang Kates (Fig. 2C.8) and Sabeh Biru (Fig. 2C.9) showing thick band intensities at 350 bp and 180 bp were identified as ABB genome whereas pisang Welut (Fig. 2C.10) and Belindang (Fig. 2C.11) identified as *Musa* AAB genome since they showed faint band intensities. However, it's not applicable to 'A' genome. All of the *M. acuminata* cultivars showed same restrictions patterns which cannot differentiate between diploid (AA) to triploid (AAA) cultivars. Ploidy determination is needed to confirm the set genome identification. Instead of using elaborated staining method chromosome analysis and expensive ploidy analyzer machine, banana plant morphology performance may also be used to determine the poidy level although it's very subjective. Banana plants which showed large and vigorous pseudostem are mostly identified as triploid whereas diploid individual plant has slender pseudostem.

Table 1. Genome identification of bananas from East Java assessed by PCR-RFLP of ITS region (Continued).

No.	Species / Cultivar name	Locality/origin	Diagnostic ITS/*Rsa*I fragments (bp)				Genome		
			530 'A'	350 'B'	180 'B'	120 'A/B'	PCR-RFLP	Morpho-logy	Final
22	Porem	Purwodadi, Pasuruan	++	+	+	+	AAB	AAB	AAB
23	Songgroito	Purwodadi, Pasuruan	++	+	+	+	AAB	AAB	AAB
24	Welut	Glagah, Banyuwangi	++	+	+	+	AAB	AAB	AAB
25	Selendang	Waru, Pamekasan	++	+	+	+	AAB	AAB	AAB
26	Osok	Batu Marmar, Pamekasan	++	+	+	+	AAB	AAB	AAB
27	Berlin	Purwosari, Pasuruan	++	-	-	+	AA/AAA	AA	AA
28	Dokare	Purwosari, Pasuruan	++	-	-	+	AA/AAA	AAB	AA
29	Jambe	Tulungagung	++	-	-	+	AA/AAA	ABB	AA
30	Orlin	Krucil, Probolinggo	++	-	-	+	AA/AAA	AA	AA
31	Grito	Krucil, Probolinggo	++	-	-	+	AA/AAA	AA	AA
32	Lilin	Kabat, Banyuwangi	++	-	-	+	AA/AAA	AA	AA
33	Satroli	Krucil Probolinggo	++	-	-	+	AA/AAA	AAB	AA
34	Raja Sri	Glagah, Banyuwangi	++	-	-	+	AA/AAA	AAB	AA
\35	Mas (BWI)	Kalibaru, Banyuwangi	++	-	-	+	AA/AAA	AA	AA
36	Berlin Kuning	Glenmore, Banyuwangi	++	-	-	+	AA/AAA	AA	AA
37	Mas Kripik	Senduro, Lumajang	++	-	-	+	AA/AAA	AA	AA
38	Gading	Bangkalan, Madura Island	++	-	-	+	AA/AAA	AA	AA
39	Mas (BGKLN)	Bangkalan, Madura Island	++	-	-	+	AA/AAA	AA	AA
40	Masang	Bangkalan, Madura Island	++	-	-	+	AA/AAA	AAB	AA
41	Ci Uci	Bangkalan, Madura Island	++	-	-	+	AA/AAA	AA	AA
42	Madu	Sumenep, Madura Island	++	-	-	+	AA/AAA	AA	AA
43	Pakak Merah	Sampang, Madura Island	++	-	-	+	AA/AAA	AAB	AA
44	Kayu	Nongkojajar, Pasuruan	++	-	-	+	AA/AAA	AAA	AAA
45	Santen	Nongkojajar, Pasuruan	++	-	-	+	AA/AAA	AAA	AAA

Notes: ++ indicates full band intensity, + indicates faint band intensity, - indicates absences of marker band. Rows highlighted with grey showed different genome identification result between PCR-RFLP and morphology method.

Banana genome identification result

The genome identification results of 68 banana accessions from East Java (Table 1) showed that about 45 accessions or about 66% of the accessions were *M. acuminata* species of AA/AAA genomes. It comprised of 3 wild species *M. acuminata* (AAw), 17 *M. acuminata* cultivars diploid (AA) and 25 *M. acuminata* cultivars triploid (AAA). This was

presumably due to Indonesia was the centre of origin of wild banana species *M. acuminata* which were the ancestors of banana cultivars contributed 'A' genome. Based on the consensus, scientific name of banana cultivar genome groups AA and AAA is *M. acuminata* (AA/AAA) cv. followed by its cultivar name (Espino *et al.*, 1992; Valmayor *et al.*, 2000).

The hybrid banana cultivars comprise of 8 *Musa* AAB accessions and 14 *Musa* ABB accessions (Table 1). The number of hybrid banana cultivars was much fewer than *M. acuminata* cultivars. Wild species *M. balbisiana* (genome donor B) was endemic in the region of northern India, China, Indochina to the Philippines (De Langhe *et al.*, 2009). It was introduced to Indonesia considered by migration of human events. Natural hybridization and domestication events in the past between *M. balbisiana* to native wild species *M. acuminata* given rise to hybrid cultivars with ABB and AAB genomes (Simmonds and Shepherd, 1955, Simmonds, 1959). Based on the consensus, scientific name of banana cultivar genome groups ABB and AAB is *M. x paradisiaca* (ABB/AAB) cv. or *M. acuminata* x *M. balbisiana* (ABB/AAB) cv. followed by its cultivar name (Espino *et al.*, 1992; Valmayor *et al.*, 2000).

Table 1. Genome identification of bananas from East Java assessed by PCR-RFLP of ITS region (Continued).

No.	Species / Cultivar name	Locality/origin	Diagnostic ITS/*Rsa*I fragments (bp)				Genome		
			530 'A'	350 'B'	180 'B'	120 'A/B'	PCR-RFLP	Morpho-logy	Final
46	Raja Kenanga	Purwodadi, Pasuruan	++	-	-	+	AA/AAA	AAA	AAA
47	Raja Molo	Purwodadi, Pasuruan	++	-	-	+	AA/AAA	AAA	AAA
48	Nangka	Purwodadi, Pasuruan	++	-	-	+	AA/AAA	AAB	AAA
49	Agung	Krucil, Probolinggo	++	-	-	+	AA/AAA	AAB	AAA
50	Kongkong	Lawang, Malang	++	-	-	+	AA/AAA	AAA	AAA
51	Morosebo	Sarangan, Madiun	++	-	-	+	AA/AAA	AAA	AAA
52	Ronggolawe	Ngawi	++	-	-	+	AA/AAA	AAB	AAA
53	Raja Ketan	Siman, Ponorogo	++	-	-	+	AA/AAA	AAB	AAA
54	Byok	Tulungagung	++	-	-	+	AA/AAA	AAA	AAA
55	Kidang	Kalisat, Jember	++	-	-	+	AA/AAA	AAA	AAA
56	Candi	Ambulu, Jember	++	-	-	+	AA/AAA	AAB	AAA
57	Embug	Ledok Ombo, Jember	++	-	-	+	AA/AAA	AAA	AAA
58	Raja Pendek	Pasrujambe, Lumajang	++	-	-	+	AA/AAA	AAA	AAA
59	Celakat	Glagah, Banyuwangi	++	-	-	+	AA/AAA	AAB	AAA
60	Sri Nyonya	Glagah, Banyuwangi	++	-	-	+	AA/AAA	AAB	AAA
61	Lumut	Sumenep, Madura Island	++	-	-	+	AA/AAA	AAA	AAA
62	Jabol	Sumenep, Madura Island	++	-	-	+	AA/AAA	AAA	AAA
63	Moseng	Sampang, Madura Island	++	-	-	+	AA/AAA	AAA	AAA
64	Rosok	Bangkalan, Madura Island	++	-	-	+	AA/AAA	AAB	AAA
65	Embuk	Pamekasan, Madura Island	++	-	-	+	AA/AAA	AAA	AAA
66	Nangkah	Pamekasan, Madura Island	++	-	-	+	AA/AAA	AAB	AAA
67	Elang	Pamekasan, Madura Island	++	-	-	+	AA/AAA	AAB	AAA
68	Pakak Santen	Pamekasan, Madura Island	++	-	-	+	AA/AAA	AAB	AAA

Notes: ++ indicates full band intensity, + indicates faint band intensity, - indicates absences of marker band. Rows highlighted with grey showed different genome identification result between PCR-RFLP and morphology method.

Genome identification results on bananas using molecular method PCR-RFLP of ITS region compared to morphological approach (Hapsari and Masrum, 2011; Hapsari, 2013; Hapsari, 2014; Hapsari *et al.*, 2015) showed different results in 19 accessions. Morphological identification errors were mostly found in banana accessions which previously thought to be hybrid AAB cultivars whilst as revealed by PCR-

RFLP of ITS region identified as AA/AAA cultivars and vice versa. Indeed, their morphological performance were almost similar to AAB cultivars although genetically were identified as AA/AAA cultivars. Morphological performance of ABB cultivars were distinguished, they were close to *M. balbisiana* species.

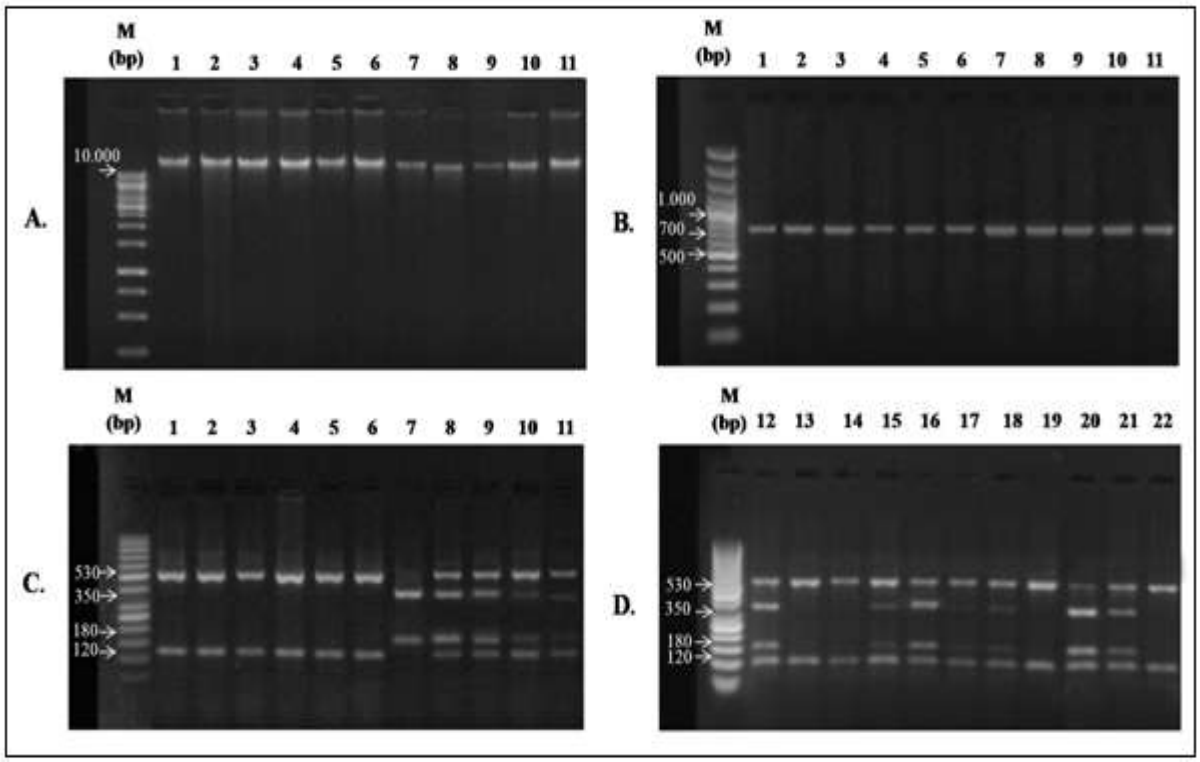

Fig. 2. Electrophoregram of (A) whole genome DNA, (B) PCR ITS products, (C and D) *Rsa*I restriction endonuclease fragments of ITS in banana accessions.

Clustering analysis result

Genetic relationship was determined by the value of similarity or distance coefficient, the higher value of similarity or fewer distance coefficients so that relationship between genome groups and/or group members are closer. Clustering analysis of 68 banana accessions based on restriction fragments of ribosomal DNA ITS produced dendogram with similarity coefficient ranges from 0,635 to 1.00.

Genome groups of BBw, ABB and AAB were closely related and clustered as sister group whereas genome group AA/AA became the out-group. The closest genetic relationship were found between hybrid genome groups ABB to AAB with distance coefficient 0,01 while the farthest relationship were found between *Musa* BBw to AA/AAA group (similarity = 0,635). Banana cultivars with AA and AAA genomes showed same restriction patterns so that they 100% identical and clustered in one group (Fig. 3).

Molecular approach using PCR-RFLP of ITS nrDNA method was effective to identify and confirms genomes composition of banana accessions with more objective and valid result than morphology method. However, morphological characterization subjected to banana accessions is still needed to conduct in order to provide more comprehensive datas and may support the weakness of molecular results. This genome identity results are become verification base to propose scientific name revision for ex-situ banana collections in Purwodadi Botanic Garden. In addition, the clustering analysis results may provide informations for a better understanding of the genetic relationship among banana accessions in East Java and may also usefull in supporting further banana breeding program.

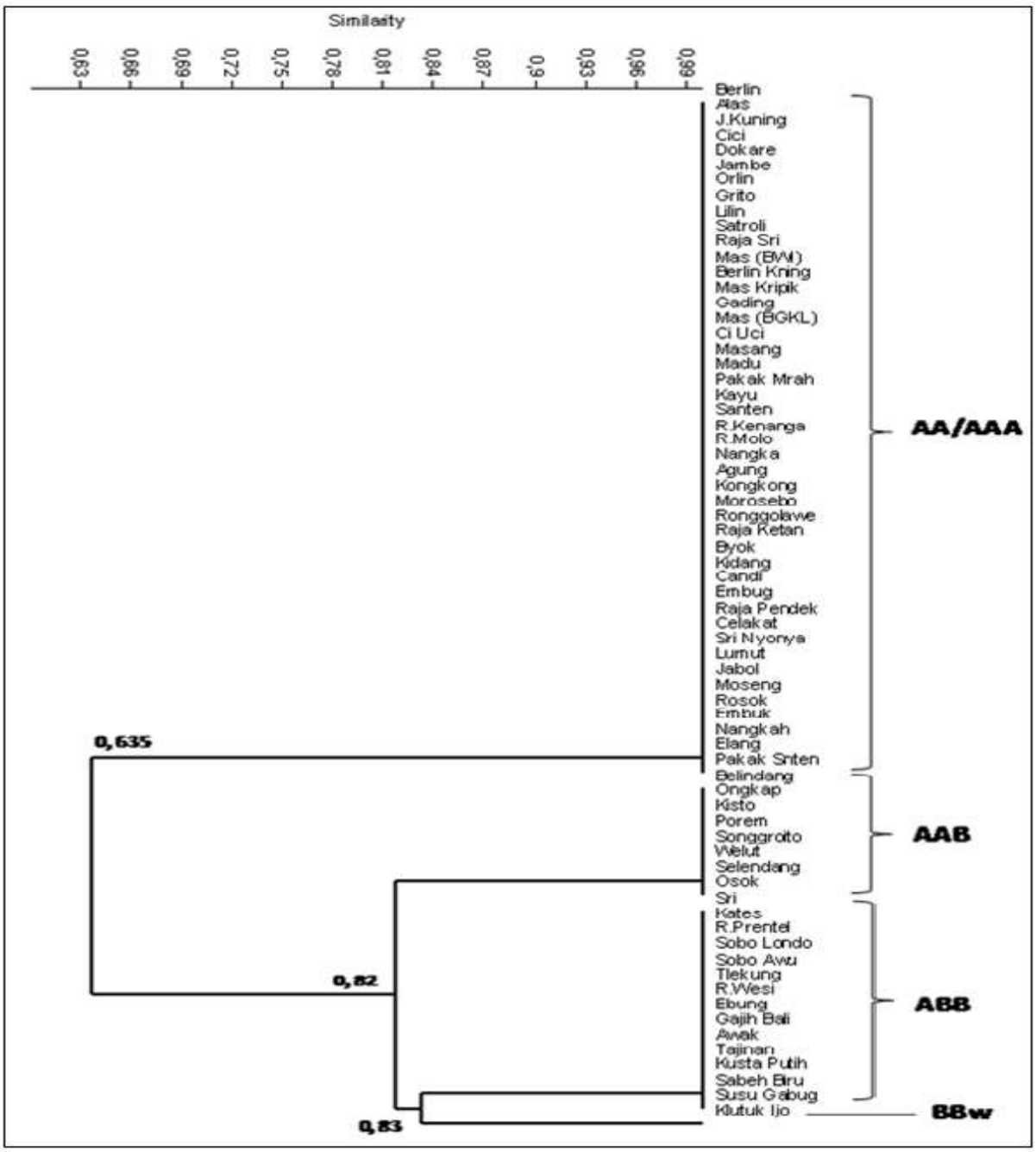

Fig. 3. Dendogram clustering analysis result of 68 banana accessions from East Java based on PCR-RFLP of ITS region.

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