PHYLOGENETIC RELATIONSHIPS OF INDONESIAN BANANA CULTIVARS INFFERED FROM TRNL-F INTERGENIC SPACER OF CHLOROPLAST DNA

Amin Retnoningsih[1)], Rita Megia[2)] & Alex Hartana[2)]
[1)] Department of Biology, Faculty of Mathematics and Sciences, Semarang State University, Jl. Raya Sekaran Gunungpati, Sekaran Campus Semarang 50229, Indonesia
[2)] Department of Biology, Faculty of Mathematics and Sciences, Bogor Agricultural University, Dramaga Campus Bogor, Indonesia

Amin Retnoningsih, Rita Megia & Alex Hartana. 2014. Hubungan kekerabatan filogenetika kultivar pisang di Indonesia berdasarkan daerah intergenik *trnL-F* DNA kloroplas. *Floribunda* 4(8): 202–211. —. Hubungan kekerabatan kultivar pisang di Indonesia dikaji berdasarkan daerah intergenik *trnL-F* DNA kloroplas. *Ingroup* terdiri atas 39 aksesi pisang dari berbagai kelompok genom di Indonesia yang meliputi 9 kultivar bergenom AA/ AAA; 11 AAA; 2 BB; 13 AAB; dan 4 ABB serta 12 sekuen DNA *Musa* sp. pada daerah yang sama yang diambil dari *GenBank* (4 pisang bergenom AA liar; 1 kultivar bergenom AA; 3 AAB; dan 4 ABB. Dua aksesi kelompok *Rhodoclamys* dan *Australimusa* digunakan sebagai *outgroup*. Seluruh aksesi *ingroup* (39 aksesi) dianalisis menggunakan primer daerah intergenik *trnL-F*. Hasil penelitian menunjukkan bahwa urutan daerah *trnL-F* dapat membedakan 40 aksesi pisang yang memiliki kloroplas tipe A (AA liar, AA, AA/ AAA; AAA; AAB, dan ABB) dari 11 aksesi pisang yang memiliki kloroplas tipe B (BB, AAB, dan ABB). Aksesi dengan kloroplas tipe A diduga diturunkan dari betina *Musa acuminata*, sedangkan aksesi dengan kloroplas tipe B diturunkan dari betina *M. balbisiana*. Namun demikian, hasil penelitian ini belum dapat mengidentifikasi nenek moyang spesifik *M. acuminata* liar dari setiap aksesi pisang tersebut.

Kata kunci: Kultivar pisang, Indonesia, hubungan kekerabatan filogenetika, daerah intergenik *trnL F* DNA kloroplas.

Amin Retnoningsih, Rita Megia & Alex Hartana. 2014. Phylogenetic relationships of Indonesian banana cultivars inffered from *trnL-F* intergenic spacer of chloroplast DNA. *Floribunda* 4(8): 202–211. —. A phylogenetic relationship of Indonesian banana cultivars was conducted using trnL-F intergenic spacer of chloroplast DNA sequences. The ingroup was 39 banana accessions from various genomic groups in Indonesia consisting of 9 AA/AAA; 11 AAA; 2 BB; 13 AAB; and 4 ABB cultivars and 12 sequences of different taxa of *Musa* downloaded from GenBank consisting of 4 AA wild; 1 AA; 3 AAB; and 4 ABB cultivars. Two accessions from section *Rhodoclamys* and *Australimusa* were used as the outgroup. The 39 accessions of the ingroup and the 2 accessions of the outgroup were analyzed using primers of the trnL-F intergenic spacer. The results showed that sequence of the trnL-F region can clearly distinguish 40 banana accessions having the A-type chloroplast (AA wild; AA; AA/AAA; AAA; AAB; and ABB) from those 11 accessions having the B-type chloroplast (BB, AAB, and ABB). The A-type chloroplast accessions are most probably derived from female *Musa acuminata*, while the B-type chloroplast accessions may be derived from female *M. balbisiana*. However, the specific *M. acuminata* ancestor of each cultivar remains unidentified.

Keywords: Banana cultivar, Indonesia, philogenetic relationship, *trnL-F* intergenic spacer of chloroplast DNA.

Most cultivated bananas are derived from inter and intraspecific hybridization between wild diploid *Musa acuminata* Colla and *M. balbisiana* Colla (Stover & Simmonds 1987). Various banana cultivars arisen from wild species were morphologically divided into AA; AAA; BB; AAB; ABB; BBB; and ABBB genomic groups (Simmonds & Shepherd 1955). Maternal and paternal origins of the banana cultivars are still unidentified as a result of the high diversity within *M. acuminata* (Pollefeys *et al*. 2004) and the low genetic diversity within *M. balbisiana* (Pillay *et al*. 2004).

Exploration of wild banana in Indonesia found 9 subspecies of *M. acuminata* (Daniells *et al*. 2001), but only 2 to 3 subspecies believed as ancestors of edible bananas. Carreel *et al*. (2002) suggested that *M. acuminata* subsp. *banskii* and subsp. *errans* are considered as the ancestral parents of most edible banana cultivars. Another study reported that *M. acuminata* subsp. *bansksii* and subsp. *malaccencis* are closely related to the starchy and the sweet banana cultivars, respec-

tively (CIRAD 2002).

Chloroplast DNA (cpDNA), mainly their noncoding regions, has been widely used to understand plant phylogenies at different taxonomic levels (Shaw *et al*. 2005; Testolin & Cipriani 1997; Zhang *et al*. 2003). The noncoding regions change more rapidly and exhibit more phylogenetically informative sites than do coding sequences. Therefore, these regions were appropriate to be used in plants phylogenetic analysis in lower taxonomic levels (Gielly & Taberlet 1994). The regions are also recognized as precious characters for studying phylogenetic relationships between closely related species (Zhang *et al*. 2003).

Chloroplast DNA of *Musa* spp. is transmitted maternally, while mitochondrial DNA is transmitted paternally. The both regions are considered an essential tool for phylogeny analysis and may suggest a powerful tool to conform hybrid origins of banana cultivars (Carreel *et al*. 2002; INIBAP 2002). Besides it is uniparentally inherited, the other characteristics of chloroplast genome are not recombining and structurally relatively stable (Barcaccia *et al*.2007).

The intergenic spacer of the *trnL* (UAA) 3' exon and the *trnF* (GAA) gene (*trnL-F* intergenic spacer) are noncoding regions in cpDNA which was suitable for DNA barcoding (Barcaccia *et al*. 2007). The intergenic spacer of the *trnL-F* also possessed phylogenetic utilities. The region may develop at rates similar to that of *rbcl* to as much as three times faster than *rbcl*, depend on the study groups (Gielly & Taberlet 1994). Besides that, the size of the *trnL-F* intergenic spacer is relatively small, ranged from 120 to 350 bp either in *Magnoliopsida* or *Liliopsida*. Therefore, this region may give reasonable resolution within *Musa,* although it was lower than that of a nuclear based phylogeny (Gielly *et al*. 1996).

The purpose of this study was to elucidate phylogenetic relationships among the Indonesian banana cultivars from various genomic groups using *trnL-F* intergenic spacer of cpDNA.

MATERIALS AND METHODS

Plant material

The ingroup examined consisted of 39 banana accessions collected from various regions in Indonesia and 12 sequences of different taxa of *Musa* downloaded from GenBank (Umali & Nakamura 2003). The 39 accessions consisted of *M. acuminata* (AA/AAA; and AAA), *M. balbisiana* (BB) and *M. x paradisiaca* (AAB and ABB). The outgroup consisted of two species of *Musa*: *M. sanguensis* from section *Rhodoclamys* (n = x =11) and 'Tongkat Langit' or the cultivar 'Fei' from section *Australimusa* (n = x =10). All accessions studied (the ingroup) and the outgroup were presented in Table 1.

Table 1. List of accessions used as the ingroup and the 2 accessions from section *Rhodoclamys* and *Australimusa* used as the outgroup.

Accession	Genomic group	Status in the study	GenBank Accession number	Region/source
Lilin	AA	Ingroup	AB095763	GenBank
subsp. *zebrina*	AAw	Ingroup	AB095767	GenBank
subsp. *banksii*	AAw	Ingroup	AB095542	GenBank
subsp. *malacensis*	AAw	Ingroup	AB095760	GenBank
subsp. *siamea*	AAw	Ingroup	AB095758	GenBank
M. balbisiana	BB	Ingroup	AB095539	GenBank
Umalag	AAA	Ingroup	AB095766	GenBank
Laknau	AAB	Ingroup	AB095759	GenBank
Cardaba	ABB	Ingroup	AB095541	GenBank
Gubao	ABB	Ingroup	AB0955757	GenBank
Pelipita	ABB	Ingroup	AB095761	GenBank
Sabakatsila	ABB	Ingroup	AB095764	GenBank
Ketan	AA/AAA	Ingroup	-	Unknown, RIF
Kole	AA/AAA	Ingroup	-	Unknown, RIF
Koumus	AA/AAA	Ingroup	-	Manokwari, RIF

Table 1. List of accessions used as the ingroup and the 2 accessions from section *Rhodoclamys* and *Australimusa* used as the outgroup (continued).

Accession	Genomic group	Status in the study	GenBank Accession number	Region/source
Lilin	AA/AAA	Ingroup	-	Palembang, Diperta
Monyet	AA/AAA	Ingroup	-	East Java, Diperta
Penjalin	AA/AAA	Ingroup	-	Sleman, Diperta
Pinang	AA/AAA	Ingroup	-	Banyuwang, Diperta
Ratu	AA/AAA	Ingroup	-	Unknown, RIF
Rejang	AA/AAA	Ingroup	-	Jasinga, Bogor
Klutuk Hijau	BB	Ingroup	-	Jasinga, Bogor
Klutuk Hitam	BB	Ingroup	-	Jasinga, Bogor
Ambon Lumut	AAA	Ingroup	-	Jasinga, Bogor
Ambon Merah	AAA	Ingroup	-	Jasinga, Bogor
Angleng	AAA	Ingroup	-	Yogyakarta, Diperta
Awomen	AAA	Ingroup	-	Unknown, RIF
Ayam	AAA	Ingroup	-	Unknown, RIF
Bole	AAA	Ingroup	-	Jayawijaya, RIF
Koja Pretel	AAA	Ingroup	-	Yogyakarta, Diperta,
Potho Ijo	AAA	Ingroup	-	Bantul, Diperta
Sebrot	AAA	Ingroup	-	Yogyakarta, Diperta
Sramfin	AAA	Ingroup	-	Manokwari, RIF
Udang	AAA	Ingroup	-	Jasinga, Bogor
Agung Pasuruan	AAB	Ingroup	-	Pasuruan, Diperta
Burlangge	AAB	Ingroup	-	Sentani Jayapura, RIF
Brentel	AAB	Ingroup	-	Malang, Diperta
Kepok Amerika	AAB	Ingroup	-	Yogyakarta, PKBT
Koumosona	AAB	Ingroup	-	Manokwari, RIF
Nangka	AAB	Ingroup	-	Unknown, PKBT
Neij Amper	AAB	Ingroup	-	Manokwari, RIF
Pisang Seribu	AAB	Ingroup	-	Unknown, Diperta
Raja Lini	AAB	Ingroup	-	Gunung Kidul, Diperta
Raja Sereh	AAB	Ingroup	-	Purworejo, Diperta
Raja	AAB	Ingroup	-	Unknown, PKBT
Susu	AAB	Ingroup	-	Unknown, PKBT
Tanduk	AAB	Ingroup	-	Jasinga, Bogor
Kepok Kuningan	ABB	Ingroup	-	Girimulyo, Diperta
Kepok Merah	ABB	Ingroup	-	Jasinga, Bogor
Selayar	ABB	Ingroup	-	Sentani Jayapura, RIF
Siam Manggala	ABB	Ingroup	-	Unknown, Diperta
M. sanguensis	-	Outgroup	-	KRB, Bogor
Tongkat langit	-	Outgroup	-	Maluku, RIF

DNA extraction

Genomic DNA was extracted from fresh young leaves by a modified protocol of Dixit (1998). The leaves were frozen in liquid nitrogen and ground using mortar and pestle, and then mixed with SDS buffer (100 mM of Tris (pH 8.0), 50 mM of EDTA (pH 8.0), 500 mM NaCl, 10 mM beta-mercaptoethanol, and 20% SDS). After purification using 10 mg/ml of RNAse free at 37ºC for 1 hour, precipitation of DNA was conducted without PEG solution treatment. Concentration and quality of DNA were evaluated by 0.8% agarose gel electrophoresis stained with ethidium bromide (EtBr) and by spectrophotometer, respectively and then diluted in TE to an estimated consentration of 10 ng μl^{-1} for PCR template.

DNA amplifications and sequencing

The *trnL-F* intergenic spacer was amplified using primers E : 5 ' G G T T C A G T C C C T C T A T C C C 3 'and F : 5'A T T T G A A C T G G T G A C A C G A G 3 ' designed by Taberlet *et al*. (1991). PCR reaction mix consisted of 5 µl 10 x PCR buffer with 25 mM $MgCl_2$, 10 µl 5 x GC-rich solution, 1 µl 10 mM dNTPs, 1 µl 10 µM of each primer, 0.4 µl 5 u μl^{-1} Taq DNA Polymerase (FastStart Taq DNA polymer-rase, Roche Applied Science®), and 7.5 µl DNA template (10 ng μl^{-1}) in 50 µl-reaction volume. The amplification was performed for 35 cycles consisting of denaturation at 94 ºC for 30 s, annealing at 52ºC for 30 s, and extention at 72ºC for 1 min. These cycles were headed by a 4 min denaturation at 94ºC, and ended by a 10 min final extention at 72ºC. After purification using Millipore system, double stran-ded PCR products were directly sequenced for one strand using ABI 330 in First Base Laboratories Sdn Bhd, Selangor Darul Ehsan, Malaysia.

Data analysis

DNA sequences obtained from the *trnL-F* intergenic spacer were aligned with CLUSTAL X version 3.1 (Thompson *et al*. 1997), and then refined manually in data matrix using Bioedit program. Short regions of sequence at the beginning and the ending of the *trnL-F* intergentic spacer were excluded from analysis due to differences of length sequences.

All data sets analyzed by heuristics search methods with tree bisection reconnection (TBR) branch swapping. Collapse of zero length branches were performed to obtain the most parsimonious trees (m.p.t). The analysis was repeated 100 times with the random addition option to minimize problems of multiple of m.p.t. Sets of equally most parsimonious tree were summarized by a strict consensus tree. Bootstrap 500 replicates with heuristic search settings identical to those of the original research were conducted to evaluate confidence of clades. For phylogenetic inference, all characters were equally weight and unordered, while gaps (insertion and deletion) were treated as missing data. Sequences were analyzed using parsimony algorithm of the software package PAUP version 4 (Swofford 1998).

RESULTS AND DISCUSSION

The *trnL-F* intergenic spacer amplifications of the 39 accessions of the ingroup and the 2 accessions of the outgroup produced clear products. Sequences from 41 accessions provided a total aligned length of 329 bp whereas the 12 sequences downloaded from GenBank have the length size of 358 bp (Umali & Nakamura 2003). It means that there were 29 bp of the *trnL-F* intergenic spacer were excluded from analyzing phylogenetic *Musa* spp. The DNA sequences ranged in size from 329 to 350 bp. DNA sequencing in this study was performed only for one strand and therefore sequences length further analyzed were determined by the shortest sequence. Two gaps were introduced in alignment of the *trnL-F* intergenic spacer; one gap contained 17 bp and another only 1 bp.

Three hundred and twenty nine characters of sequences were compared in the *trnL-F* intergenic spacer, of which 318 (96.7%) were constant and 11 (3.3%) were variable. Five of the 11 variable characters were parsimony-uninformative (autapomorphic), and the remaining 6 characters were potentially informative in parsimony analysis. The parsimony analysis of the *trnL-F* sequences resulted in 200 m.p.t., one of which is presented in Figure 1, with a tree length of 13 steps, a consistency index (CI) excluding uninformative characters of 0.75 and a retention index (RI) of 0.93. The strict consensus tree demonstrated that the phylogenetic tree was recovered in the ingroup with bootstrap support less than 61%.

All banana accessions of the ingroup formed a clade supported by a bootstrap value of 60% (Figure1).'Penjalin'(AA/AAA),'Raja Sereh' (AAB), 'Susu' (AAB), 'Pinang' (AA/AAA), and a small clade (55% bootstrap) consisting of' Brentel' (AAB) and 'Kepok Amerika'(AAB) formed a clade with the 56% bootstrap. This clade was separated from two sister clades consisting of accessions containing the A genome alone in the first

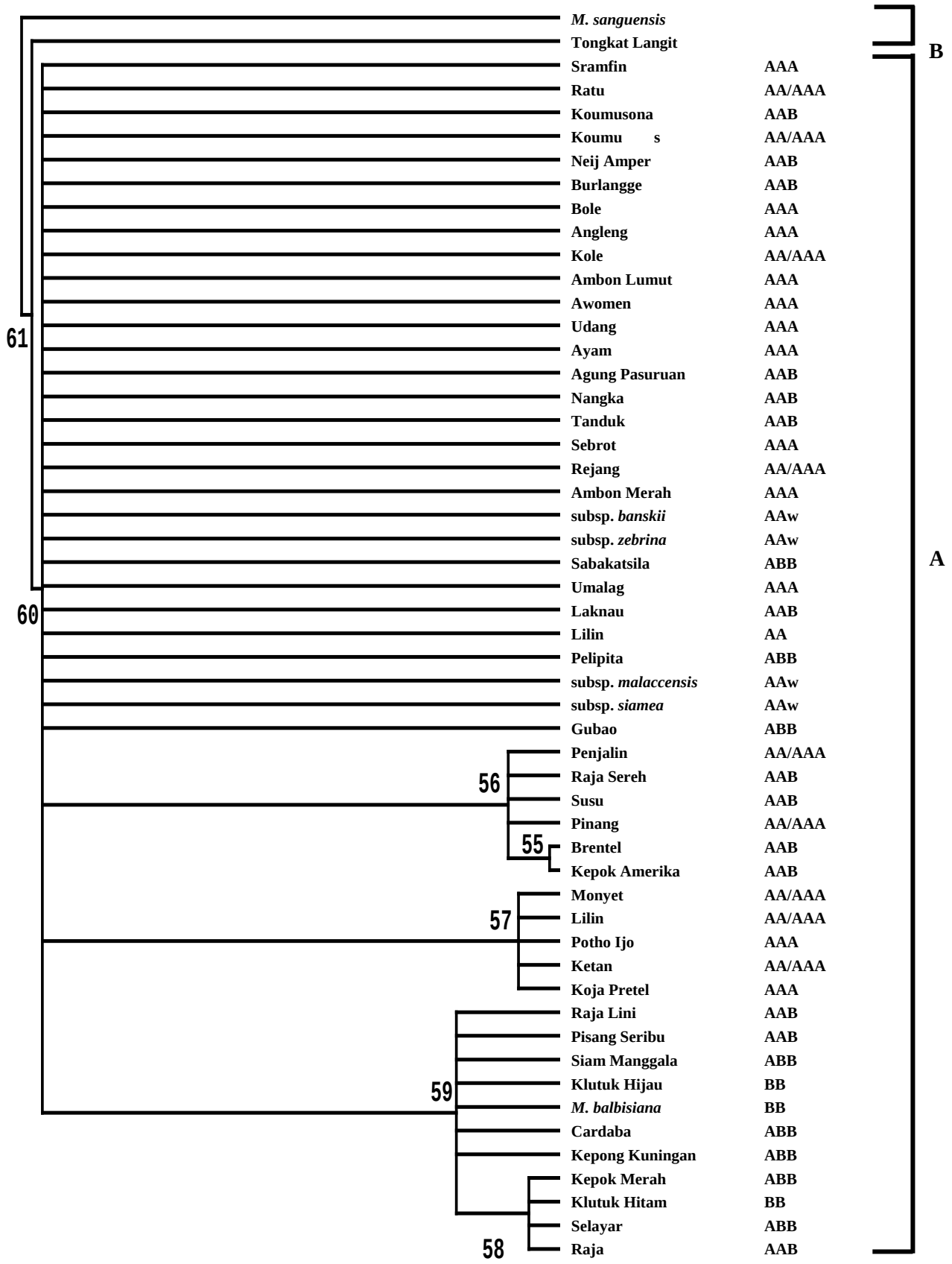

Figure 1. A strict consensus tree from analysis data sets in the *trnL-F* intergenic spacer of cpDNA. A accessions of the ingroup, B accessions of the outgroup.

sister clade, and those the B genome in the second sister clade. The first sister clade consisting of 'Monyet' (AA/AAA), 'Lilin' (AA/AAA), 'Potho Ijo' (AAA), 'Ketan' (AA/AAA) and 'Koja Pretel' (AAA) formed a clade with 57% bootstrap.

Several accessions containing the B genome formed a clade with the 58% bootstrap within a large clade also containing the B genome with 59% bootstrap. The result showed that one accession of BB from Indonesia ('Klutuk Hitam') formed a clade separating from the other BB accessions either from GenBank or Indonesia ('Klutuk Hijau'). The analysis suggested that the B genome exhibited variation in the *trnL-F* intergenic spacer region which perhaps had contributed variation in BB, AAB and ABB cultivars.

Banana accessions of the ingroup consisting of various genomic group used in this study formed group with the 60% bootstrap value. *M. balbisiana* and 'Cardaba' formed a clade with the bootstrap 59% separated from the other accessions from GenBank, included accessions considered to be the ancestral parents of most banana cultivars namely *M. acuminata* subsp. *banksii* and subsp. *malaccensis.*

Evolutionary relationships in the genus *Musa* have not been fully elucidated due to the occurrences of hybridization and polyploidy (Pillay *et al*. 2004). *Musa* genetic improvement programs in the future crucially need the knowledge of these natural relationships. The development of biotechnologies allowed many molecular techniques to be employed to study evolutionary and genetic relationships in plants. Swangpol *et al*. 2007 reported that the utility of 2 introns and 2 intergenic spacers of cpDNA regions could clearly distinguish *M. acuminata* Colla (A) from *M. balbisiana* Colla (B)-containing genomes. They reported that several triploid interspecific hybrids such as the AAB or ABB contained A-type chloroplasts, whereas others, the BBA or BBB contained B-type chloroplasts.

Noncoding regions of cpDNA were originally thought to be potentially for phylogenetic analysis due to their high variation (Curtis & Clegg 1984; Clegg *et al*. 1994). However in this study showed that the region is conserved and it has not high variation as exhibited in conifers (Raubenson & Jansen 2004). Therefore, the resolution of phylogenetic tree of banana accessions in the present study exhibited clades with the bootstrap ranging only from 55% to 60%. Although the resolution of phylogenetic tree is low, the result commonly supported previous study by Swangpol *et al*. (2007). The present study detected two trends. The first trend showed accessions derived from female of *M. acuminata* and the second trend showed accessions arisen from female of *M. balbisiana*. Forty banana accessions which consisted of either pure *acuminata* of AA wild; AA; AA/AAA; and AAA cultivars; or hybrids AAB and ABB cultivars were indicated to contain the A-type chloroplast, while the remaining 11 banana accessions of BB, AAB (BAA) and ABB (BBA) contained the B-type chloroplast (Figure 2).

The group of banana accessions of A-type chloroplast may be derived from female ancestors of *M. acuminata* subsp. *banskii*, *mallacensis*, *siamea*, or *zebrina* due to the existence of these subspecies in the clade of the A-type chloroplast. The result supported the study by Carreel *et al*. (2002) describing that *M. acuminata* subsp. *banksii* is related to most cultivars of the starchy bananas through their mitochondrial genomes. It means that the starchy bananas were perhaps originated from male *M. acuminata* subsp. *banksii*.

Accessions of starchy bananas or subgroup Plantain such as 'Tanduk' (AAB), 'Nangka' (AAB), and the other AAB grouped within the A-type chloroplast clade may due to the occurrence of hybridization between triploid female AAB or ABB and diploid male AA parents. The A-type chloroplast is donated by AB gamet produced from normal chromosome segregation of AAB and ABB parents (Pillay *et al*. 2004). The AAB cultivars with the A-type chloroplast also may be originated from crossing between diploid female AA through abnormal chromosome segregation and diploid male BB through normal segregation or between triploid female AAA and diploid male BB parents. The triploid AAA contributed AA gamet through normal chromosome segregation. Those AAB accessions may be included in dessert bananas or dual purpose bananas as reported by Valmayor *et al*. (2000).

Accessions of ABB containing the A-type chloroplast were perhaps developed from crossing between triploid female AAB or ABB and diploid male BB parents. Theoretically, female AAB or ABB donated AB gamet from normal chromosome segregation (Pillay et al. 2004). All of the cases, origin of the A-type chloroplast did not precisely identified, from *M. acuminata* subsp. *banksii*, subsp. *malaccensis*, subsp. *zebrina* or subsp. *siamea*. The clade containing 'Penjalin'(AA/AAA), 'Raja Sereh' (AAB), 'Susu' (AAB),' Pinang' (AA/AAA), 'Brentel' (AAB) and 'Kepok Amerika (AAB) and the clade of 'Monyet' (AA/AAA), 'Li-

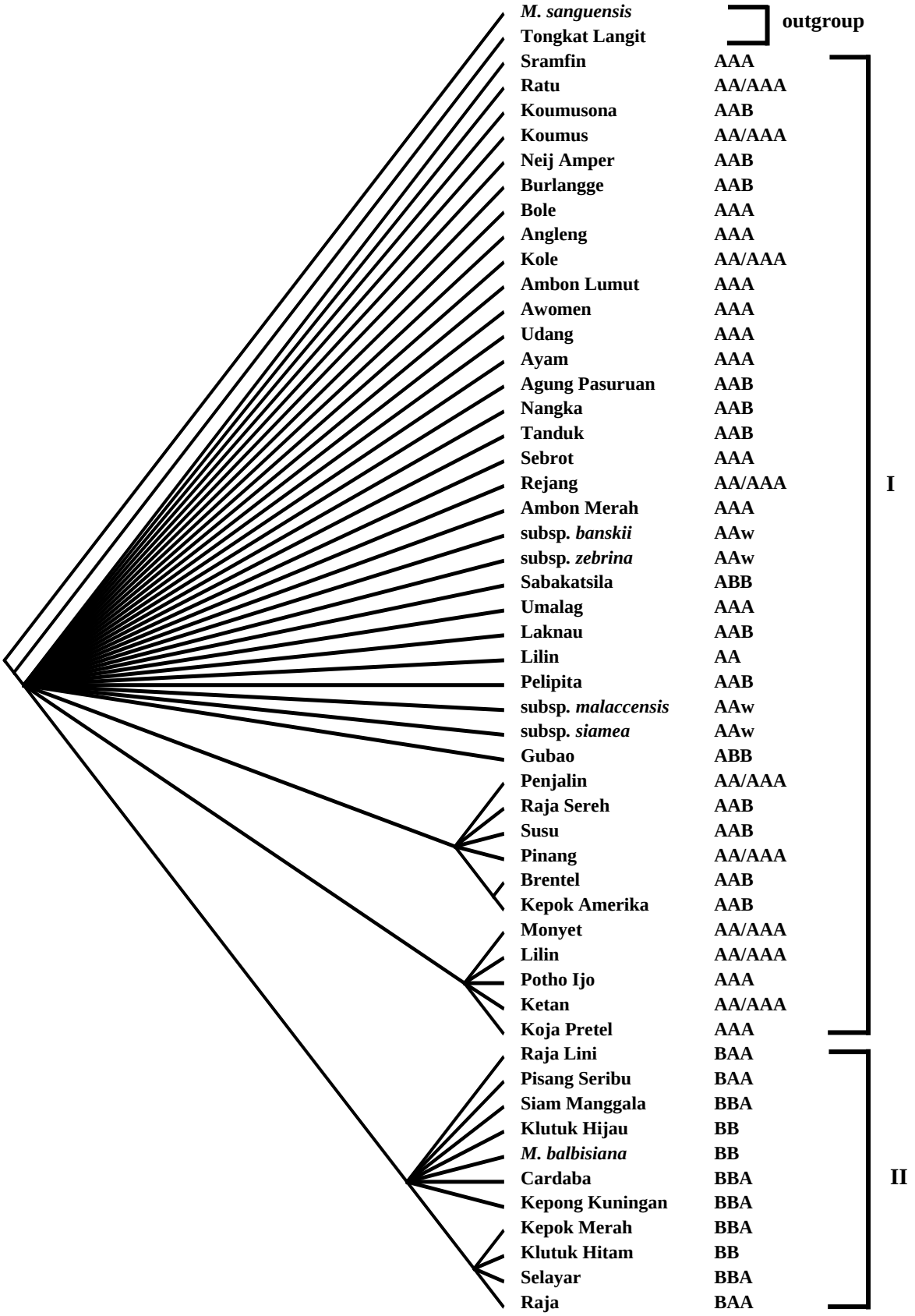

Figure 2. Slanted cladogram depicting relationships among banana accessions supposed have the A- and the B-type chloroplast using the *trnL-F* intergenic spacer of cpDNA. I. A-type chloroplast, II. B-type chloroplast.

lin' (AA/AAA), 'Potho Ijo' (AAA), 'Ketan' (AA/AAA) and 'Koja Pretel' (AAA) may be derived from outside of subspecies of AA wild diploid used in this study. Those accessions may be derived from female *M. acuminata* subsp. *errans* or subsp. *burmannica* which were not used in the study.

Commonly, genetic diversity in the A-type chloroplast group probably connected with the existence of 9 subspecies of *M. acuminata* found in Indonesia region (Daniells *et al*. 2001). Most banana accessions with the A-type chloroplast in this study were known belong to the sweet bananas (dessert bananas). The result showed that all subspecies of *M. acuminata* used in this study were closely related to most banana accessions of *M. acuminata* and hybrids AAB as reported by Racharak & Eiadthong (2007). The pure BB banana cultivar genomic group was resulted from crosses between diploid BB female and diploid BB male parents. The BB cultivars also could be derived from triploid ABB female with normal segregation and diploid BB male with normal segregation or between triploid AAB female with abnormal segregation and diploid BB male with normal segregation (Pillay *et al*. 2004). The information suggested that the BB genomic groups were more closely related to the ABB (BBA) than AAB (BAA) groups, and therefore they tend to form a clade together with the ABB (BBA) banana accessions containing the B-type chloroplast (Swangpol *et al*. 2007).

Variation in the *trnL-F* intergenic spacer sequences, especially at the cultivar levels of *Musa* spp. were very low because the regions have to be conserved and non recombined (Gielly *et al*. 1996). In this region, alteration of only one nucleotide perhaps required many hundreds years of times. Several techniques could be used to increase resolution of phylogenetic trees. The *trnL-F* intergenic spacers were sequenced for two strands in order to produce full length fragments, so entire sequences could be analyzed. Phylogenetic analysis also could be performed using the other non coding sequences which possessed longer size than the *trnL-F* regions. Small *et al*. (2005) reported the association between sequence length and the number of phylogenetically informative characters. As sequence length increase, the number of both variable and phylogenetically informative characters also increases. The utility of noncoding chloroplast DNA regions in *Musa* spp. would provide an exceptional opportunity for studying the maternal lineage of clones or cultivars (Carreel *et al*. 2002). It is hoped that future study will be able to elucidate the exact progenitors of edible bananas (Pillay *et al*. 2004).

In the following study, sequence comparison of the *trnL-F* region of the chloroplast genome was employed to obtain an independent estimation of relationships among banana accessions in Indonesia. This mainly noncoding sequence region is especially suitable for sequence comparisons due to its moderate size with the length less than 1500 bp depending of plants and the fact that it is bounded by the transfer RNA genes for *trnL* (UAA) and *trnF* (GAA) which give highly conserved primer sites for PCR amplification and sequencing (Taberlet *et al*. 1991). The region between *trnL* and *trnF* is more variable in length among groups of flowering plants. Other noncoding sequences of the chloroplast genome also having phylogenetic potential are *atpB-rbcL* intergenic region (Manen *et al*. 1994), *trnL* (UAA) intron (Taberlet *et al*. 1991), *trnC-trnD* region (Lee & Wen 2004), and *trnH-psbA* intergenic spacer (Kress *et al*. 2005).

CONCLUSIONS

The data and analysis presented in this study showed that the *trnL-F* intergenic spacer of cpDNA sequences were potential to be used to infer phylogenetic relationship of *Musa* spp. Based on the regions, 40 banana accessions containing the A-type chloroplast consisting of AA wild; AA; AA or AAA; AAA; AAB; and ABB cultivars were clearly distinguished from those 11 accessions of B-type chloroplast containing of BB, AAB and ABB cultivars. The A-type chloroplast accessions are most probably derived from female *M. acuminata*, while the B-type chloroplast accessions may be derived from female *M. balbisiana.* However, the exact progenitors of each edible banana have not been identified yet.

ACKNOWLEDGEMENT

This study was funded by DIKTI through Basic research program. Comment and suggestions from Prof. Dr. Mien A. Rifai are sincerely appreciated. We would like to express our thanks to Ir. Agus Sutanto, MSc, Ir. HS Edison, MSc. and the other staff of RIF Solok, West Sumatera and staff of Diperta Yogyakarta for providing plant materials and information about the accessions.

REFERENCES

Barcaccia G, Nicole S & Lucchin M. 2007. Assessing distinctiveness of crop plat species and varieties through DNA barcoding. *Proceeding of the 51st Italian Sociaty of Agricultural Genetics Annuals Cronggress Riva del Garda*, Italy 23–26 September 2007. ISBN 978-88-900622-7-8. Poster Abstract –A.06

Carreel F, Leon DG, Lagoda PJL, Lanaud C, Jenny C, Horry JP & du Montcel. 2002. Ascertaining maternal and paternal lineage within *Musa* by chloroplas and mitochondrial DNA RFLP analyses. *Genome* 45: 679–692.

Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD). 2002. Banana plantain molecular analysis of genetic diversity. Rome: CIRAD.

Clegg MT, Gaut BS, Learn Jr GH & Morton BR. 1994. Rates and patterns of chloroplast DNA evolution. *Proc. Nat. Acad. Sci*. 91: 6795–6801.

Curtis SE & Clegg MT. 1984. Molecular evolution of chloroplast DNA sequences. *Mol. Biol. Evol*. 1: 291–301.

Daniells J, Jenni C, Karamura D & Tomelpe K. 2001. Musalogue: A Catalogue of *Musa* Germplasm, Diversity in the Genus *Musa*. Montpellier: INIBAP.

Dixit A. 1998. A simple and rapid procedure for isolation of *Amaranthus* DNA suitable for fingerprint analysis. *Plant. Mol. Biol. Rep*. 16: 1–8.

Gielly L & Tarbelet P. 1994. The use of chloroplast DNA to resolve plant phylogenetic: noncoding versus *rbcL* requences. *Mol. Biol. Evol*. 11: 769–777.

Gielly L,Yuan YM, Kupper P & Tarbelet P. 1996. Phylogeny use of noncoding regions in the genus *Gentiana* L: chloroplast trnL (UAA) intron versus nuclear ribosomal internal transcribe spacer sequences. *Mol. Phylogenet. Evol*. 5: 460–466.

International Network for the Improvement of Banana and Plantain (INIBAP). 2002. Banana genomics-the genes beneath the peel. Montpelier: INIBAP.

Kress WJ, Wurdack KJ, Zimmer EA, Weight LA & Jansen DH. 2005. Use of the DNA barcodes to identity flowering plants. *Proc. Natl. Acad. Sci*. 102: 8369–8374.

Lee C & Wen J. 2004. Phylogeny of *Panax* using chloroplast *trnC-trnD* intergenic region and utility of *trnC-trnD* interspecific studies of plants. *Mol. Phylogenet. Evol.* 31: 894–903.

Manen JF, Savolainen V & Simon P. 1994. The *atpB* and *rbcL* promoters in plastid DNAs of a wide dicot range. *J. Mol. Evol*. 38: 577–582.

Pillay M, Tenkouano A, Ude G & Ortiz R. 2004. Molecular characterization on genomes in *Musa* and its applications. *In*: Jain SM & Swennen R (Eds), *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations*. Science Publishers, Inc. Enfield (NH), USA. Plymouth, UK. pp. 271–286.

Pollefeys P, Sharrock S & Arnaud E. 2004. Preliminary Analysys of the Literature on the Distribution on Wild *Musa* Species Using MGIS and DIVA-GIS. INIBAP-IPGRI. INIBAP

Racharak P & Eiadthong W. 2007. Genetic relationship among subspecies of *Musa acuminata* Colla and A-genomeconsisting edible cultivated bananas assayed with ISSR markers. *Songkl. J. Sci. and Technol*. 29: 1479–1489.

Raubeson LA & Jansen RK. 2004. A rare chloroplast-DNA structural mutation is shared by all conifers. *Biochem. Syst. Ecol*. 20: 17–24.

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder Ct, Schilling E & Small RL. 2005. The tortoise and the hare II: relativee utility of 21 noncoding chloroplast DNA sequences for phylogenetics analysis. *Am. J. Bot*. 92: 142–166.

Simmods NW & Sheperd K. 1955. Taxonomy and origins of the cultivated bananas. *Bot. J. of the Linn. Soc*. 55: 302–312.

Small RL, Lickey EB, Shaw J & Hauk WD. 2005. Amplification of noncoding chloroplast DNA for phylogenetic studies in lycophytes and monilophytes with a comparative example of relative phylogenetic utility from *Ophioglosaceae*. *Mol. Phylogenet. Evol.* 36: 509–522

Stover RH & Simmonds NW. 1987. Bananas, 3rd ed. Harlow, UK: Longman.

Swangpol S, Volkaert H, Sotto RC & Seelanan T. 2007. Utility of selected non-coding chloroplast DNA sequences for lineage assessment of *Musa* interspecific hybrids. *J. Biochem. Mol. Biol.* 40: 577–587.

Swofford DL. 1998. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods) version 4. Sunderland, MA: Sinaeur Associates.

Taberlet P, Gielly L, Pautou G & Bouvet J. 1991. Universal primers for amplification of three non coding regions of chloroplast DNA. *Plant. Mol. Biol.* 17: 1105–1109.

Testolin R & Cipriani G. 1997. Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in the genus *Actinidia*. *Theor. Appl. Genet.* 94: 897–903.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F & Higgins DG. 1997. The clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analisis tools. *Nucl. Ac.Researc.* 24: 4876–4882.

Umali RP & Nakamura I. 2003. http://www. ncbi. nlm.nih.gov/. July 12, 2005.

Valmayor RV, Jamaluddin SH, Silayoi B, Kusumo S, Danh LD, Pascua OC & Espino RRC. 2000. Banana cultivar names and synonyms in Southeast Asia. INIBAP-Asia and the Pasific Office, Laguna, Philippines, available on line http://bananas. Bioversity-international.org/files/files/pdf/publications /synonyms.pdf July 12, 200.

Zhang WH, Chen ZD, Li JH, Chen YC & Anndtang HB. 2003. Phylogeny of the Dipsacales s.l. based on chloroplast trnL and ndhF sequrnces. *Mol. Phylogen. Evol.* 26: 176–189.