Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 5, p. 266-272, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Classification of local banana *Mulu Bebe* North maluku based on rpoC genes and trnF–trnH intron

Adriana Hiariej[1*], Estri Laras Arumingtyas [2], Arik Arubil Fatina[2]

[1]*Department of Biology, Faculty of Mathematic and Natural Sciences, Pattimura University, Ambon, Maluku, Indonesia*

[2]*Department of Biology, Faculty of Mathematic and Natural Sciences, University of Brawijaya, Malang, Indonesia*

Article published on May 20, 2014

Key words: Classification, *Mulu Bebe* banana, North Maluku, rpoC gene sequences and trnF-trnH intron.

Abstract

North Maluku has a distinctive banana plant, the name of the plant is determined by the local community. The local name is *Mulu Bebe* due to the shape of the fruit's edges resembles a duck's mouth. Previously, there are no data on the existing *Mulu Bebe* bananas in other parts of Indonesia, which means *Mulu Bebe* banana fathomed as typical species of North Maluku. Information on *Mulu Bebe* banana are limited and the newest report was only on physical and morphological variability. Morphological features observed on previous study in 2012, determined *Mulu Bebe* as *Musa* AA group banana. This study aimed to classify *Mulu Bebe* banana based on its molecular characters. This study was conducted from October to December 2013 in the Laboratory of Molecular Biology, University of Brawijaya. Molecular markers used in this study is the chloroplast DNA (cpDNA), especially the sequence coding region (rpoC1) and non-coding regions (trnF-trnH introns). The results of molecular analysis using gene rpoC1 and trnF - trnH Intron indicate local banana *Mulu Bebe* in North Maluku include in *Musa acuminata* group with a similarity value of 100%.

***Corresponding Author:** Adriana Hiariej ✉ hiariejd@yahoo.com

Introduction

All provinces in Indonesia is growing both wild and cultivated bananas. Sunyoto (2011) estimated that there are more than 230 types of bananas in Indonesia. Each 100 g of banana provide 90 calories, contains no cholesterol, rich in vitamins A, C, B6, minerals calcium, potassium, and phosphorus (Megia, 2005) as well as magnesium, iron and serotonin (Sunyoto, 2011). Considering the importance of bananas for human life, not many studies were assessed either the wild type or public consumption banana. All bananas are edible, except *Fei* bananas, derived from *Musa acuminata* Colla crossed with (AA genome) and *Musa balbisiana* Colla (BB genome) in the section of Eumusa (Purseglove, 1985). Wahyuningtyas *et al*. (2009) raised a variety of genetic variation through a process that plays an important role in the evolution of the banana plant. Evolution of wild bananas produce banana cultivars with different ploidy levels with genomic variation AA, BB, AB, AAA, AAB, ABB, AAAA, ABBB, AAAB and AABB (Stover and Simmonds, 1987).

North Maluku Province is part of the eastern region of Indonesia with a very large banana germplasm include wild and cultivated bananas, including *Mulu Bebe* bananas. *Mulu Bebe* bananas are local in North Maluku (BPTP North Maluku).

The main objective of systematics or phylogeny theory is outlining efforts to reconstruct the evolutionary history of a particular group of taxa through the evidence in the evolutionary process. Such evidence can be in a form of possessed characters of each species in a group. Many characters can be formulated in an attempt to reconstruct the evolution, such as molecular characters (Ubaidilah *et al*., 2009).

Phylogeny is the main aim of the systematic, referring to the evolutionary history of groups of organisms. Phylogeny is generally represented in the form of a cladogram (phylogenetic tree). Conceptually, branching in the diagram is an evolutionary pattern of progeny. Line in the cladogram indicates lineage progeny populations through time to time (Simpson, 2006).

Molecular markers can be used in the taxa identification of both inter and intra species because its application is easy and objective (Judd *et al*., 2002; Chakravarthi and Naravaneni, 2006; Jonah *et al*., 2011). Chloroplast genome/plastome is a widely used molecular marker in the identification of taxa in plants (Clegg and Zurawski, 1991). Chloroplast is the plant organelle that contains all the enzymatic inquiry for photosynthesis (Saski, 2005). Chloroplast genome consists of DNA double spiral, circular-shaped, measuring 110-200 kb and encodes 30-50 genes RNA which divided into tRNA and rRNA, and approximately 100 protein-coding genes. The genes that encoding these proteins divided into two groups with translational function (rpoB, rpoC1, rpoC2, rpsl6, and matK) and photosynthesis (afpB, rbcL, PSAA, and petB) (Baumgartner *et al*., 1993; De Las Rivas *et al*., 2002).

Terrestrial plants have approximately 40-50% of each chloroplast genome that contains non - coding spacers (Saski, 2005). trnF-trnH intron is one of non-coding region in the chloroplast, an area that mainly prone to mutations, insertions and deletions. Intronic areas with mutations (Roy and Penny, 2007) and deletions or insertions (Wolfe *et al*., 1987) have relevance to the evolution of plants that essential especially in the classification of organisms.

Systematics taxonomy includes traditional description, identification, nomenclature, and classification of the organisms with the main purpose of phylogeny life reconstruction (evolutionary history) (Simpson, 2006). Information on *Mulu bebe* taxonomy has been collected by BPTP of North Maluku, on physical and morphological variability. However, there are no phylogenetic studies with molecular markers yet. Therefore, we conducted molecular studies for this local banana to reconstruct the phylogeny.

In phylogenetic studies, molecular markers that commonly used is DNA, which derived from the nucleus, mitochondria and chloroplasts. *Mulu bebe* banana phylogeny studies use chloroplast DNA (cp DNA) because the total DNA extracted and analyzed with easily overflow, containing a single gene, sufficient conservative rate of nucleotide substitution, and ideal for the evolution of molecular phylogenetic studies (Clegg *et al.*, 1994).

According to Judd *et al.* (2002), phylogenetic studies of plant chloroplast DNA sequences are widely used because of the genes in the chloroplast DNA is able to accumulate mutations faster than the genes in the nucleus and mitochondria DNA. Based on the above concept, the phylogeny analysis was performed using *Mulu Bebe* banana cpDNA gene. The purpose is to assess the similarity between sequences and taxonomic status of the *Musa* genus of *Mulu Bebe* Banana. This was done with multigene molecular markers of chloroplast DNA - rpoC gene sequences and trnF- trnH intron. Furthermore, rpoC gene sequence and trnF-trnH intron compared with sequences from the National Center for Biotechnology Information (NCBI) for setting the position of taxa in phylogeny tree.

Materials and methods

Sampling was conducted on the island of Halmahera (Jailolo) North Maluku, and molecular analysis carried out from October to December 2013, at the molecular and cellular biology laboratory, Brawijaya University. Molecular analysis of DNA samples use leaves isolation based on Ferdous *et al.* (2012) method. Electrophoresis was conducted on results of DNA isolation by spectrophotometry to determine the quality of subsequent DNA, and then amplified with gene rpoC and trnF - trnH intron. Purified PCR results were sequenced with the ABI Prism sequencer Automatic machine XL 3730 in Korea.

DNA isolation

DNA isolation was performed according to the method of Ferdous *et al.* (2012). Young leaves was weighed for 0.2 grams and put in a mortar then added by 0.1 grams of PEG to be crushed by Pestel to obtain leaves powder. The powder entered into microtube containing 600 µl of extraction buffer (10% CTAB, 0.5 M EDTA pH 8.1, M Tris HCl pH 8.5, M NaCl, sterile distilled water) and 1% β-mercaptoethanol of 60°C temperature, the mixture was homogenized with a vortex and transferred to new microtube. Homogenates of 2x CTAB buffer was added to 400 µl, was incubated in a water bath at a temperature of 65˚C. The homogenate was cooled to room temperature and add 400 µl of PCI. Homogenates vortexed and centrifuged at 8000 rpm, 25˚C for 10 minutes. Centrifugation of the supernatant was then transferred to the results of a new microtube and added 400 mL of isopropanol, inverted and incubated for 15 minutes at room temperature. The mixture was centrifuged at 12,000 rpm, 4˚C for 10 minutes. The supernatant was discarded and the pellet was washed with 70% ethanol, then dried at a temperature of 55˚C incubator until the ethanol dry. Subsequently, Pellet / DNA rehydrated with ± 100 mL ddH2O. DNA can be stored at - 20˚C to be used.

DNA Confirmation by Agarose Gel Electrophoresis

DNA isolation was confirmed by agarose electrophoresis. 1% agarose gel made by weighing the 0:20 g agarose plus 20 ml of TBE buffer heated 2 minutes in the microwave, when it's getting cold, we added ethidium bromide (EtBr) 0.5 mg/ml. The solution then poured into the plate (mold gel) which had been installed. Poured into the electrophoresis chamber with TBE buffer. 2 µl of the isolated DNA is inserted into the well using a micropipette. DNA running was use 100V voltage for 30 minutes. Observation of the UV band profiles using transiluminator and photographed.

Table 1. Amplification Region and Primers

Amplification Region	Primary	Reference
rpoC	GGCAAAGAGGGAAGATTTCG CCATAAGCATATCTTGAGTTGG	CBOL,2009
trnF – trnH intron	GTTATGCATGAACGTAATGCTC CGCGCATGGTGGATTCACAATCC	CBOL,2009

PCR of rpoC gene and trnF- trnH Intron

PCR was conducted using primers of rpoC and trnF-trnH intron. PCR was performed by making a PCR solution containing 6 mL ddH2O, 15 mL master mix, forward and reverse primer 5 pmol (Macrogen) – 3 mL each, and isolated DNA samples for 3 mL. The solution was processed in the PCR thermal cycler (Applied Bioscience) with initial denaturation 95˚C for 5 min, followed by 35 cycles of denaturation at 95˚C for 45 seconds, annealing at 56.8˚C for 45 seconds, and denaturation of 72˚C for 45 seconds. The PCR program ended with a final extension at 72˚C for 10 minutes.

rpoC gene and trnF- trnH Intron Confirmation by Agarose Gel Electrophoresis

PCR results of rpoC gene amplicons and trnF – trnH intron were also confirmed by agarose electrophoresis. agarose gel of 1.5% made from 0.3 g of agarose added with 30 ml of TBE buffer, and heated for 2 minutes in the microwave. After getting colder, 0.5 µl ethidium bromide (EtBr) was added into the agarose gel. This solution then poured into the plate and inserted into the electrophoresis chamber, then added TBE buffer. Each 2 µl of rpoC gene amplicons and trnH- trnF intron sample is inserted into the well using a micropipette. DNA running was done by connecting the cathode and anode at 100V voltage . UV band profiles observed using transiluminator and then photographed.

Purification and sequencing

PCR products (25 mL) were purified by adding 200 mL of cold absolute ethanol and 20 mL of Na-Acetate. The mixture was incubated at -20˚C for 1 hour - overnight. The results were centrifuged at 12,000 rpm, 4˚C for 10 minutes. The supernatant was discarded and the pellet was washed with 70% ethanol 200 mL, and centrifuged at 12,000 rpm, 4˚C for 10 minutes. The supernatant was discarded and the pellet re-dried at a temperature of 55˚C in incubator until ethanol free state. The dried pellet was added to 25 mL of ddH2O and continued for sequencing. Sequencing was performed by Macrogen sequencing methods standard in Korea using automatic machine sequencer (ABI 3730 XL).

Analysis

Phylogenetic analysis were done by comparing gene sequences of *Mulu Bebe* banana studied with reference sequences from the NCBI. Reference sequences are used to determine the position of studied taxa on the phylogenetic tree. Construction of phylogenetic tree was performed using MEGA5 software with Jukes - Cantor model of evolution and Neighbor Joining algorithm.

Results and discussion

The reconstruction of phylogenetic tree (cladogram) of *Mulu Bebe* banana with two molecular and compare to sequences from NCBI showed that *Mulu Bebe* taxa similar to *Musa acuminata* (Fig. 1). Hidayat *et al*. (2012) stated that variability in physical and morphological features characterized *Mulu Bebe* banana as Musa AA group. According to Purseglove (1985), *M. acuminata* Colla with AA genome is include in eumusa, as well as the hybridization with *M. balbisiana* Colla (BB genome). Otherwise, the level variation of ploidy genome is the evolution of wild banana (Stover and Simmond, 1987; de Jesus, 2013). Thus supporting the research with molecular markers determination of AA genome by BPTP North Maluku based on key reference of Stover and Simmonds (1987).

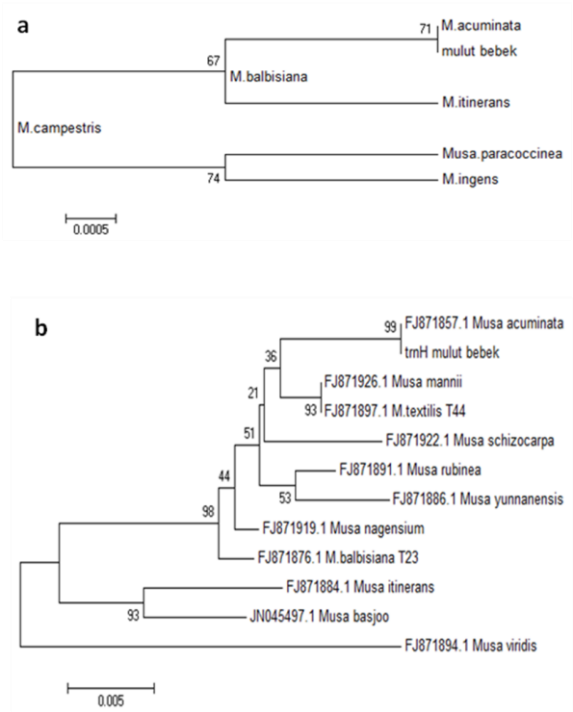

Fig. 1. *Mulu Bebe* Banana Cladogram

a. using rpoC gene sequences; b. using trnF-trnH intron

Phylogenetic tree (cladogram) of *Mulu Bebe* banana by rpoC gene sequences (Fig. 1a) posite *Mulu Bebe* taxa in the same position with *M. Acuminata* with a bootstrap value of 71. *Mulu Bebe* cladogram based on trnF-trnH intron sequences also posite *Mulu Bebe* same with *M. Acuminata* (bootstrap value of 99). According to Susan (2003), bootstrap values expressed the genetic relationship between the organisms. Thus the phylogeny of *Mulu Bebe* banana based on rpoC gene sequences and trnF-trnH intron determined 70% and 99% familial relationship with *M. Acuminata*.

Similarity Value banana mulu bebe well with markers rpoC and the trnF –trnH intron value 100% similarity with *M. Acuminata* (Table 1). This implied that it is possible that *Mulu Bebe* banana is varieties of *M. Cuminata*, with similarity matrix of rpoC gene ranging between 99.14 - 100% (Table 2.), while similarity matrix based on trnF-trnH intron ranged from 95.40-100% (Table 3).

Comparison between the results of molecular analysis of *Mulu Bebe* using rpoC gene and the trnF-trnH intron, with the results of phylogenetic reconstruction sequence with the reference sequence from NCBI showed that *Mulu Bebe* occupy the same taxa with *M. acuminata* with the similarity value of 100% (Fig.1; Table 2, 3). Therefore, we concluded that Mulu bebe banana is a variant of *M. acuminata*.

Table 2. Similarity matrix based on rpoC gene

	Musa paracoccinea	**Musa ingens**	**Musa intinerans**	**Musa campestris**	**Musa balbasiana**	**Musa acuminata**
Musa paracoccinea						
Musa ingens	99,57142741					
Musa itinerans	99,14039174	99,1403917				
Musa campestris	99,57142741	99,5714274	99,5714274			
Musa balbasiana	99,3561922	99,3562192	99,7860198	99,78602		
Musa acuminata	99,14039174	99,1403917	99,5714274	99,571427	99,78602	
Mulu bebe	99,14039174	99,1403917	99,5714274	99,571427	99,78602	100

Table 3. Similarity matrix based on trnF- trnH intron

	Musa acuminata	*Musa rubinea*	*Musa mannii*	*Musa yunnanensis*	*Musa nagensium*	*Musa chizocarpa*	*Musa itinerans*	*Musa Basjoo*	*Musa viridis*	*Musa Balbasiana*	*Musa textilis*
M.acuminata											
M.rubinea	98,92										
M. mannii	99,07	99,23									
M. yunnanensis	98,13	99,23	99,07								
M.nagensium	98,92	99,38	99,23	98,92							
M. chizocarpa	98,6	98,76	98,92	98,6	99,07						
M. itinerans	96,86	97,02	97,18	96,54	97,5	97,02					
M. basjoo	97,34	97,18	97,34	96,7	97,66	97,18	98,6				
M. viridis	95,4	95,73	96,38	95,89	96,21	95,56	96,38	96,38			
M. balbasiana	98,6	99,07	99,23	98,92	99,69	98,76	97,5	97,66	96,54		
M. textilis	99,07	99,23	100	99,07	99,23	98,92	97,18	97,34	96,38	99,23	
Mulu bebe	100	98,92	99,07	98,13	98,92	98,6	96,86	97,34	95,4	98,6	99,07

References

Baumgarthner BJ, Rapp JC, Mullet JE. 1993. Plastid genes encoding the transcription/translation apparatus are differentially transcribes early in barley (*Hordeum vulgare*) chloroplast development. Plant Physiology **101**, 781-791.

CBOL. 2009. CBOL approves *matK* and *rbcL* as the DNA barcode regions for land plants. Executive Committee, CBOL Proceedings of the National Academy of Science.

Chakravarthi BK, Nuravaneni R. 2006. SSR marker based DNA fingerprinting and diversity study ini rice (*Oryza sativa* L.). African Journal of Biotechnology **5** (9), 684-688.

Clegg MT, Gautt BS, Learn GHJr, Morton BR. 1994. Rates and pattern of chloroplast DNA evolution. Proceeding of National Academy of Science USA **91**, 6795-6801.

Clegg MT, Zurawski G. 1991. Chloroplast DNA and study of plant phylogeny. In: Soltis PS, Soltis DE, Doyle JJ, Eds. Molecular systematics of plants. New York: Champman and Hall.

De Jesus ON, de O. Silva S, Amorin EP, Ferreira CF, de Campos JMS, de G. Silva G, Figueira A. 2013. Genetic diversity and population structure of *Musa* accessions in ex situ conservation. BMC Plant Biology **13,** 41.

De Las Rivas J, Lozano JJ, Ortiz AR. 2002. Comparative analysis of chloroplast genomes: functional annotation, genome based phylogeny, and deduced evolutionary patterns. Genome Research **2 (30)**, 567-583.

Ferdous J, Hanafi MM, Rafi MY, Muhammad K. 2012. A quick DNA extraction protocol without liquid nitrogen in ambient temperature. African Journal of Biotechnology **11 (27),** 6956 – 6964

Hidayat Y, Indra HH, Miskat R, Syahbuddin H. 2012. Investigation on physical and morphology performance of *Mulu Bebe* Banana. North Maluku: BPTP.

Jonah PM, Bello LL, Lucky O, Midau A, Moruppa SM. 2011. Review: The importance of molecular markers in plant breeding programmes. Global Journal of Science Frontier Research **11 (5),** 1-9.

Judd WS, Campbell CS, Kellog EA, Stevens PF, Donoghue MJ. 2002. Plant Systematics: A Phylogenetic approach, 2nd Edition. Sunderland: Sinauer Associates.

Megia R. 2005. Musa as a genome model. Jurnal Hayati **12 (4)**, 167-170.

Purseglove JW. 1985. Tropical crops Monocotyledons. London: Logman.

Roy SW, Penny D. 2007. Patterns of intron loss and gain in plants: intron loss-dominated evolution and genome-wide comparison of *O. sativa* and *A. thaliana*. Molecular Biology evolution **24 (1),** 171-181.

Saski C, Seung-Bum L, Dniell H, Wood TC, Tomkins J, Hyi-Gyung K, Jansen RK. 2005. Complete chloroplast genome sequence of *Glycine max* and comparative analyses with other legume genome. Plant Molecular Biology **59**, 309–322.

Simpson MG. 2006. Plant systematics. Canada: Elsevier Academic Press.

Stover RH, Simmonds NW. 1987. Banana. 3rd Ed. London: Logman.

Sunyoto A. 2011. Cultivation of Cavendish Banana. Jogjakarta: Berlian Media.

Susan H. 2003. Bootstrapping Phylogenetic trees: theory and method. Statistical Science **18 (2)**, 241–255

Ubaidillah R, Sutrisno H. 2009. Introduction to Biosystematics theory and practices. Biology Research Center. Bogor: Indonesian Institute of Science.

Wahyuningtyas W, Retno A, Rahayu SE. 2009. Genetics diversity of B genome banana based on Microsatellite Markers. Biosaintifika **1 (1)**, 1-10.

Wolfe KH, Li WH, Sharp PM. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proceeding of National Academy of Science USA **84**, 9054-9058.