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Variation of *Musa* spp. in West Kalimantan, Indonesia, Based on *rbc*L Chloroplast DNA

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ABSTRACT

West Kalimantan is home to *Musa* spp. conservation and genetic assessment of wild banana relatives are important for future breeding purposes. The present study aims to evaluate the genetic relationship of *Musa* spp. in West Kalimantan by analyzing the *rbc*L chloroplast DNA using phylogenetic analysis. The methods in this study were sampling, DNA extraction, PCR of *rbc*L fragment, and data analysis. The specific primer was used to amplify the *rbc*L chloroplast DNA of ten accessions of *Musa* spp. in West Kalimantan. The results showed that the area of the *rbc*L region of *Musa* spp. in this study was estimated at 587-591 bp. It showed high variability with a conservation level A+T content of 56.95%. The *rbc*L sequences of *Musa* spp. have polymorphic sites on 13 numbers of nucleotides. The phylogenetic analysis with an ML algorithm of 35 *Musa* spp. from West Kalimantan and GenBank data was successfully divided into 4 main clades, and the bootstrap value was 80-81%. This study is expected beneficial for taxonomic, conservation, and banana breeding efforts.

1. Introduction

Zingiberales, an order of flowering plants, encompasses several families that possess significant economic and ornamental value. The families of Musaceae and Zingiberacea are highly regarded for their economic importance, while Heliconiaceae and Strelitziaceae are esteemed for their ornamental value (Kress and Specht 2006). Among these, Musaceae comprises two genera, namely *Musa* and *Ensente*, with Musa being the largest genus. The *Musa* genus is further classified into two sections: sect. *Musa* and sect. *Callimusa* (Häkkinen 2013). Indonesia boasts several species of wild bananas. West Java is home to eight wild banana species, and two new wild banana species were discovered on Sulawesi Island (Sulistyaningsih *et al.* 2014). Additionally, Hastuti *et al.* (2019) the discovery of eight wild banana species was reported in Sulawesi, while a new species, *Musa borneensis* var. donggalaensis, was confirmed after its discovery in Central Sulawesi (Sulistyaningsih 2017). Furthermore, West Kalimantan is known to have three species of wild banana i.e. *Musa campestris* var. *sarawakensis* (Sulistyaningsih and Irawanto 2011; Sunandar 2017; Sunandar and Kurniawan 2020), *Musa borneensis* var. *sarawakensis* (Sunandar and Kurniawan 2020).

The diversity of wild banana species in Indonesia is a valuable asset that can be leveraged to enhance the quality of cultivated bananas. In order to ensure the future of modern bananas, it is critical to support banana improvement through genomic research, which involves collecting and evaluating the diversity of wild banana relatives (Hapsari *et al.* 2020). These wild banana species are rich sources of resistance genes that offer protection against both biotic and abiotic stresses, as well as other desirable traits (Heslop-Harrison 2011). One notable example of a wild banana species that provides essential genetic resources for banana breeding is *M. balbisiana* Colla, which boasts cold and disease resistance (Wang *et al.* 2007).

Recent studies have demonstrated that molecular markers are the latest and most effective

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technique for identifying the genome composition and grouping of banana cultivars. By utilizing the DNA sequence from the chloroplast genome, such as *rbc*L, scientists have been able to identify the banana cultivar genome in *Musa troglodytarum, Musa acuminata*, and *Musa balbisiana* (Hiariej *et al.* 2015; Ainiyah *et al.* 2020; Hapsari *et al.* 2020). Most chloroplast genes evolve at a slower rate compared to nucleus genes and are considered the best genes for systematic studies (Steane 2005). The *rbc*L exon size, which is approximately 1,400 bp long, provides several characters that can be utilized in phylogenetic analysis (Hapsari *et al.* 2019). The universal primer *rbc*L exhibits a high conservation level and evolves at a slow rate, which is valuable for studies of genetic diversity and phylogenetics (Hollingsworth *et al.* 2009; Hapsari *et al.* 2020). The *rbc*L has also been related to the evolutionary process of environmental adaption and climate change (Hasegawa *et al.* 2009). The present study aims to evaluate the genetic relationship of *Musa* spp. in West Kalimantan by analysing the *rbc*L chloroplast DNA using phylogenetic analysis.

2. Materials and Methods

2.1. Sample Collection

Ten accessions of *Musa* spp. were collected from Mempawah Regency, Landak Regency, Kayong Utara Regency, Ketapang Regency, Melawi Regency, and Kapuas Hulu Regency. It consisted of three species i.e., *Musa acuminata, Musa campestris*, and *Musa borneensis*. Five accessions of *M. acuminata* were comprised of one variety. Two accessions of *M. campestris*. Three accessions of *M. borneensis* were comprised of three varieties (Table 1 and Figure 1). Twenty-four sequences of wild bananas were collected from Genbank. *Heliconia chartacea* (MK238301) was used as an outgroup, retrieved from Genbank. Young leaves of 10 accessions of *Musa* spp. were collected for DNA extraction.

2.2. DNA Extraction, Amplification, and Sequencing

The total genome of each sample was isolated from the leaves. Total genome extraction was used the Genomic DNA Mini Kit (Plant) protocol from

Table 1. Ten accessions of *Musa* spp. in West Kalimantan

Species name	Population locality	Morphological caracteristics
Musa acuminata subsp. *microcarpa*	Mempawah, West Kalimantan (1)	Petiole canal leaf wide with erect margins, male bud intermediate, bract color purple yellow externally, slim fruits, straight, curved shape, bottleneck apex, seed black, angular, wrinkled
Musa acuminata subsp. *microcarpa*	Mempawah, West Kalimantan (2)	Petiole canal leaf wide with erect margins, male bud intermediate, bract color purple yellow externally, fruits straight curved shape, bottleneck apex, seed black, angular, wrinkled
Musa acuminata subsp. *microcarpa*	Landak, West Kalimantan (1)	Petiole canal leaf wide with erect margins, male bud intermediate, bract color purple yellow externally, slim fruits, straight, curved shape, bottleneck apex, seed black, angular, wrinkled
Musa acuminata subsp. *microcarpa*	Landak, West Kalimantan (2)	Petiole canal leaf wide with erect margins, male bud intermediate, bract color purple yellow externally, fruits straight curved shape, bottleneck apex, seed black, angular, wrinkled
Musa acuminata subsp. *microcarpa*	Ketapang, West Kalimantan	Male bud intermediate, bract color purple yellow externally, fruits straight curved shape, bottleneck apex, seed brown, angular, wrinkled
Musa acuminata subsp. *microcarpa*	Kapuas Hulu, West Kalimantan	Leaf bases asymmetric; both sides rounded, petiole canal leaf straight with erect margin, petiole bases winged and corrugated auricles, male bud cordate, tinted with green color, bract color yellow externally, compound tepal upper part light green, seed obpyriform
Musa borneensis var. *alatuceae*	Kapuas Hulu, West Kalimantan	Leaf bases asymmetric; both sides rounded, petiole canal leaf straight with erect margin, petiole bases winged and corrugated auricles, male bud cordate, tinted with green color, bract color yellow externally, compound tepal upper part light green, seed obpyriform

Table 1. Continued

Species name	Population locality	Morphological caracteristics
Musa borneensis var. *lutea*	Kapuas Hulu, West Kalimantan	Leaf bases asymmetric; both sides pointed, petiole canal leaf straight with erect margin, petiole bases winged and corrugated auricles, male bud cordate, bract color yellow externally, seed obpyriform
Musa borneensis var. *phoenica*	Melawi, West Kalimantan	Petiole canal leaf straight with erect margin, petiole bases winged and corrugated auricles, male bud cordate, tinted with green color, bract color yellow externally, seed obpyriform
Musa campestris	Ketapang, West Kalimantan	Petiole bases winged with heavily wrinkled auricles, petiole canal leaf wide with erect margin, rachis position erect, male bud ovoid, bract color pink-purple externally, fruits straight, fruit apex blunt-tipped, seed cylindrical obpyriform
Musa campestris	Singkawang, West Kalimantan	petiole bases winged with heavily wrinkled auricles, petiole canal leaf wide with erect margin, rachis position erect, male bud ovoid, bract color pink-purple externally, fruits straight, fruit apex blunt-tipped, seed cylindrical obpyriform

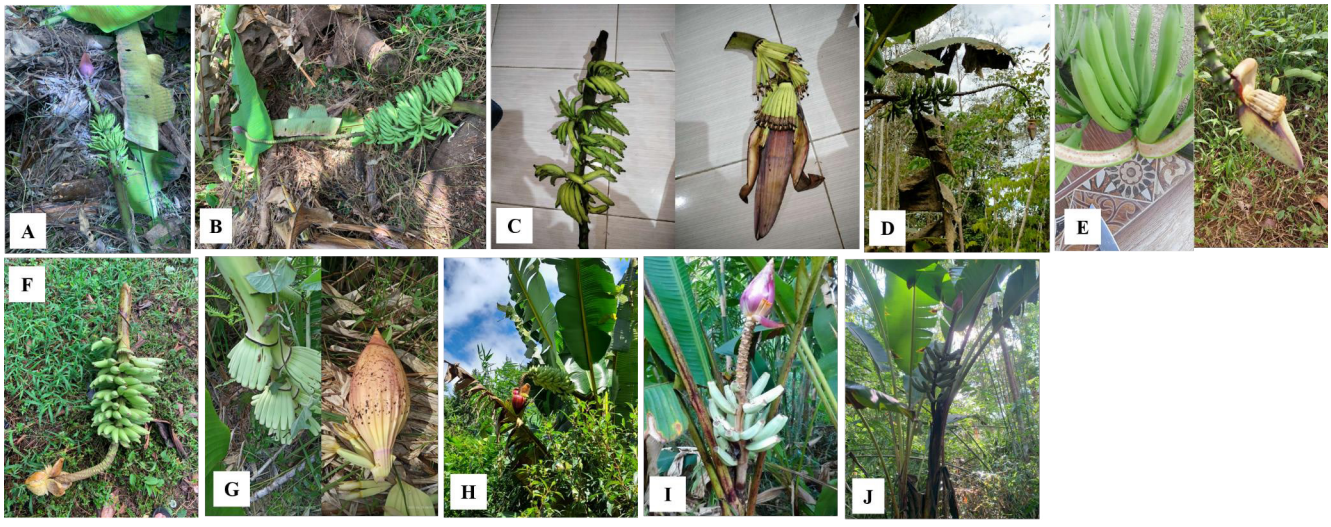

Figure 1. Morphological appearances of wild banana examined. (A) *Musa acuminata* subsp. *microcarpa* (Mempawah 1), (B) *Musa acuminata* subsp. *microcarpa* (Mempawah 2), (C) *Musa acuminata* subsp. *microcarpa* (Landak 1), (D) *Musa acuminata* subsp. *microcarpa* (Landak 2), (E) *Musa acuminata* (Ketapang), (F) *Musa borneensis* var. *alatuceae* (Kapuas Hulu), (G) *Musa borneensis* var. *lutea* (Kapus Hulu), (H) *Musa borneensis* var. *phoenica* (Melawi), (I) *Musa campestris* (Ketapang), (J) *Musa campestris* (Singkawang)

Geneaid. Amplification of *rbc*L gene sequences was conducted by PCR using *rbc*L forward primer (5'–ATG TCA CCA CAA ACA GAG ACT AAA GC–3') and *rbc*L reverse primer (5'– GTA AAA TCA AGT CCA CCR CG– 3') (Candramila *et al.* 2023). The PCR conditions consist of pre-denaturation at 95°C for 2 minutes, denaturation at 92°C for 1 minute, annealing at 50°C for 30 seconds, extension at 72°C for 1 minute, and post-extension at 72°C for 5 minutes. The denaturation, annealing, and DNA extension stages were repeated for up to 35 cycles. PCR products were performed by DNA electrophoresis process using 1% gel agarose. Sequencing of the *rbc*L gene was conducted by Sanger DNA Sequencing using capillary electrophoresis at Apical Scientific Laboratory, Malaysia. Each sample was sequenced with *rbc*L forward and reverse primers.

2.3. Data Analysis

The sequencing results were analyzed using MEGA11 software. Forward and Reverse sequences of each sample were aligned to get the contig sequences. The *rbc*L gene sequences of ten accession *Musa* spp. were aligned with the data in NCBI GenBank using Basic Local Alignment System Tools (BLAST). The taxonomic relationship between ten

*rbc*L gene sequences of *Musa* spp. in West Kalimantan and the GenBank data was analyzed. The *rbc*L gene sequences of research data and GenBank data were aligned using clustalW. The phylogenetic tree was performed using Maximum Likelihood (ML) analysis with a number of bootstrap applications of 1,000.

3. Results

3.1. Molecular Analysis

Visualization of PCR results in 1% agarose gel revealed a single band, indicating that the primers successfully amplified the *rbc*L fragment from the genome of ten *Musa* spp. The results showed that the fragment of the *rbc*L of *Musa* spp. in this study was estimated at 587-591 bp (Figure 2). All of the samples were good quality, with thick DNA bands, indicating the *rbc*L gene amplification processes of ten samples were successful. The current study's findings indicate that the use of universal primers (*rbc*L) is effective for amplification, identification, and discriminating of *Musa* spp. The nucleotide composition of the *rbc*L region in *Musa* spp. was high in A+T bases content. On average, the A+T base content of 34 *Musa* spp. was 56.95% (Table 2). The Single Nucleotide Polymorphism (SNP) detection was obtained from the *rbc*L sequences, 13 SNPs were detected at 1, 2, 3, 11, 12, 16, 71,141, 240, 285, 431, 519, 585 number of nucleotide (Table 3).

3.2. Phylogenetic Tree Analysis

Genetic relationship analysis of *Musa* spp. based on *rbc*L sequences using ML algorithm resulted in a tree which separated into 4 mains clades (Figure 3). *Musa acuminata* subsp. *microcarpa* from West Kalimantan has a bootstrap value of 81%, which is

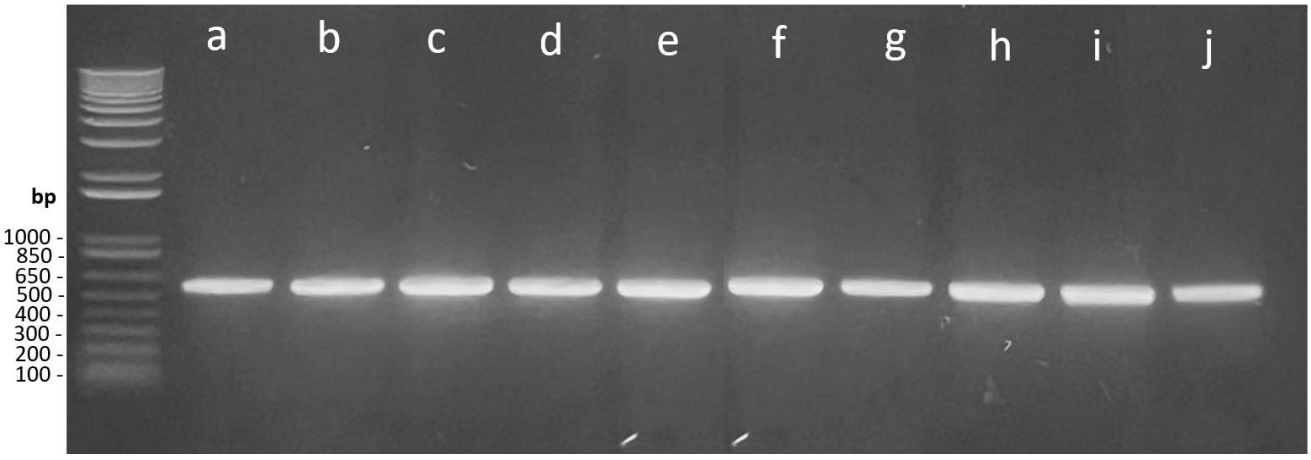

Figure 2. DNA amplification results of *rbc*L gene of ten accession of wild banana (a) *Musa borneensis* (Kapuas Hulu), (b) *Musa acuminata* subs. *microcarpa* (Ketapang), (c) *Musa borneensis* var. *phoenica* (Melawi), (d) *Musa borneensis* var. *lutea* (Kapuas Hulu), (e) *Musa acuminata* subs. *microcarpa* (Landak 1), (f) *Musa acuminata* subs. *microcarpa* (Landak 2), (g) *Musa acuminata* subs. *microcarpa* (Mempawah 1), (h) *Musa acuminata* subs. *microcarpa* (Mempawah 2), (i) *Musa campestris* (Ketapang), and (j) *Musa campestris* (Singkawang)

Table 2. Nucleotide composition of *rbc*L sequences of *Musa* spp.

Sample (origin/accession)	Nucleotide composition (%)				Total of nucleo tide
	T	C	A	G	
Musa borneensis (Kapuas Hulu)	28.81	20.34	27.97	22.88	590
Musa acuminata subs. *microcarpa* (Ketapang)	28.76	20.30	28.09	22.84	591
Musa borneensis var. *phoenica* (Melawi)	28.98	20.17	27.97	22.88	590
Musa borneensis var. *lutea* (Kapuas Hulu)	28.93	20.30	27.92	22.84	591
Musa acuminata subs. *microcarpa* (Landak 1)	28.76	20.47	27.92	22.84	591
Musa acuminata subs. *microcarpa* (Landak2)	28.64	20.51	27.97	22.88	590
Musa acuminata subs. *microcarpa* (Landak 3)	28.62	20.27	28.11	23.00	587
Musa acuminata subs. *microcarpa* (Landak 4)	28.76	20.47	27.92	22.84	591
Musa campestris (Ketapang)	28.98	20.51	27.63	22.88	590
Musa campestris (Singkawang)	28.93	20.47	27.75	22.84	591
Musa coccinea (MH603431)	29.03	20.03	28.01	22.92	589

Table 2. Continued

Sample (origin/accession)	Nucleotide composition (%)				Total of nucleotide
	T	C	A	G	
Musa balbisiana var. *balbisiana* cultivar Pisang Klutuk Wulung (NC039815)	29.15	20.00	27.97	22.88	590
Musa troglodytarum (OK323374)	29.15	20.00	27.97	22.88	590
Musa peekelii subsp. *angustigemma* (NC 058958)	29.03	20.03	28.01	22.92	589
Musa barioensis (NC 058955)	29.15	20.00	27.97	22.88	590
Musa borneensis (NC058952)	29.15	20.00	27.97	22.88	590
Musa beccarii (NC058953)	29.03	20.03	28.01	22.92	589
Musa cheesmanii (NC058950)	29.03	20.03	28.01	22.92	589
Musa ingens (OK012356)	29.15	20.00	27.97	22.88	590
Musa salaccensis (NC058934)	29.15	20.00	27.97	22.88	590
Musa textilis (NC022926)	29.15	20.00	27.97	22.88	590
Musa balbisiana (LC609623)	29.15	20.00	27.97	22.88	590
Musa aurantiaca (NC058957)	28.86	20.20	28.01	22.92	589
Musa lolodensis (OK012350)	29.03	19.86	28.18	22.92	589
Musa ornata (OK012345)	28.98	20.17	27.97	22.88	590
Musa schizocarpa (NC 058932)	28.98	20.17	27.97	22.88	590
Musa yunnanensis (OK012327)	28.98	20.17	27.97	22.88	590
Musa acuminata subsp. *halabanensis* (NC 058945)	28.86	20.03	28.18	22.92	589
Musa rubra (NC058936)	28.86	20.03	28.18	22.92	589
Musa acuminata subsp. *truncata* (NC058928)	28.86	20.03	28.18	22.92	589
Musa acuminata var. *zebrina* (NC058925)	28.98	20.00	28.14	22.88	590
Musa laterita (NC056828)	28.86	20.03	28.18	22.92	589
Musa acuminata subsp. *microcarpa* (LC609621)	28.86	20.03	28.18	22.92	589
Musa acuminata subsp. *malaccensis* strain Doubled-haploid Pahang (HG996477)	28.86	20.03	28.18	22.92	589
Heliconia chartacea (MK238301)	28.55	20.41	27.96	23.08	676
Avg.	28.94	20.15	28.01	22.90	592.17

Table 3. Nucleotide polymorphic site of *Musa* spp.

Sample (origin/accession)	Number of nucleotide												
	1	2	3	11	12	16	71	141	240	285	431	519	585
Musa borneensis var. *lutea* (Kapuas Hulu)	-	T	A	C	A	A	C	T	T	A	C	T	C
Musa acuminata subs. *microcarpa* (Ketapang)	T	T	A	C	A	A	A	T	T	A	C	C	C
Musa borneensis var. *phoenica* (Melawi)	T	T	A	-	A	A	C	T	T	A	C	T	C
Musa borneensis var. *alatuceae* (Kapuas Hulu)	T	T	A	C	A	A	C	T	T	A	C	T	C
Musa acuminata subs. *microcarpa* (Landak 1)	T	T	A	C	C	A	A	T	T	A	C	C	C
Musa acuminata subs. *microcarpa* (Landak 2)	-	T	A	C	C	A	A	T	T	A	C	C	C
Musa acuminata subs. *microcarpa* (Mempawah 1)	-	-	-	-	A	A	A	T	T	A	C	C	C
Musa acuminata subs. *microcarpa* (Mempawah 2)	T	T	A	C	C	A	A	T	T	A	C	C	C
Musa campestris (Ketapang)	T	T	A	C	C	-	C	T	T	A	C	T	C
Musa campestris (Singkawang)	T	T	A	C	C	A	C	T	T	A	C	T	C
Musa coccinea (MH603431)	-	T	A	-	A	A	C	T	T	A	C	T	T
Musa balbisiana var. *balbisiana* cultivar Pisang Klutuk Wulung (NC039815)	T	T	A	-	A	A	C	T	T	A	C	T	T
Musa troglodytarum (OK323374)	T	T	A	-	A	A	C	T	T	A	C	T	T
Musa peekelii subsp. *angustigemma* (NC 058958)	-	T	A	-	A	A	C	T	T	A	C	T	T
Musa barioensis (NC 058955)	T	T	A	-	A	A	C	T	T	A	C	T	T
Musa borneensis (NC058952)	T	T	A	-	A	A	C	T	T	A	C	T	T
Musa beccarii (NC058953)	-	T	A	-	A	A	C	T	T	A	C	T	T
Musa cheesmanii (NC058950)	-	T	A	-	A	A	C	T	T	A	C	T	T
Musa ingens (OK012356)	T	T	A	-	A	A	C	T	T	A	C	T	T

Table 3. Continued

Sample (origin/accession)	Number of nucleotide												
	1	2	3	11	12	16	71	141	240	285	431	519	585
Musa salaccensis (NC058934)	T	T	A	-	A	A	C	T	T	A	C	T	T
Musa textilis (NC022926)	T	T	A	-	A	A	C	T	T	A	C	T	T
Musa balbisiana (LC609623)	T	T	A	-	A	A	C	T	T	A	C	T	T
Musa aurantiaca (NC058957)	-	T	A	-	A	A	C	T	T	A	C	C	T
Musa lolodensis (OK012350)	-	T	A	-	A	A	A	T	T	A	C	T	T
Musa ornata (OK012345)	T	T	A	-	A	A	C	T	T	A	C	C	T
Musa schizocarpa (NC 058932)	T	T	A	-	A	A	C	T	T	A	C	C	T
Musa yunnanensis (OK012327)	T	T	A	-	A	A	C	T	T	A	C	C	T
Musa acuminata subsp. *halabanensis* (NC 058945)	-	T	A	-	A	A	A	T	T	A	C	C	T
Musa rubra (NC058936)	-	T	A	-	A	A	A	T	T	A	C	C	T
Musa acuminata subsp. *truncata* (NC058928)	-	T	A	-	A	A	A	T	T	A	C	C	T
Musa acuminata var. *zebrina* (NC058925)	T	T	A	-	A	A	A	T	T	A	C	C	T
Musa laterita (NC056828)	-	T	A	-	A	A	A	T	T	A	C	C	T
Musa acuminata subsp. *microcarpa* (LC609621)	-	T	A	-	A	A	A	T	T	A	C	C	T
Musa acuminata subsp. *malaccensis* strain Doubled-haploid Pahang (HG996477)	-	T	A	-	A	A	A	T	T	A	C	C	T

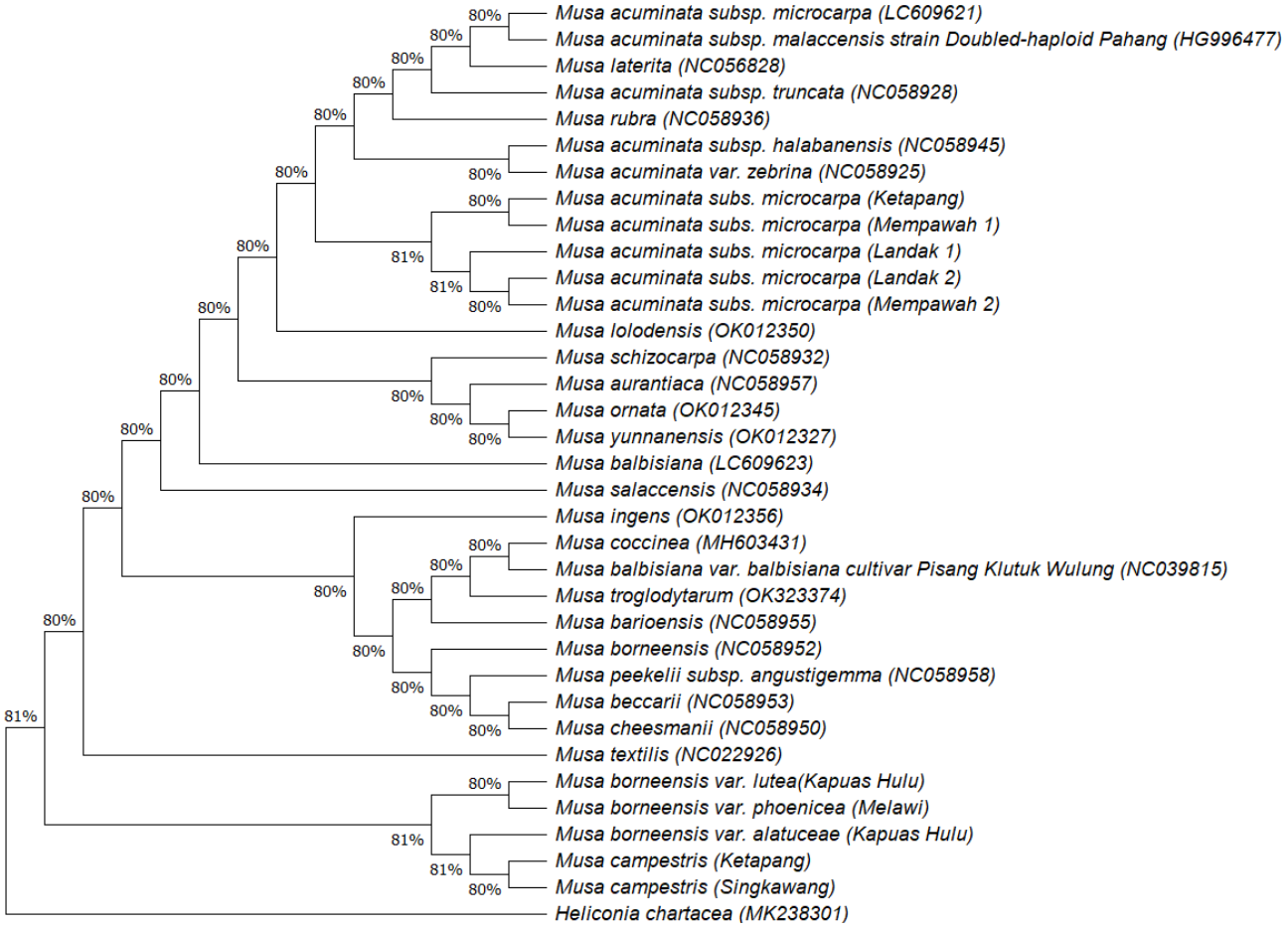

Figure 3. Phylogenetic tree of 10 sequences of *Musa* spp. in this study and 24 sequences of *Musa* spp. based on *rbc*L marker from GenBank using ML algorithm

divided into two groups. The first group, namely *Musa acuminata* subsp. *microcarpa* from Ketapang and Mempawah 1 district. The second group consists of Musa acuminata subsp. *microcarpa* from Landak 1, Landak 2, and Mempawah 2 districts. *Musa borneensis* and *Musa campestris* from West Kalimantan has a bootstrap value of 81% which is divided into two groups, the first group namely *Musa borneensis* var. *lutea* (Kapuas Hulu) and *Musa borneensis* var. *phoenica* (Melawi). Interestingly, the second group consists of *Musa borneensis* var. *alatuceae* (Kapuas Hulu) and *Musa campestris* (Ketapang and Singkawang), which, based on morphological characters, are two different species (Table 1, Figure 1 and 3).

4. Discussion

The results showed that the area of the *rbc*L region *Musa* spp. in this study was estimated at 590 bp length (Figure 1). Ainiyah *et al.* (2020) reported the sequence length of *rbc*L in *Musa* spp. was 743 bp. Similar results were reported by Hapsari *et al.* (2020), who stated that the *rbc*L region band size of *Musa* spp. was 713 to 723 bp. The full length of *rbc*L sequences is 1,400 bp (Newmaster *et al.* 2006). The current study's findings indicated the use of universal primers (*rbc*L) is effective for amplification, identification, and discriminating of *Musa* spp. The *rbc*L primer system for banana identification is a reliable and rapid technique (Ainiyah *et al.* 2020).

The nucleotide composition of the *rbc*L region in *Musa* spp. was high in A+T bases content. On average, the A+T base content of 34 *Musa* spp. was 56.95% (Table 2). A similar result was also obtained by nucleotide composition, which was higher in AT (56.99%) than in GC (43.01%) in wild bananas. Chloroplast genes (including *rbc*L) are dominated by a genomic bias towards a higher AT than GC. The average AT nucleotide composition for *rbc*L *Musa acuminata* was found higher than GC content (Martin *et al.* 2013). The possible explanation of higher AT contents than GC contents in both markers could be due to high variability in nucleotide composition and higher nucleotide substitution rate in these genes (Ismail *et al.* 2020).

Thirteen SNPs were detected at 1, 2, 3, 11, 12, 16, 71,141, 240, 285, 431, 519, and 585 number of nucleotide (Table 3). The SNPs were also detected in *Musa textilis* (Barbosa *et al.* 2023). Several studies have shown that the SNPs and InDels are highly abundant and present throughout the genome in various species, including plants (Nasu *et al.* 2002). SNP genotyping is a valuable tool for gene mapping, map-based cloning, and marker-assisted selection (MAS) in crops (Hayashi *et al.* 2004). Singleton variation sequences unique to individual species could be considered as identifying barcodes (Hapsari *et al.* 2018).

In this study, the phylogenetic analysis of the ingroup species inferred by *rbc*L sequences data showed moderate (80-81) (Figure 3). The higher the bootstrap values (70-100), the greater the confidence in the phylogenetic trees, but the lower the bootstrap values, the greater the chance of branching rearrangement (Hapsari *et al.* 2019). The *rbc*L sequences were highly conserved with low parsimony informative, but it is powerful to separate the dataset at families, genera, and species level, and suggested that it is moderately suitable to separate within species (intraspecies) level of wild bananas (Hapsari *et al.* 2020). In this study, *Musa borneensis* var. alatuceae and *Musa campestris* from West Kalimantan are in the same clade, both are separated with a bootstrap value of 81%. This occurs because of the differences of a single nucleotide in the 12th nucleotide (A -> C). Based on morphological characters, *Musa borneensis* var. *alatuceae* and *Musa campestris* are two different species. *Musa borneensis* var. *alatuceae* has leaf bases asymmetric, both sides rounded, petiole canal leaf straight with erect margin, petiole bases winged and corrugated auricles, male bud cordate, tinted with green color, bract color yellow externally, compound tepal upper part light green, seed obpyriform (Table 1 and Figure 1). However, *Musa campestris* has petiole bases winged with heavily wrinkled auricles, a petiole canal leaf wide with an erect margin, a rachis position erect, a male bud ovoid, bract color pink-purple externally, fruit straight, fruit apex blunt-tipped, seed cylindrical obpyriform (Table 1 and Figure 1).

Indonesia has a diverse range of banana pathogens, including Panama wilt (*Fusarium oxysporum* var. *cubesence*), Sigatoka (*Mycospaerella musicola*), *Pseudomonas* sp., nematodes, and viruses (Banana Bunchy Top Virus/BBTV, Banana Streak Virus/BSV, Cucumber Mosaic Virus/BMV, and Banana Bract Mosa) (Poerba *et al.* 2017). The disease-resistant banana varieties are one method for controlling disease in bananas. Banana breeding

focuses on disease-resistant triploid hybrids due to their superior vigor and yield. The wild bananas in this study are valuable genetic resources with high potential. Especially, *Musa acuminata* has the potential to be used in banana breeding programs (Poerba *et al.* 2019). *Musa acuminata* provides a genetic source of disease resistance (Sutanto *et al.* 2014; Fraser-Smith *et al.* 2016). *Musa acuminata* was selected as the male parent to produce disease-resistant triploid hybrids. *Musa acuminata* var. *malaccensis* was employed as a male parent to create disease-resistant triploid hybrids (Poerba *et al.* 2017). Another potential of *Musa acuminata* is medicine (Begashaw *et al.* 2023). However, there is no information regarding the use of *Musa borneensis* and *Musa campestris*. In Indonesia, the potential uses of wild banana species have yet to be fully explored (Hapsari 2014).

The results showed that the area of the *rbc*L region *Musa* spp. in this study was estimated at 587-591 bp length. It showed high variability with a conservation level A+T content of 56.95%. The *rbc*L sequences of *Musa* spp. have polymorphic sites on 13 numbers of nucleotides. The phylogenetic analysis with ML algorithm of 35 *Musa* spp. from West Kalimantan and GenBank data was successfully divided into 4 main clades, and the bootstrap value is moderate (80-81).

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