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Phylogenetic analysis of wild bananas (*Musa* spp.) in West Kalimantan, Indonesia, based on *maturase* K (*mat*K) genes

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Abstract. *Fajri H, Sunandar A, Qurbaniah M. 2024. Phylogenetic analysis of wild bananas* (Musa *spp.*) *in West Kalimantan, Indonesia, based on* maturase *K* (mat*K*) *genes. Biodiversitas 25: 2612-2618.* West Kalimantan is home to wild bananas (*Musa* spp.). Genetic assessment of wild banana relatives is important for future breeding purposes. This study aims to analyze the phylogenetic relationship of wild banana species in West Kalimantan based on *maturase* K (*mat*K) gene sequence data to understand better the relationship among wild banana species in West Kalimantan. A total of 30 samples, including 23 *mat*K sequences from GenBank, were used. Species of *Heliconia schiedeana* were incorporated as an outgroup. The phylogenetic analysis was conducted using the Maximum Likelihood (ML) algorithm in MEGA 11 application. The size of PCR-amplified *mat*K is estimated at 540-561 bp. It showed high variability with conservation level A+T, with an average value was 66.26%. Some unique nucleotides were found in *Musa acuminata* subsp. *microcarpa*, *Musa campestris*, and *Musa borneensis*. The *mat*K sequences of *Musa* spp. have a polymorphic site on 23 nucleotide numbers. The phylogenetic analysis with ML algorithm of 30 *Musa* spp. from West Kalimantan and GenBank data was successfully divided into two main clades in 62-69 bootstrap value. Our study showed that *mat*K genes are able to describe the differences in *M. acuminata*, *M. campestris*, and *M. borneensis* in West Kalimantan. This study will benefit taxonomic, genetic conservation strategy, and banana breeding efforts. It will provide valuable insights and tools for these fields, thereby contributing to the conservation and breeding of bananas.

Keywords: *Mat*K gene, wild banana genetic diversity, wild banana in West Kalimantan, wild banana phylogeny

INTRODUCTION

The Zingiberales order of flowering plants includes several economically and ornamentally valuable families. Musaceae and Zingiberaceae are valued for their economics, while Heliconiaceae and Strelitziaceae are valued for their beauty (Kress and Specht 2006). Musaceae has two genera, *Musa* and *Ensente*, with *Musa* being the biggest. *Musa* is divided into sects: *Musa* and *Callimusa* (Häkkinen 2013). Several wild banana species grow in Indonesia. Sulistyaningsih (2016) reported eight wild banana species in West Java and two on Sulawesi Island (Sulistyaningsih et al. 2014). Hastuti et al. (2019) found eight wild banana species in Sulawesi, while *Musa borneensis* var. *donggalensis*. was found in Central Sulawesi (Sulistyaningsih 2017). West Kalimantan has three wild banana species (Sulistyaningsih and Irawanto 2011; Sunandar 2017; Sunandar and Kurniawan 2020).

The diversity of wild banana species in Indonesia can improve cultivated banana quality. Genomic research on wild banana ancestors is essential to the future of modern bananas (Hapsari et al. 2020). Wild banana species include several resistance genes that defend against biotic and abiotic stressors and other beneficial features (Heslop-Harrison 2011). *Musa balbisiana*, a cold and disease-resistant wild banana species, supplies genetic resources for banana breeding (Wang et al. 2007). According to Ravi et al. (2013), it has been observed that the majority of *M. balbisiana* banana cultivars exhibit a higher level of tolerance to drought conditions compared to *M. acuminata* cultivars.

Therefore, elucidating the taxonomy and phylogeny of wild bananas was critical since it can provide useful information for collection and characterization for future improvement (Ardiyani et al. 2023). Despite being at the center of banana origin and variety, there have been few systematic studies to uncover the taxonomy and phylogeny of wild bananas in Indonesia. Borneo Island is one of Indonesia's plant biodiversity hotspots, including bananas, although comprehensive research into the plant's diversity, potential, and genetic resources is still quite restricted. The identification and characterization of wild banana species in Borneo Island, especially in West Kalimantan, are very urgent for many purposes, such as genetic conservation, breeding programs, and elucidation of socio-economic and cultural significance, while the genetic erosion due to land conversion, mining, and forest fire is increasing in recent years. The genetic links between wild, local, and commercially grown bananas are particularly fascinating to disclose the genetic transfer and exchange among them and to look for the contribution of the wild cultivars to the commercially developed cultivars (Sunaryo et al. 2020).

The molecular markers are the latest method for identifying and categorizing banana cultivars by genomic

makeup. The molecular approach, based on DNA sequence, is more accurate and efficient than morphological and PCR-based methods (Probojati et al. 2021). Banana cultivar genomes have been identified using DNA sequences from chloroplast genomes, including *rbc*L, *trn*K intron, *trn*L-F, and *mat*K (*maturase* K) (Wahyudi et al. 2013; Bieniek et al. 2014; Janssens et al. 2016; Nikmah et al. 2016; Udensi et al. 2017; Hariyanto et al. 2021; Probojati et al. 2021). Chloroplast DNA is inherited from the maternal and is stable and non-recombinant (Costion et al. 2011; Yuan et al. 2015; Shekhar et al. 2019). Several researches have been done on the *mat*K gene-based molecular systematics in plants as a possible method to identify species and assess genetic diversity (Shekhar et al. 2019).

The *mat*K is a gene found in the chloroplast genome of plants. It exhibits little genetic recombination and is solely inherited maternally, making it useful for studying an organism's evolutionary history (Wang et al. 2013). The total length of the *mat*K sequence is estimated to be roughly 1,570 base pairs (bp), and it is situated inside the plastid genome. Specifically, it is found within the intron region of the *trn*K gene (Harnelly et al. 2018). The *mat*K gene is commonly used for plant identification due to its efficacy, slow mutation rate, and higher accuracy than other genes (Probojati et al. 2021). Several prior investigations have documented the utilization of the *mat*K marker in investigating genetic differences in several plant families, including Dipterocarpaceae (Harnelly et al. 2018), Solanaceae and Euphorbiaceae (Wattoo et al. 2016), Acanthaceae (Arif et al. 2019), Liliopsida (Kolondam 2015), Orchidaceae (Barthet et al. 2015), Fabaceae (Sil et al. 2021) and Poaceae (Osman and Ramadan 2019). The *mat*K gene successfully reconstructed phylogenetic trees in banana cultivars and is sufficient to discriminate at the intraspecific level of desert bananas (Hariyanto et al. 2021; Probojati et al. 2021). The *mat*K gene sequence has not been used to study the genetic relationship of wild bananas in West Kalimantan.

This study aims to analyze the phylogenetic relationship of wild banana species in West Kalimantan based on *maturase* K (*mat*K) gene sequence data to understand better the relationship among wild banana species in West Kalimantan. Results from this study are expected to benefit taxonomic, genetic conservation strategy, and banana breeding efforts.

MATERIALS AND METHODS

Sample collection

Seven accessions of *Musa* spp. were collected from Mempawah Regency, Landak Regency, Singkawang Regency, Ketapang Regency, and Kapuas Hulu Regency, Indonesia. It consisted of three species, i.e., *Musa acuminata*, *Musa campestris*, and *Musa borneensis*. Four accessions of *M. acuminata* comprised one and one unidentified variety (Table 1). One accessions of *M. campestris*. One species of *M. borneensis* var. *lutea* (Table 1). Twenty-two sequences of wild bananas were collected from Genbank. *Heliconia schiedeana* (OQ289825.1) was retrieved from Genbank as an outgroup. Young leaves of 7 accessions of *Musa* spp. were collected for DNA extraction.

DNA extraction, amplification, and sequencing

The total genome of each sample was isolated from the leaves. Total genome extraction was used in the Genomic DNA Mini Kit (Plant) protocol from Geneaid. Amplification of matK gene sequences was conducted by PCR using universal matK forward primer (5'- `CTA CAT ATC CGG CCA AAT CG` -3') and matK reverse primer (5'- `CTT CTC ATT TGC GAT CAA CAT C` -3'). The PCR conditions consist of pre-denaturation at 95°C for 2 minutes, denaturation at 92°C for 1 minute, annealing at 53.5°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 product examination was performed by DNA electrophoresis process using 1% agarose gel. Sequencing of matK genes was conducted by Sanger DNA Sequencing using capillary electrophoresis at Apical Scientific Laboratory, Malaysia. Each sample was sequenced with both matK forward and reverse primers.

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 *matK* gene sequences of seven accession *Musa* spp. were aligned with the data in NCBI GenBank using Basic Local Alignment System Tools (BLAST). The taxonomic relationship was analyzed between seven *mat*K gene sequences of *Musa* spp. in West Kalimantan and the GenBank data. The *mat*K gene sequences of research and GenBank data were aligned using clustalW. The phylogenetic tree was performed using Maximum Likelihood (ML) analysis with the number of bootstrap applications was 1,000.

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

Species name	Population locality	Sampling coordinate
Musa acuminata subsp. *microcarpa*	Mempawah, West Kalimantan (1)	0021’14.3"N 109015’51.5"E
Musa acuminata subsp. *microcarpa*	Mempawah, West Kalimantan (2)	0021’14.3"N 109015’51.5"E
Musa acuminata subsp. *microcarpa*	Landak, West Kalimantan (1)	0°19'54.0"N 109°24'22.9"E
Musa acuminata subsp. *microcarpa*	Landak, West Kalimantan (2)	0°19'10.4"N 109°22'30.1"E
Musa acuminata	Ketapang, West Kalimantan	1°15'16.7"S 110°31'42.0"E
Musa borneensis var. *lutea*	Kapuas Hulu, West Kalimantan	0°50'11.2"N 112°57'45.5"E
Musa campestris	Singkawang, West Kalimantan	0°51'54.2"N 109°02'27.3"E

RESULTS AND DISCUSSION

Molecular analysis

Visualization of PCR results in 1% agarose gel revealed a single band, indicating that the primers successfully amplified the *mat*K fragment from the genome of seven *Musa* spp. The results showed that the fragment length of the *mat*K of *Musa* spp. in this study was estimated at 540 bp (Figure 1). All of the samples were good quality, with thick DNA bands, indicating that seven samples' *mat*K genes amplification processes were successful. The matK gene sequences within *M. acuminata* cultivars were estimated at 724 to 731 bp (Hariyanto et al. 2021), and wild *Musa* spp. was estimated at 720-950 bp (Li et al. 2013). The *mat*K gene sequences of *Musa* spp. from West Kalimantan were estimated at 540-561 bp.

The difficulty in PCR amplification and sequencing in the *mat*K area has resulted in developing novel taxon-specific primers or modificatying to existing primers (Heckenhauer et al. 2016). The current study's findings indicate that the newly designed *mat*K primer effectively amplifies wild bananas on *M. acuminata* subsp. *microcarpa*, *M. borneensis*, and *M. campestris* with a 100% success rate. The *mat*K genes found in the chloroplast genome were very variable and can be used to identify and verify species (Roslim et al. 2016). The *mat*K marker can distinguish the species level of Indonesian banana cultivars and their wild relatives (Ardiyani et al. 2023). The *mat*K gene barcoding method is also a powerful tool for identifying inter-genera and inter-specific palms (Abbas et al. 2020).

The nucleotide composition of *the matK* region in *Musa* spp. was high in A+T base content. On average, the A+T base content of seven accessions of wild bananas was 66.26% (Table 2). The *mat*K gene, as a coding region or exon, was known to have a high AT base concentration because of its roles in transcription and protein translation. It is thought to aid in splicing seven separate chloroplast group IIA introns found within precursor RNAs for critical chloroplast function (Barthet et al. 2015). Low GC concentration indicates low rates of mutation and recombination. Mutation or loss of the *mat*K gene, the primary target for *mat*K protein activity, resulted in a co-evolutionary decrease (Barthet et al. 2015). However, *M. acuminata* cv. AAA group and pisang kates (*Musa* cv. ABB) have higher GC content (Hariyanto et al. 2021; Hapsari et al. 2022). DNA sequences with higher GC content are mutation hotspots; the C base is often methylated and causes errors during multiplication (Hapsari et al. 2018).

The Single Nucleotide Polymorphism (SNP) detection was obtained from the *mat*K sequences, 23 SNPs were detected at nucleotides number 9,11, 14, 20,73, 105, 118, 124, 158, 215, 303, 365, 368, 380, 383, 384, 393, 413, 414, 422, 428, 445, 452 of our amplified fragments (Table 3). Previous study was reported that *Musa* spp. have 13 polymorphic sites based on *rbc*L chloroplast marker (Sunandar et al. 2024). This indicated that the *mat*K marker is more comprehensive to use as DNA barcoding for *Musa* spp. because the very high variability in SNPs. SNPs are commonly employed for genetic mapping, evolutionary studies, genome-wide association studies, and detecting genetic diversity (Du et al. 2019; Guo et al. 2019; Liu et al. 2019). SNPs have a significant role in phenotypic variation and disease resistance by affecting protein structure and function, transcription factor binding affinity, and alternative splicing (Mustilli et al. 1999; Zhou et al. 2012; Wang et al. 2015; Guo et al. 2018). In *Musa* spp., AAA group, most SNPs strongly linked with bunch weight and its component attributes were grouped on chromosome 3 (Nyine et al. 2019).

Phylogenetic tree analysis result

The study of phylogenetic relationships is fundamental to the study of biological evolution. Evolution is the slow process by which an organism accumulates changes over many generations, making simple species more complex. Phylogenetic analysis is performed by building an evolutionary history and determining the relationship between progenitors and ancestors using character similarity as a benchmark. The MEGA 11 program and the Maximum Likelihood (ML) method created the phylogenetic tree. Constructing a phylogenetic tree aims to understand the relationships between different *Musa* spp. species. The phylogenetic tree was statistically tested with 1,000 replications using the bootstrap method.

Genetic relationship analysis of *Musa* spp. based on *mat*K fragment sequences using the ML algorithm, a tree separated into 2 main clades (Figure 2). Clade 1 comprises *M. acuminata* subsp. *microcarpa* (Mempawah 1, Mempawah 2, Landak 1, and Landak 2) and *M. acuminata* (Ketapang) with a bootstrap value of 68%. Clade II comprises *M. campestris* (Ketapang) has a bootstrap value of 69%, and *M. borneensis* var. *lutea* (Kapuas Hulu) has a bootstrap value of 62% (Figure 2). The number of times the branching pattern appears at a node in the original tree and causes the bootstrap value indicates repeats. A high degree of confidence in the node can be inferred if the bootstrap value is more than 95% (Dharmayanti 2011). All *M. acuminata* species are in a branch, indicating that five species are very close based on the *mat*K gene fragment (Figure 2). Based on the nucleotide sequences, the species of *M. acuminata* from Ketapang differ from other *M. acuminata*. The difference was because of two substitutions: A to T on the 9th nucleotide and T to C on the 11th nucleotide (Table 3). *Musa borneensis* var. *lutea* from Kapuas Hulu on a branch with *M. borneensis* (NC 058955.1) from NCBI indicated that the two species are very close based on the *mat*K gene fragment (Figure 2).

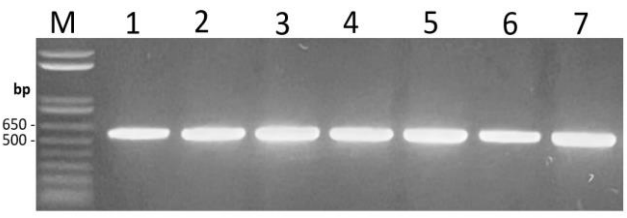

Figure 1. DNA amplification results of *mat*K genes fragment seven accessions of wild bananas from West Kalimantan. M: 1 kb DNA ladder; 1. *Musa acuminata* subsp. *microcarpa*, Mempawah 1; 2. *Musa acuminata* subsp. *microcarpa* Mempawah 2; 3. *Musa acuminata* subsp. *microcarpa*, Landak 1; 4. *Musa acuminata* subsp. *microcarpa*, Landak 2; 5. *Musa acuminata*, Ketapang; 6. *Musa borneensis* var. *lutea*, Kapuas Hulu; 7. *Musa campestris*, Singkawang

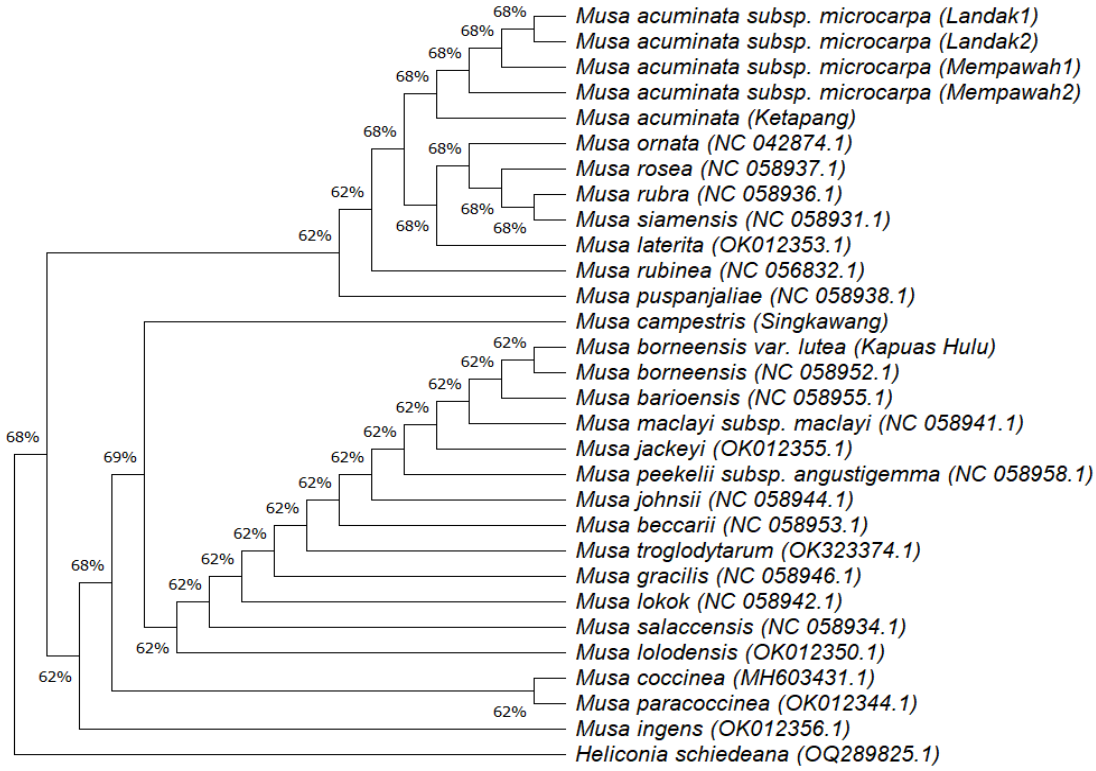

Figure 2. Phylogenetic tree of 7 sequences of *Musa* spp. in this study and 22 sequences of *Musa* spp. based on *mat*K marker from GenBank using ML algorithm

Table 2. Nucleotide composition of *mat*K sequences of *Musa* spp.

Species	T(U)	C	A	G	A+T	G+C	Total
Musa acuminata subsp. *microcarpa* (Landak1)	29.41	17.47	36.36	16.76	65.78	34.22	561
Musa acuminata subsp. *microcarpa* (Landak2)	29.52	16.97	36.53	16.97	66.05	33.95	542
Musa acuminata subsp. *microcarpa* (Mempawah1)	29.39	17.01	36.60	17.01	65.99	34.01	541
Musa acuminata subsp. *microcarpa* (Mempawah2)	29.26	17.22	36.48	17.04	65.74	34.26	540
Musa campestris (Singkawang)	29.34	17.34	37.27	16.05	66.61	33.39	542
Musa acuminata (Ketapang)	29.39	17.01	36.78	16.82	66.17	33.83	541
Musa borneensis (Kapuas Hulu)	28.97	17.34	36.90	16.79	65.87	34.13	542
Musa barioensis (NC 058955.1)	29.18	17.96	37.14	15.71	66.33	33.67	490
Musa troglodytarum (OK323374.1)	29.39	17.96	37.14	15.51	66.53	33.47	490
Musa peekelii subsp. *angustigemma* (NC 058958.1)	29.39	17.96	37.14	15.51	66.53	33.47	490
Musa beccarii (NC 058953.1)	29.39	17.96	37.14	15.51	66.53	33.47	490
Musa borneensis (NC 058952.1)	28.98	18.16	37.14	15.71	66.12	33.88	490
Musa gracilis (NC 058946.1)	29.39	17.96	37.14	15.51	66.53	33.47	490
Musa jackeyi (OK012355.1)	29.39	17.96	37.14	15.51	66.53	33.47	490
Musa lokok (NC 058942.1)	29.39	17.96	37.14	15.51	66.53	33.47	490
Musa maclayi subsp. *maclayi* (NC 058941.1)	29.39	17.96	37.14	15.51	66.53	33.47	490
Musa salaccensis (NC 058934.1)	29.39	17.96	37.14	15.51	66.53	33.47	490
Musa lolodensis (OK012350.1)	29.39	17.96	36.94	15.71	66.33	33.67	490
Musa johnsii (NC 058944.1)	29.39	17.96	37.14	15.51	66.53	33.47	490
Musa coccinea (MH603431.1)	28.70	17.59	37.41	16.30	66.11	33.89	540
Musa paracoccinea (OK012344.1)	28.98	18.16	37.14	15.71	66.12	33.88	490
Musa ingens (OK012356.1)	29.39	17.76	37.96	14.90	67.35	32.65	490
Musa puspanjaliae (NC 058938.1)	29.39	17.76	36.53	16.33	65.92	34.08	490
Musa ornata (NC 042874.1)	29.26	17.04	36.85	16.85	66.11	33.89	540
Musa laterita (OK012353.1)	29.26	17.04	36.85	16.85	66.11	33.89	540
Musa rosea (NC 058937.1)	29.26	17.04	36.85	16.85	66.11	33.89	540
Musa rubra (NC 058936.1)	29.26	17.04	36.85	16.85	66.11	33.89	540
Musa siamensis (NC 058931.1)	29.26	17.04	36.85	16.85	66.11	33.89	540
Musa rubinea (NC 056832.1)	29.59	17.76	36.33	16.33	65.92	34.08	490
Heliconia schiedeana (OQ289825.1)	37.20	17.96	29.04	15.80	66.24	33.76	785
Average	29.69	17.60	36.56	16.15	66.26	33.74	522.47

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

Species	Number of nucleotide																						
	9	**11**	**14**	**20**	**73**	**105**	**118**	**124**	**158**	**215**	**303**	**365**	**368**	**380**	**383**	**384**	**393**	**413**	**414**	**422**	**428**	**445**	**452**
Musa acuminata var. *microcarpa* (Landak1)		T	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	T	G	T	A
Musa acuminata var. *microcarpa* (Landak2)	A	T	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	T	G	T	A
Musa acuminata var. *microcarpa* (Mempawah1)		T	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	T	G	T	A
Musa acuminata var. *microcarpa* (Mempawah2)		C	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	T	G	T	A
Musa campestris (Singkawang)		C	A	T	C	G	T	G	C	A	C	T	C	A	T	A	T	G	A	C	G	G	A
Musa acuminata (Ketapang)	T	C	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	T	G	T	A
Musa borneensis (Kapuas Hulu)		T	G	T	C	G	T	G	C	A	C	T	C	A	G	A	T	G	A	C	G	G	A
*Musa barioensi*s (NC_058955.1)		C	A	T	C	G	T	G	C	A	C	T	C	A	G	A	T	G	A	C	G	G	A
Musa troglodytarum (OK323374.1)		C	A	T	C	G	T	G	C	A	C	T	C	A	T	A	T	G	A	C	G	G	A
Musa peekelii subsp. *angustigemma* (NC_058958.1)		C	A	T	C	G	T	G	C	A	C	T	C	A	T	A	T	G	A	C	G	G	A
Musa beccarii (NC_058953.1)		C	A	T	C	G	T	G	C	A	C	T	C	A	T	A	T	G	A	C	G	G	A
Musa borneensis (NC_058952.1)		C	A	C	C	G	T	G	C	A	C	T	C	A	G	A	T	G	A	C	G	G	A
Musa gracilis (NC_058946.1)		C	A	T	C	G	T	G	C	A	C	T	C	A	T	A	T	G	A	C	G	G	A
*Musa jackey*i (OK012355.1)		C	A	T	C	G	T	G	C	A	C	T	C	A	T	A	T	G	A	C	G	G	A
Musa lokok (NC_058942.1)		C	A	T	C	G	T	G	C	A	C	T	C	A	T	A	T	G	A	C	G	G	A
Musa maclayi subsp. *Maclayi* (NC_058941.1)		C	A	T	C	G	T	G	C	A	C	T	C	A	T	A	T	G	A	C	G	G	A
Musa salaccensis (NC_058934.1)		C	A	T	C	G	T	G	C	A	C	T	C	A	T	A	T	G	A	C	G	G	A
Musa lolodensis (OK012350.1)		C	A	T	C	G	T	G	C	A	C	T	C	G	T	A	T	G	A	C	G	G	A
Musa johnsii (NC_058944.1)		C	A	T	C	G	T	G	C	A	C	C	T	A	T	A	T	G	A	C	G	G	A
Musa coccinea (MH603431.1)		C	A	C	C	G	T	G	A	A	C	C	C	A	T	G	C	G	A	C	G	G	A
Musa paracoccinea (OK012344.1)		C	A	C	C	G	T	G	A	A	C	C	C	A	T	G	C	G	A	C	G	G	A
Musa ingens (OK012356.1)		C	A	T	C	A	T	A	A	A	C	T	C	A	T	G	T	A	A	C	A	G	A
Musa puspanjaliae (NC_058938.1)		C	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	C	G	G	A
Musa ornata (NC_042874.1)		C	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	T	G	G	A
Musa laterita (OK012353.1)		C	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	T	G	G	A
Musa rosea (NC_058937.1)		C	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	T	G	G	A
Musa rubra (NC_058936.1)		C	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	T	G	G	A
Musa siamensis (NC_058931.1)		C	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G		G	G	A
Musa rubinea (NC_056832.1)		C	G	T	G	G	C	G	A	T	T	T	C	A	C	G	T	G	G	C	G	G	T

Our study showed a tree separated into 2 main clades (Figure 2). *Musa acuminata* subsp. *microcarpa* from Mempawah and Landak and *M. acuminata* from Ketapang are in the same Clade. Geographically, Mempawah and Landak districts are close together. However, Ketapang district is located further away from Mempawah and Landak districts. Different geographic origins led to the introduction and naturalization of cultivars from their original places (Kiran et al. 2015). Isolation in the same geographic zone reduces the genetic distance between populations, leading to the emergence of traits with similar genetic features. In contrast, distinct geographical environments also result in other adaption patterns and genetic features (Karuwal et al. 2024).

The *mat*K gene could describe the differences between *M. acuminata* and *M. borneensis* (Figure 2). The *mat*K gene, found in the chloroplast genome, is very variable and can be used to identify and verify species (Roslim et al. 2016). The *mat*K marker can be used to distinguish at the species level of Indonesian banana cultivars and their wild relatives (Ardiyani et al. 2023). The *mat*K sequences are commonly employed in plant phylogenetic studies and molecular identification because of their rapid evolution and diverse sequences (Guo et al. 2016). Marker sensitivity refers to their capacity to classify accessions of various species into phylogenetic groups. More sensitive markers produce more relevant cluster analysis results (Song et al. 2022).

The findings from this research contribute to addressing the disease-related issues that pose a threat to banana plants. Disease-resistant banana varieties are utilized to manage diseases affecting bananas. Banana breeding focuses on creating disease-resistant triploid hybrids, which are favored for their vigorous growth and high yields. The wild bananas studied here represent valuable genetic resources with significant potential, particularly *M. acuminata*, which is highly regarded for its potential in banana breeding programs (Poerba et al. 2019). *Musa acuminata* serves as a genetic reservoir for disease resistance (Sutanto et al. 2014; Fraser-Smith et al. 2016). It has been specifically chosen as the male parent to produce disease-resistant triploid hybrids, with *M. acuminata* var. *malaccensis* also used for this purpose (Poerba et al. 2017). The use of genomic technologies in banana breeding allows

for the identification and evaluation of genes responsible for certain features, leading to the development of novel traits. Further research using functional DNA-based markers is suggested to evaluate the diversity and precise properties of banana germplasm, including disease resistance, stress-related genes, and quantitative trait loci (Heslop-Harrison 2011).

Assessing genetic diversity in wild bananas in West Kalimantan can guide conservation strategies for wild banana breeding and development. Ex-situ conservation of banana that are genetically similar is useless and inefficient (Hariyanto et al. 2021). In our study, wild banana clustered in clade 1 were revealed identical, if resources are limited, it is recommended to select one of the identical samples as a representative group.

In conclusion, the results showed that the primer amplified *mat*K of *Musa* spp samples with fragment lengths ranging from 540 bp to 561 bp. It showed high variability with a conservation level A+T content of 66.26%. The *mat*K sequences of *Musa* spp. have a polymorphic site on 23 numbers of nucleotides. The phylogenetic analysis with the ML algorithm of 30 *Musa* spp. from West Kalimantan and GenBank data was successfully divided into 2 main clades, and the bootstrap value is 62-69. Our study showed that *mat*K gene sequences used could describe the differences in species of *M. acuminata*, *M. campestris*, and *M. borneensis* in West Kalimantan.

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