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Phylogenetic Relationships of Selected Raja Banana (*Musa* × *paradisiaca L.*) Cultivars from Java Based on Chloroplast rbcL Sequences and Their Implications for Germplasm Conservation

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ABSTRACT

Purpose of the study: This study aimed to investigate the phylogenetic relationships, genetic variation, and geographic distribution patterns of Raja banana (*Musa* × *paradisiaca L.*) cultivars distributed across Java Island using chloroplast rbcL gene sequences and to provide molecular information supporting germplasm conservation and sustainable management of banana genetic resources in Indonesia.

Methodology: Young leaf samples of seven Raja banana cultivars were collected from Java Island. Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, USA). The chloroplast rbcL gene was amplified by PCR and sequenced by First BASE Laboratories (Malaysia). Sequence alignment was performed using ClustalX v2.1. Genetic diversity was analyzed with DnaSP v6.12.03, phylogenetic reconstruction with MEGA v6.0, haplotype network analysis with Network v5.0, and tree visualization with FigTree v1.4.3.

Main Findings: The rbcL sequences showed high conservation with limited nucleotide variation among cultivars. Of the 553 nucleotide positions analyzed, 11 variable sites were detected, resulting in two haplotypes with haplotype diversity (Hd) of 0.571 and nucleotide diversity (π) of 0.0043. Maximum Likelihood phylogenetic analysis grouped the cultivars into two major clades, indicating close evolutionary relationships among the examined cultivars. Haplotype network analysis supported the phylogenetic structure and revealed distinct genetic groupings among cultivars distributed across Java Island.

Novelty/Originality of this study: The integration of chloroplast rbcL-based phylogenetic analysis with geographic distribution interpretation to investigate Raja banana cultivars across Java Island. This research evaluates phylogenetic relationships, haplotype structure, and cultivar distribution patterns, providing additional insights into the conservation of Indonesian banana germplasm.

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1. INTRODUCTION

Banana (*Musa* spp.) is one of the most important fruit crops in tropical and subtropical regions, contributing substantially to food security, agricultural economies, and cultural practices [1], [2]. In Indonesia, banana cultivation has long been integrated into traditional agricultural systems and serves as an important source of carbohydrates, vitamins, minerals, and household income. Among the cultivated banana groups, Raja banana (*Musa* × *paradisiaca L.*) is highly valued due to its superior fruit quality, distinctive flavor, and high market demand [3], [4]. The widespread cultivation of Raja banana across Java Island has resulted in the emergence of numerous local cultivars that exhibit variations in fruit morphology, bunch characteristics, growth performance, and environmental adaptability [5], [6]. This diversity represents a valuable genetic resource that supports crop improvement, conservation programs, and sustainable agricultural development.

Understanding genetic diversity among cultivated banana populations is essential for maintaining genetic resources and ensuring long-term crop resilience. Genetic variation provides the foundation for adaptation to environmental changes, resistance to pests and diseases, and the development of superior cultivars [7], [8]. The diversity of Raja banana cultivars found throughout Java Island is believed to have arisen through a combination of natural evolutionary processes, farmer-mediated selection, and long-term domestication practices. However, morphological characteristics alone are often insufficient to accurately determine genetic relationships among cultivars because phenotypic traits may be influenced by environmental conditions and cultivation practices [9], [10]. Consequently, molecular approaches have become increasingly important for resolving taxonomic ambiguities and understanding evolutionary relationships among cultivated bananas [11], [12].

Previous studies have employed various molecular markers to investigate genetic diversity and phylogenetic relationships within the genus *Musa* [13], [14]. Molecular approaches based on RAPD, AFLP, SSR, ITS, *matK*, and chloroplast DNA regions have demonstrated considerable effectiveness in identifying genetic variation and reconstructing evolutionary histories among banana cultivars [15], [16]. These studies have shown that DNA-based analyses provide more reliable information than morphological observations alone, particularly for distinguishing closely related cultivars and assessing patterns of genetic differentiation. Furthermore, molecular phylogenetic analyses have contributed significantly to understanding the domestication, diversification, and dispersal processes of cultivated bananas in different geographic regions [17], [18].

Previous molecular studies on Indonesian banana cultivars have primarily focused on genetic identification, cultivar classification, and diversity assessment using various molecular markers such as matK, ITS, RAPD, SSR, ISSR, and AFLP [13], [19]-[21]. For example, Probojati et al. [19] investigated phylogenetic relationships among banana cultivars from Java Island using the matK gene, while Ardiyani et al. [21] employed DNA barcoding approaches to examine genetic relationships between Indonesian banana cultivars and their wild relatives. Other studies have also utilized SSR and ISSR markers to evaluate genetic diversity among local Musa germplasm in Southeast Asia [13], [20]. These studies have significantly contributed to the molecular characterization of banana diversity and provided important insights into the taxonomy and domestication history of Musa cultivars.

However, despite these advances, information regarding Raja banana cultivars specifically remains relatively limited, particularly for cultivars distributed across different regions of Java Island. Previous studies generally emphasized broader Musa diversity, local-scale cultivar identification, or multilocus diversity assessments without specifically evaluating phylogenetic relationships among selected Raja banana cultivars using chloroplast rbcL sequences. In addition, comparative information regarding haplotype structure and geographic distribution patterns among Raja banana cultivars remains insufficiently documented. Consequently, molecular evidence supporting the evolutionary relationships and conservation importance of Raja banana germplasm across Java is still incomplete.

The present study does not aim to provide comprehensive phylogenomic or biogeographic reconstruction of Musa evolution. Instead, this study provides a preliminary phylogenetic assessment of selected Raja banana cultivars using the chloroplast rbcL marker as a widely used and highly conserved DNA barcode region suitable for initial molecular characterization and cultivar relationship analysis. The findings are expected to complement previous studies employing other molecular markers and contribute additional baseline molecular information for germplasm documentation and conservation planning of Indonesian banana resources.

Despite substantial progress in molecular characterization of banana germplasm, information regarding the phylogenetic relationships and geographic distribution of Raja banana cultivars across Java Island remains limited [19], [22]. Most previous studies have focused primarily on cultivar identification, genetic diversity assessments at local scales, or taxonomic classification without integrating phylogenetic and biogeographic perspectives [14], [23]. As a result, the evolutionary relationships, historical dispersal pathways, and genetic connectivity among Raja banana cultivars distributed throughout Java remain poorly understood [19], [24]. This lack of comprehensive information restricts efforts to develop effective conservation strategies and limits the understanding of how geographic and anthropogenic factors have shaped the present distribution of Raja banana diversity.

The chloroplast *rbcL* (*ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit*) gene is one of the most widely used molecular markers in plant phylogenetic studies due to its conserved structure, ease of amplification, and suitability for DNA barcoding applications [25], [26]. As a fundamental component of the photosynthetic machinery, the *rbcL* gene provides valuable evolutionary information that can be used to infer genetic relationships among plant taxa. Previous studies have demonstrated the effectiveness of *rbcL* sequences in species identification, phylogenetic reconstruction, biodiversity assessment, and molecular systematics [27], [28]. Therefore, the application of *rbcL*-based analyses provides an opportunity to clarify genetic relationships among Raja banana cultivars and to explore the evolutionary processes underlying their distribution across different regions of Java Island.

The contribution of this study lies in providing preliminary molecular phylogenetic information on selected Raja banana cultivars from different regions of Java Island using chloroplast rbcL sequences, thereby complementing previous studies employing alternative molecular markers for Musa germplasm characterization. Unlike previous studies that primarily focused on cultivar identification or localized genetic diversity assessments, this research simultaneously evaluates phylogenetic relationships, genetic divergence, and potential dispersal patterns among Raja banana cultivars originating from different geographic regions [13], [20]. This integrated approach provides a more comprehensive understanding of the evolutionary history and spatial distribution of Raja banana germplasm within one of Indonesia's most important agricultural landscapes.

The urgency of this research is closely related to the increasing threats faced by local banana genetic resources in Indonesia, including genetic erosion, land-use change, agricultural intensification, pest and disease outbreaks, and the gradual replacement of traditional cultivars by commercially preferred varieties [24], [29], [30]. The loss of genetic diversity may reduce the adaptive capacity of banana populations to future environmental challenges and limit opportunities for crop improvement programs. Furthermore, the absence of comprehensive molecular and geographic distribution information may hinder the development of evidence-based conservation strategies for Raja banana germplasm. Therefore, understanding the phylogenetic relationships and distribution patterns of Raja banana cultivars is essential for supporting germplasm conservation, sustainable agricultural management, and the preservation of Indonesia's plant genetic resources.

Therefore, this study aimed to (1) determine the phylogenetic relationships among Raja banana (Musa × paradisiaca L.) cultivars from different regions of Java Island based on chloroplast rbcL sequences, (2) assess their genetic variation and evolutionary divergence, and (3) investigate geographic distribution patterns that may explain their present distribution. The findings of this study are expected to contribute to molecular taxonomy, phylogenetic research, biodiversity conservation, and the sustainable management of banana genetic resources in Indonesia.

2. RESEARCH METHOD

2.1. Study Design and Sample Collection

This study employed a descriptive molecular phylogenetic approach to evaluate the genetic relationships among selected Raja banana (*Musa × paradisiaca L.*) cultivars distributed across Java Island, Indonesia [21], [31]. Seven cultivars were selected purposively based on their local availability, distinct cultivar names recognized by local farmers, and representation of several geographical regions across Java Island. The selected cultivars were intended to provide preliminary molecular information regarding Raja banana diversity rather than to comprehensively represent the entire genetic diversity of Raja banana germplasm in Java.

Each cultivar was represented by one healthy adult individual sampled from a cultivated population. Young fresh leaf tissues were collected for molecular analysis because young leaves generally provide high-quality genomic DNA for PCR amplification. The use of single representatives for each cultivar was considered sufficient for preliminary phylogenetic assessment using the conserved chloroplast rbcL marker; however, this sampling design may not fully capture intraspecific genetic variation within each cultivar population. Therefore, the present findings should be interpreted as preliminary molecular evidence of phylogenetic relationships among selected Raja banana cultivars [21], [32].

2.2. DNA Extraction and Quality Assessment

Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. Young, curled leaves were used as the tissue source because they generally have better DNA quality than mature tissue. DNA concentration and purity were evaluated using a NanoDrop spectrophotometer based on the A260/A280 absorbance ratio [33], [34]. DNA quality was also verified by electrophoresis on a 1% agarose gel run in 1× TBE buffer and visualized using an ultraviolet gel documentation system. DNA samples with a purity ratio between 1.8–2.0 and showing clear genomic DNA bands were used for further analysis. The quality and integrity of the extracted genomic DNA were further confirmed by electrophoresis on 1% agarose gel stained with ethidium bromide under UV illumination.

Representative agarose gel images demonstrating DNA quality and integrity are provided in the Supplementary Material.

2.3. PCR Amplification and DNA Sequencing

The chloroplast rbcL (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) gene fragment was amplified using the primer pair rbcL1 (5'-TGTCACCAAAAACAGAGACT-3') and rbcL2 (5'-TTCCATACTTCACAAGCAGC-3'). The PCR reaction was carried out in a total volume of 30 µL consisting of PCR Master Mix (Intron Biotechnology, South Korea), DNA template, forward and reverse primers, and nuclease-free water [35], [36]. Amplification conditions included pre-denaturation at 98°C for 45 seconds, followed by 35 cycles of denaturation at 98°C for 45 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 40 seconds, and final extension at 72°C for 10 minutes. PCR products were verified by 1.5% agarose gel electrophoresis and subsequently sequenced using First BASE Laboratories Sdn. Bhd. (Selangor, Malaysia) [37], [38].

2.4. Sequence Alignment and Genetic Diversity Analysis

The obtained *rbcL* sequences were edited and assembled using Sequence Scanner v1.0. Multiple sequence alignment was performed using ClustalX v2.1 with default parameters. Genetic variation among Raja banana cultivars was evaluated using DnaSP v6.12.03. The analyzed parameters included the number of conserved sites, variable sites, parsimony-informative sites, haplotype diversity (*Hd*), nucleotide diversity (π), and the average number of nucleotide differences [39], [40]. These parameters were used to assess the level of genetic diversity among cultivars collected from different regions of Java Island.

2.5. Phylogenetic Analysis

Phylogenetic relationships among Raja banana cultivars were reconstructed using the Maximum Likelihood (ML) method implemented in MEGA v6.0. The best-fit nucleotide substitution model was selected based on the lowest Bayesian Information Criterion (BIC) value [41], [42]. Node support was evaluated using 1,000 bootstrap replications to assess the reliability of the inferred phylogenetic relationships. Two *Ensete* species were included as outgroups to root the phylogenetic tree and facilitate evolutionary interpretation. The resulting phylogenetic tree was visualized and edited using FigTree v1.4.3.

2.6. Haplotype Network and Geographic Distribution Analysis

A haplotype network was constructed using the Median-Joining algorithm implemented in Network v5.0 to evaluate genealogical relationships among cultivars. The network was used to identify haplotype groups and infer potential evolutionary connections among geographically distributed populations [43], [44]. Geographic distribution interpretation was performed by integrating phylogenetic relationships, haplotype structure, and sampling locations across Java Island. The distribution patterns were subsequently analyzed to evaluate the potential influence of environmental factors and human-mediated dispersal on the diversification of Raja banana cultivars.

3. RESULTS AND DISCUSSION

3.1. DNA Quality Assessment

Analysis of chloroplast rbcL sequences revealed a high degree of sequence conservation among the seven Raja banana cultivars examined in this study. Multiple sequence alignment indicated that most nucleotide positions were conserved, whereas only a limited number of variable sites were detected. The low level of sequence variation is consistent with the highly conserved nature of the rbcL gene, which encodes the large subunit of the Rubisco enzyme and plays a fundamental role in photosynthetic carbon fixation [45], [46]. Although sequence divergence was relatively low, several polymorphic sites were sufficient to distinguish phylogenetic groupings among the cultivars and to infer their evolutionary relationships.

The limited genetic variation observed among Raja banana cultivars suggests that these cultivars may share a relatively recent common ancestry or have undergone extensive gene flow through human-mediated dispersal. Similar patterns have been reported in cultivated bananas, where vegetative propagation and long-term domestication often reduce genetic differentiation among geographically separated populations [47], [48]. Nevertheless, the polymorphic sites detected in this study provide valuable information for understanding the evolutionary diversification of Raja banana germplasm across Java Island.

Table 1. DNA Quality and Concentration of Raja Banana Cultivars Used in This Study

Cultivar	Origin	DNA Concentration (ng/µL)	A260/A280 Ratio
Raja Bali	Bantul, Yogyakarta	128.45	1.91
Raja Kisto	Banyuwangi, East Java	136.72	1.89
Raja Lini	Gunungkidul, Yogyakarta	121.63	1.93
Raja Delima	Malang, East Java	142.18	1.88
Raja Gareng	Temanggung, Central Java	133.51	1.90
Raja Kutuk	Purworejo, Central Java	126.27	1.92
Raja Brentel	Sukoharjo, Central Java	130.44	1.89

The quality assessment of genomic DNA extracted from the seven Raja banana cultivars demonstrated that all samples possessed adequate purity and concentration for downstream molecular analyses. DNA concentrations ranged from 121.63 to 142.18 ng/µL, indicating successful DNA extraction from young leaf tissues. The A260/A280 ratios varied between 1.88 and 1.93, which fall within the acceptable range for high-quality DNA suitable for PCR amplification and sequencing. No evidence of significant protein contamination was observed among the analyzed samples. These results confirm that the extracted DNA met the quality requirements for reliable amplification and phylogenetic analysis based on chloroplast *rbcL* sequences.

3.2. Genetic Variation of *rbcL* Sequences

Table 2. Genetic Variation Parameters of the *rbcL* Sequences

Parameter	Value
Sequence length	553 bp
Conserved sites	542
Variable sites	11
Singleton variable sites	4
Parsimony-informative sites	7
Number of haplotypes	2
Haplotype diversity (Hd)	0.571
Nucleotide diversity (π)	0.0043
Average number of nucleotide differences (K)	2.37

Analysis of the aligned *rbcL* sequences revealed a high degree of nucleotide conservation among the examined Raja banana cultivars. Of the 553 nucleotide positions analyzed, 542 sites were conserved and only 11 sites exhibited variation, indicating relatively low sequence divergence. The presence of seven parsimony-informative sites suggests that the detected polymorphisms were sufficient to provide phylogenetic information for cultivar differentiation. Haplotype diversity (Hd = 0.571) and nucleotide diversity (π = 0.0043) indicated moderate levels of genetic variation within the studied germplasm. Overall, these findings suggest that while the cultivars remain genetically closely related, detectable sequence polymorphisms have contributed to their evolutionary differentiation.

The relatively low nucleotide variation observed among Raja banana cultivars is consistent with previous studies reporting limited sequence divergence in chloroplast genes of cultivated bananas. Chloroplast markers such as *rbcL* are generally characterized by slow evolutionary rates and high sequence conservation because of their essential role in photosynthesis and plant metabolism [49], [50]. Similar findings have been reported in several studies on *Musa* germplasm, where chloroplast regions successfully resolved broad phylogenetic relationships but exhibited limited polymorphism at the cultivar level. Nevertheless, the presence of variable and parsimony-informative sites detected in this study demonstrates that even highly conserved chloroplast genes retain sufficient evolutionary signals to distinguish major genetic groups [51], [52]. These findings support the continued use of *rbcL* as an effective molecular marker for preliminary phylogenetic assessments and germplasm characterization in cultivated banana populations.

The moderate haplotype diversity identified among the examined cultivars suggests that Raja bananas in Java retain a measurable level of genetic variation despite extensive vegetative propagation. Such variation may have accumulated through somatic mutations, historical hybridization events, and long-term domestication processes occurring under diverse environmental conditions [53], [54]. In cultivated crops, the persistence of genetic diversity is essential for maintaining adaptive potential and supporting breeding programs aimed at improving resistance to biotic and abiotic stresses. Therefore, the observed genetic variation represents an important component of the genetic resources available for future banana improvement initiatives. The maintenance of this diversity should be considered a priority within national germplasm conservation strategies.

The relatively low genetic variation observed in this study, characterized by only 11 variable sites, two haplotypes, and low nucleotide diversity (π = 0.0043), highlights the limited discriminatory power of the

chloroplast rbcL marker at the cultivar level. Although rbcL is widely recognized as an effective DNA barcode for plant identification and higher-level phylogenetic reconstruction because of its conserved sequence structure, its evolutionary rate is generally too slow to resolve fine-scale genetic differentiation among closely related cultivars [49], [50]. Consequently, the low sequence divergence detected among Raja banana cultivars was expected and reflects the conservative nature of chloroplast coding regions.

Similar limitations of rbcL for intraspecific or cultivar-level discrimination have been reported in previous studies on Musa and other cultivated plant species. Molecular markers with higher mutation rates, such as matK, ITS, SSR, ISSR, and SNP-based approaches, generally provide greater phylogenetic resolution and stronger discriminatory capacity among closely related cultivars [13], [14], [19], [20] . For example, Probojati et al. [19] demonstrated that matK sequences provided improved phylogenetic resolution among banana cultivars from Java Island, while SSR-based studies revealed higher levels of polymorphism and genetic differentiation among Musa germplasm [13], [20]. Similarly, ITS regions and genome-wide SNP analyses have been shown to detect finer-scale evolutionary divergence and domestication patterns in cultivated bananas [18], [55].

Despite these limitations, the rbcL marker remains useful for preliminary molecular characterization because of its high amplification success, sequence reliability, and broad applicability in plant DNA barcoding studies. In the present study, the detected polymorphic sites were still sufficient to distinguish two major phylogenetic groups among the examined Raja banana cultivars. Nevertheless, future studies should incorporate multilocus approaches and more rapidly evolving molecular markers to improve phylogenetic resolution and provide a more comprehensive understanding of genetic diversity and evolutionary relationships among Indonesian banana cultivars.

3.3. Phylogenetic Relationships among Raja Banana Cultivars

Phylogenetic reconstruction using Neighbor Joining, Maximum Likelihood, Maximum Parsimony, and Bayesian Inference consistently revealed the formation of two major clades among the Raja banana cultivars. The first clade comprised Raja Bali, Raja Kisto, and Raja Lini, whereas the second clade consisted of Raja Delima, Raja Gareng, Raja Kutuk, and Raja Brentel. The congruence among different phylogenetic methods indicates that the observed grouping pattern is robust and reflects genuine evolutionary relationships among the cultivars.

The close relationship between Raja Bali, Raja Kisto, and Raja Lini suggests that these cultivars may have originated from a common ancestral lineage or experienced frequent exchange of planting materials among regions. Interestingly, Raja Kisto from Banyuwangi clustered with Raja Bali and Raja Lini from Yogyakarta despite their geographical separation. This pattern indicates that geographical distance alone does not fully explain the observed phylogenetic relationships [56], [57]. Historical movement of planting materials through trade networks, farmer exchanges, and traditional cultivation practices may have contributed to the dissemination of genetically similar cultivars across different regions of Java.

In contrast, Raja Delima, Raja Gareng, Raja Kutuk, and Raja Brentel formed a separate evolutionary lineage characterized by relatively closer genetic affinity among its members. The grouping of these cultivars may reflect a different domestication history or adaptation trajectory. The separation between the two major clades suggests that diversification within Raja banana cultivars has likely been influenced by both evolutionary divergence and human selection processes operating over extended periods.

The low genetic distance values observed among all cultivars indicate that Raja bananas in Java remain genetically related despite their morphological and geographical differences. Such findings are expected because cultivated bananas are predominantly propagated vegetatively, a reproductive strategy that generally preserves ancestral genetic characteristics while allowing the accumulation of limited mutations over time [58], [59]. Consequently, even small nucleotide substitutions in conserved chloroplast genes can provide useful phylogenetic signals for distinguishing cultivar groups.

Table 3. Pairwise Genetic Distance Matrix Based on *rbcL* Sequences

Cultivar	Bali	Kisto	Lini	Delima	Gareng	Kutuk	Brentel
Raja Bali	0.000	0.002	0.001	0.008	0.009	0.009	0.010
Raja Kisto	0.002	0.000	0.002	0.009	0.010	0.009	0.010
Raja Lini	0.001	0.002	0.000	0.008	0.009	0.008	0.009
Raja Delima	0.008	0.009	0.008	0.000	0.002	0.001	0.002
Raja Gareng	0.009	0.010	0.009	0.002	0.000	0.002	0.001
Raja Kutuk	0.009	0.009	0.008	0.001	0.002	0.000	0.002
Raja Brentel	0.010	0.010	0.009	0.002	0.001	0.002	0.000

Pairwise genetic distance analysis demonstrated relatively low levels of divergence among the Raja banana cultivars distributed across Java Island. Genetic distance values ranged from 0.001 to 0.010, indicating close evolutionary relationships among all examined cultivars. The smallest genetic distance was observed

between Raja Delima and Raja Kutuk, suggesting a particularly close genetic affinity between these cultivars. Conversely, the largest genetic distance was detected between Raja Brentel and Raja Bali, reflecting a greater degree of genetic differentiation. Despite these differences, the overall low distance values indicate that the cultivars likely share a relatively recent common ancestry and have experienced limited sequence divergence within the chloroplast genome [60], [61].

The low genetic distance values among Raja banana cultivars indicate that these cultivars share a relatively close evolutionary origin despite being distributed across different regions of Java Island. Such patterns are commonly observed in clonally propagated crops, where vegetative reproduction tends to preserve ancestral genetic characteristics over long periods [62], [63]. Previous molecular studies on cultivated bananas have similarly reported low levels of chloroplast sequence divergence among geographically separated cultivars, highlighting the influence of domestication and human-mediated dispersal on genetic structure [64], [65]. The close affinity observed between Raja Delima and Raja Kutuk may reflect a shared domestication history or the historical exchange of planting materials between agricultural communities. Conversely, the greater genetic differentiation observed between Raja Brentel and Raja Bali may indicate independent evolutionary trajectories or localized adaptation processes.

From a conservation perspective, the identification of varying levels of genetic distance among cultivars provides valuable information for germplasm management. Cultivars exhibiting greater genetic divergence may contain unique alleles or evolutionary histories that contribute disproportionately to overall genetic diversity. Preserving representatives from genetically distinct groups is therefore important for maximizing the conservation value of ex situ and in situ collections [66], [67]. Furthermore, genetic distance data can support the selection of parental materials in breeding programs by identifying cultivars that potentially harbor complementary genetic characteristics. Consequently, these findings contribute not only to understanding evolutionary relationships but also to the sustainable utilization of banana genetic resources.

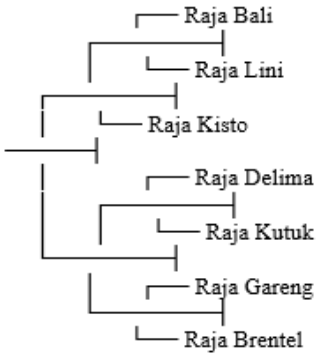

Figure 1. Maximum Likelihood phylogenetic tree inferred from chloroplast *rbcL* sequences showing two major clades among Raja banana cultivars distributed across Java Island

The Maximum Likelihood phylogenetic tree revealed the existence of two major clades among the Raja banana cultivars analyzed in this study. The first clade consisted of Raja Bali, Raja Kisto, and Raja Lini, whereas Raja Delima, Raja Gareng, Raja Kutuk, and Raja Brentel formed the second clade. This clustering pattern was supported by bootstrap values ranged from 76% to 98% across major nodes.and was consistent with the evolutionary relationships inferred from sequence variation analyses. The grouping of cultivars originating from geographically distant regions suggests that historical dispersal and human-mediated movement of planting materials may have contributed to their current distribution [68], [69]. These findings indicate that the diversification of Raja banana cultivars in Java has been influenced by both evolutionary processes and anthropogenic factors.

The formation of two major phylogenetic clades suggests that Raja banana cultivars in Java have undergone a degree of evolutionary differentiation despite maintaining overall genetic similarity. Similar phylogenetic structures have been reported in other cultivated banana groups, where domestication history, farmer selection, and geographic isolation collectively contributed to lineage diversification [59], [70]. The clustering of Raja Bali, Raja Kisto, and Raja Lini within a single clade may indicate a common ancestral origin or prolonged exchange of planting materials among communities inhabiting different regions of Java. Such patterns illustrate the importance of anthropogenic factors in shaping the genetic composition of cultivated banana populations. The role of traditional agricultural practices should therefore be considered when interpreting the evolutionary history of local banana cultivars.

The second clade, comprising Raja Delima, Raja Gareng, Raja Kutuk, and Raja Brentel, may represent an alternative domestication pathway or a lineage that experienced distinct selective pressures during cultivation. Differences in environmental conditions, cultivation systems, and farmer preferences can drive divergence

among cultivars even when originating from closely related ancestral populations. The phylogenetic separation observed in this study therefore reflects the combined influence of natural evolutionary processes and long-term human management [71], [72]. Such findings contribute to a broader understanding of crop diversification within agricultural landscapes. Moreover, they highlight the dynamic interactions between biological evolution and cultural practices in shaping plant genetic diversity.

3.4. Geographic Distribution Patterns and Haplotype Relationships

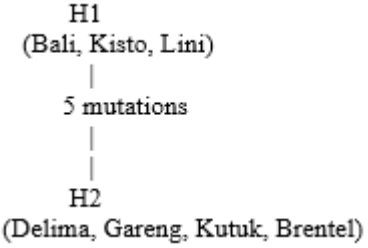

Figure 2. Median-joining haplotype network showing two major haplotypes among Raja banana cultivars. Circle size is proportional to haplotype frequency, while connecting lines represent mutational steps

The haplotype network analysis identified two major haplotypes among the Raja banana cultivars, corresponding closely to the clades observed in the phylogenetic reconstruction. Raja Bali, Raja Kisto, and Raja Lini were grouped within the first haplotype, whereas Raja Delima, Raja Gareng, Raja Kutuk, and Raja Brentel shared a second haplotype. The separation between the two haplotypes by several mutational steps suggests the occurrence of evolutionary divergence within Raja banana germplasm. The consistency between haplotype and phylogenetic analyses strengthens the reliability of the inferred genetic relationships among cultivars. These results indicate that the cultivars represent distinct evolutionary lineages that have undergone differentiation despite remaining genetically related.

The identification of two principal haplotypes reinforces the phylogenetic patterns observed in the Maximum Likelihood analysis and provides additional evidence for the existence of distinct evolutionary lineages within Raja banana germplasm. Haplotype structure is often used to infer historical demographic processes because closely related haplotypes typically reflect recent evolutionary connections [73], [74]. The separation between the two haplotypes by multiple mutational steps suggests that genetic divergence has accumulated over an extended period rather than resulting from recent differentiation alone. Similar haplotype patterns have been documented in cultivated bananas and other clonally propagated crops, where diversification occurs through mutation accumulation and localized selection. These processes may have contributed to the development of distinct cultivar groups observed across Java Island.

The distribution of haplotypes also provides insight into historical dispersal dynamics. The presence of genetically related cultivars in geographically separated regions suggests that human-mediated movement of planting materials has played an important role in shaping current distribution patterns. Farmers have traditionally exchanged planting materials through trade, migration, and social networks, facilitating gene flow across broad geographic areas. Consequently, the observed haplotype structure likely reflects both historical dispersal events and subsequent local adaptation. Understanding these processes is important for reconstructing the evolutionary history of banana cultivation in Indonesia and for developing conservation strategies that preserve representative genetic lineages.

The observed geographic distribution pattern suggests that environmental and geographical factors may have contributed to shaping the present distribution of Raja banana diversityth. Banyuwangi, Bantul, and Gunungkidul are generally characterized by relatively warmer lowland environments, whereas Malang and Temanggung include higher-elevation areas with cooler climatic conditions. Such ecological differences may influence local adaptation processes and contribute to the accumulation of genetic variation over time [75], [76].

However, the distribution pattern cannot be explained solely by environmental conditions. Human activities have likely played a major role in the dispersal of Raja banana cultivars across Java. For centuries, the island has been connected by extensive agricultural, cultural, and trade networks that facilitated the movement of planting materials among regions. The clustering of cultivars from geographically distant locations therefore suggests that anthropogenic dispersal has contributed substantially to gene flow and cultivar distribution patterns.

From an evolutionary perspective, the haplotype structure observed in this study supports the hypothesis that Raja banana diversification in Java has resulted from an interaction between environmental adaptation and human-mediated domestication. While ecological conditions may have promoted local differentiation, continuous exchange of planting materials likely maintained genetic connectivity among populations. This

combination of natural and anthropogenic processes has contributed to the complex geographic distribution structure currently observed in Raja banana cultivars.

Table 4. Geographic Distribution of Raja Banana Cultivars in Java Island

Cultivar	Location
Raja Kisto	Banyuwangi
Raja Delima	Malang
Raja Gareng	Temanggung
Raja Kutuk	Purworejo
Raja Brentel	Sukoharjo
Raja Bali	Bantul
Raja Lini	Gunungkidul

The geographic distribution of Raja banana cultivars demonstrated a broad spatial pattern extending across eastern, central, and southern regions of Java Island. Cultivars belonging to the same phylogenetic group were not always located in adjacent geographic areas, suggesting that geographic proximity alone does not explain their genetic relationships. This pattern indicates that historical exchange of planting materials among farming communities may have facilitated the spread of genetically similar cultivars across distant locations. Environmental factors, including differences in elevation, climate, and agricultural practices, may also have contributed to the observed distribution patterns [77], [78]. Consequently, the present distribution of Raja banana cultivars appears to reflect a combination of natural ecological processes and long-term human-mediated dispersal.

The spatial distribution of Raja banana cultivars demonstrates that genetic relationships do not always correspond directly with geographic proximity. This finding suggests that factors beyond simple geographic distance have influenced the diversification and dispersal of cultivars throughout Java. Similar patterns have been reported in many cultivated plant species where human activities frequently override natural barriers to gene flow. The movement of planting materials through agricultural trade networks can establish genetically similar populations in distant regions, thereby weakening the association between genetic and geographic distance. Consequently, historical human activities appear to have played a substantial role in shaping the present distribution of Raja banana cultivars.

Environmental heterogeneity across Java Island may have further contributed to cultivar diversification following their introduction into different regions. Variations in temperature, rainfall, elevation, and soil characteristics can create distinct selective pressures that promote local adaptation and phenotypic differentiation. Although the chloroplast *rbcL* marker revealed relatively limited genetic divergence, ecological factors may still influence the development of cultivar-specific characteristics over time. This interaction between environmental conditions and human-mediated dispersal has likely generated the complex biogeographic patterns observed in the present study. Such findings emphasize the importance of integrating molecular, ecological, and geographic approaches to better understand the evolution and conservation of cultivated banana diversity.

3.5. Implications for Germplasm Conservation and Sustainable Agriculture

The identification of distinct phylogenetic and haplotype groups has important implications for the conservation of banana genetic resources in Indonesia. Cultivars representing different evolutionary lineages may contain unique genetic variations that are valuable for future breeding programs. Therefore, conservation strategies should prioritize the preservation of cultivars from both major clades to maximize the retention of genetic diversity.

The findings also contribute to the development of molecular databases for Indonesian banana germplasm and provide baseline information for cultivar authentication. Such information is increasingly important in the context of climate change, emerging diseases, and agricultural intensification, which collectively threaten the persistence of traditional banana cultivars. By integrating phylogenetic and biogeographic approaches, this study provides a scientific foundation for evidence-based germplasm management and supports the long-term sustainability of banana production systems in Indonesia.

Despite the successful identification of phylogenetic relationships among selected Raja banana cultivars, this study has several limitations that should be acknowledged. The use of a single chloroplast marker (rbcL) provided only limited genetic resolution because of the highly conserved nature of the gene region, as reflected by the low nucleotide variation and small number of haplotypes detected among cultivars. Consequently, the present findings should be interpreted as a preliminary molecular assessment rather than a comprehensive phylogenetic reconstruction of Raja banana diversity. Future studies are therefore strongly recommended to incorporate multilocus approaches and higher-resolution molecular markers such as matK, ITS, SSR, and SNP-based analyses to improve cultivar-level discrimination and provide a more detailed

understanding of genetic diversity, domestication history, and evolutionary relationships among Indonesian banana germplasm.

Future research should also integrate population genetics, genomic approaches, and spatial analyses to reconstruct historical dispersal routes and evaluate the relative influence of environmental and anthropogenic factors on banana diversification across Indonesia. Such studies would provide a more comprehensive understanding of the evolutionary dynamics of *Musa* germplasm and strengthen conservation planning for local banana resources.

4. CONCLUSION

This study successfully elucidated the phylogenetic relationships and geographic distribution patterns of selected Raja banana (*Musa* × *paradisiaca L.*) cultivars distributed across Java Island using chloroplast rbcL gene sequences. The results revealed low genetic variation among cultivars, with 11 variable sites, two haplotypes, haplotype diversity (Hd) of 0.571, and nucleotide diversity (π) of 0.0043. Phylogenetic analysis grouped the cultivars into two major clades, providing preliminary molecular information that may support germplasm conservation strategies for Indonesian banana resources. The observed phylogenetic patterns may indicate potential influences of environmental conditions and historical human activities on the distribution of Raja banana cultivars across Java Island; however, further studies incorporating population genetic, ecological, and spatial analyses are required to verify these relationships.

Despite the successful identification of phylogenetic relationships among selected Raja banana cultivars, this study has several limitations that should be acknowledged. The use of a single chloroplast marker (rbcL) provided limited genetic resolution because of the highly conserved nature of the gene region, as reflected by the low nucleotide variation and small number of haplotypes detected among cultivars. Consequently, the present findings should be interpreted as a preliminary molecular assessment rather than a comprehensive phylogenetic or biogeographic reconstruction. Future studies are therefore strongly recommended to incorporate broader sampling coverage, multilocus approaches, and higher-resolution molecular markers such as matK, ITS, SSR, and SNP-based analyses to obtain a more comprehensive understanding of the genetic diversity, evolutionary relationships, and distribution dynamics of Indonesian banana germplasm.

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AUTHOR CONTRIBUTIONS

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

Not applicable.

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