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In Silico Phylogeny of Magelang Taro (*Colocasia esculenta*) Using *rbcL* and *matK* DNA Barcodes

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Abstract: *Colocasia esculenta* (taro) is an important tropical crop with high morphological diversity, particularly in Magelang Regency and City, Indonesia. However, morphologically based identification is often unreliable due to strong environmental influences, which limit its effectiveness in resolving genetic relationships among local varieties. Therefore, molecular approaches such as DNA barcoding are required to obtain more accurate and consistent genetic information. This study aimed to analyse the phylogenetic relationships among C. esculenta varieties from Magelang and to evaluate the effectiveness of the chloroplast genes rbcL and matK as DNA barcodes using an in silico approach. Secondary nucleotide sequence data for rbcL and matK were retrieved from the NCBI GenBank database and analysed using multiple sequence alignment with ClustalW in MEGA X. Phylogenetic trees were reconstructed using the Neighbour-Joining method with the Tamura-3-Parameter model and 1,000 bootstrap replications. At the same time, genetic distances were calculated using pairwise distance analysis. The results showed that matK exhibited a high level of sequence conservation, effectively resolving interspecific relationships within the genus Colocasia, but showed limited resolution at the intraspecific level. The rbcL gene displayed slightly higher nucleotide variation than matK, yet remained insufficient to discriminate local C. esculenta varieties clearly. Genetic distance analysis confirmed very low divergence among several varieties, indicating close evolutionary relationships. In conclusion, the use of rbcL or matK as single markers is inadequate for distinguishing local taro varieties. It is recommended that future studies employ multilocus approaches by combining rbcL–matK with faster-evolving markers, such as ITS, ITS2, or trnH–psbA, to achieve higher resolution at the intraspecific level and to support conservation and breeding programs of local taro germplasm.

Keywords: *Colocasia esculenta*, DNA barcoding, Magelang, phylogenetics.

Introduction

Colocasia esculenta (L.) or taro is a tropical plant primarily cultivated for tuber production, while its leaves and stems are underutilized (Mitharwal et al., 2022). There are at least three genera, namely Alocasia, Colocasia, and Xanthosoma, that grow widely in Indonesia. *Colocasia esculenta* is the most grown in Indonesia and other Southeast Asian countries. *Colocasia esculenta*, commonly known as taro, is one of the primary varieties widely cultivated in numerous countries.

The diversity of taro varieties in Indonesia is unique. Previous studies have demonstrated striking morphological variations among different taro accessions, which have a significant impact on germplasm development (Setyowati et al., 2016). This is due to one of its superior characteristics,

namely its ability to adapt to various extreme conditions, such as waterlogged soil and saline soil, as well as the discovery of several metabolites related to drought tolerance. In previous studies, high diversity in taro morphological characteristics was observed, particularly in plant type, tubers, and some leaf characteristics, on Magelang and Java (Andarini et al., 2018; Yunika et al., 2024).

Morphological characters can be used as essential indicators in analysing genetic diversity, which includes variation within species (Kaur et al., 2012). This variation is crucial for plant breeding, enabling the improvement of various plant traits, such as resistance to pests, productivity, and adaptability to the environment (Delsuc et al., 2006). However, morphology-based identification methods have limitations because the observed characteristics are highly susceptible to changes due to environmental factors, plant growth stages, and cultivation techniques. These conditions often make it difficult to determine species identity accurately. Therefore, a DNA-based approach is crucial for obtaining more consistent and objective information that can more accurately represent genetic diversity and kinship relationships between varieties. Above all, taro is used as a local food crop in Indonesia; therefore, taro conservation is necessary to maintain diversity and ensure the sustainability of this plant as an essential food source (Delsuc et al., 2006).

In previous studies, various varieties of taro have been found growing in Magelang City and Regency, exhibiting a wide range of morphological diversity (Yunika et al., 2024: Lestari et al., 2024). To understand the genetic relationships between taro varieties that support conservation and the development of superior varieties, phylogenetic analysis is necessary to identify close kinship relationships with exceptional characteristics. Understanding the phylogenetics of taro varieties can be used to select the right parental seeds to produce new, superior varieties that are, for example, more resistant to pests and diseases, faster to harvest, and more adaptable to changing environmental conditions. This will certainly be a solution in preserving local taro varieties in Magelang City and Regency, as well as in increasing food security.

Phylogenetic studies of plants can be conducted using DNA barcoding techniques with molecular markers in silico. DNA barcoding is a molecular approach that utilises short DNA sequence fragments to identify species quickly, accurately, and consistently. This technique is a crucial tool in biodiversity and taxonomy studies, as it enables the recording of genetic variation within and between species (Rahayu dan Jannah, 2019). In plants, plastid DNA sequences (rbcL, matK, trnHA-psbA regions) and nuclear DNA (ITS and ITS2) are often used for phylogenetic studies, with rbcL and matK being the most recommended by the Consensus Barcode of Life (CBOL) (Kress et al., 2005; Fazekas et al., 2008). The rbcL and matK molecular markers have high amplification and sequencing efficiency in genetic diversity analyses of taro conducted in India (Devi et al., 2022). Based on previous studies, matK is more effective in detecting intraspecific variation in taro than rbcL. However, the combination of rbcL and matK can produce more precise species resolution results than using a single gene(Ho et al., 2020). Therefore, in this study, the rbcL and matK molecular markers will be used to confirm the relationship between taro varieties, thereby constructing a phylogenetic tree that provides a more accurate species grouping. However, the matK marker is recommended as the primary marker, while rbcL is used as a complementary marker for plant phylogenetic studies (Cahyaningsih et al., 2022).

To address the need for a deeper understanding of genetic diversity and kinship relationships among taro varieties in Magelang Regency and City, this study employed an in silico approach through the analysis of rbcL and matK chloroplast gene sequences. This study specifically aims to analyse the phylogenetic relationships between Colocasia esculenta taro varieties and evaluate the effectiveness of the rbcL and matK molecular markers, which serve as effective DNA barcodes for identifying and distinguishing taro varieties at the species and variety levels.

Materials and Methods

Time and Place

This study was conducted in silico using secondary data obtained from the National Center for Biotechnology Information (NCBI) database. Secondary data refers to information collected and published by parties other than those directly involved in the research process (Hidayat et al., 2018). In this study, the data used were nucleotide sequences of the rbcL and matK chloroplast genes from various varieties of *Colocasia esculenta* found in the Magelang Regency and City. The ingroup sequences consisted of varieties that had been identified in both regions and were available on NCBI. At the same time, two species from the genus Xanthosoma, namely *Xanthosoma sagittifolium* and *Xanthosoma helleborifolium,* were used as the outgroup. These outgroups were selected because they are close relatives within the Araceae family but belong to different genera, thus serving as a sufficient rooting point for the phylogenetic tree. Sequence searches were conducted by entering the species name and the gene of interest (matK and rbcL), for example: *Colocasia esculenta* rbcL.

Data Analysis

The rbcL and matK gene DNA sequence data obtained were then selected and evaluated for quality before further analysis. The sequence alignment process (multiple sequence alignment) was performed using ClustalW software integrated in MEGA X software. At this stage, the ends of the sequences that showed many mismatches or gaps were trimmed to produce a uniform alignment that could be analysed optimally (Anafarida, 2020).

Phylogenetic analysis was performed using the Neighbour-Joining (NJ) method with the Tamura-3-Parameter (T92) substitution model to construct the phylogenetic relationships among taro varieties and species. Branch strength testing was performed using bootstrapping with 1000 replications. The phylogenetic tree was constructed using MEGA X software. In addition, genetic distance calculations were performed by calculating pairwise distance using the Compute Pairwise Distance method to evaluate the genetic proximity between taro varieties and species analysed based on the same model.

Results and Discussion

Collection of DNA Sequences from GenBank NCBI

The rbcL and matK gene sequences were obtained from the NCBI (National Center for Biotechnology Information) nucleotide database. The downloaded sequences represent Colocasia esculenta from various varieties and isolates originating from different geographical regions. Based on the findings of a previous study on taro morphology in the city and regency of Magelang, five cultivars of *Colocasia esculenta* L. Schott were found at the sampling locations, namely *Pratama* 1 (Pra1), *Pratama* 2 (Pra2), *Pratama* 3 (Pra3), *Talas Bogor* (TB), and *Talas Hitam* (TH). Based on these findings, the data used for analysis included *C. esculenta, C. esculenta var. antiquorum, C. esculenta var. esculenta, Colocasia esculenta var. fontanesii*, and several other additional species as comparators.

Table 1. Colocasia species and accession numbers used in the study were obtained from the NCBI database

Species Name	*matK*		*rbcL*	
	Accession	**Length (bp)**	**Accession**	**Length (bp)**
Colocasia esculenta	LT995105	2828	MH270468	1443
Colocasia esculenta var antiquorum	JF828113	676	PV978336	531
Colocasia esculenta var esculenta	N/A	–	PV978335	514
Colocasia esculenta var fontanesii	JQ238892	2411	JN105557	478
Colocasia menglaensis	JQ238894	2390	KF284543	415
Colocasia affinis	JQ238889	2396	PV954744	557
Colocasia lihengiae	JF828116	676	JF828094	649
Colocasia fallax	JQ23889	2386	PV978337	515
Leucocasia gigantea	JF828120	676	JF828102	649

Table 2. Species used as outgroups obtained from the NCBI database

Species Name	*matK*		*rbcL*	
	Accesssion	**Length (bp)**	**Accession**	**Length (bp)**
Xanthosoma helleborifolium	AM920612	1771	AM905790	1391

Sequence alignment using ClustalW

Based on the alignment results using the ClustalW method with MEGA X on 9 ingroup sequences and one outgroup sequence in the target matK gene, it can be observed that there are many conserved regions marked with the symbol (*), indicating a closer relationship between species. There are several differences between Colocasia species, making it challenging to use them as a barcode. This is because the matK gene has a high level of conservation and a low mutation rate. After all, the matK gene is located in the chloroplast genome, which generally evolves more slowly than some marker genes such as ITS, so that the variation between individuals or newly diverged varieties can be minimal (Yan et al., 2018). The results of sequence alignment in the rbcL gene reveal nucleotide variation in several positions, likely the result of accumulated mutations and potentially influenced by environmental factors and mutagenic compounds (Anzani et al., 2021). In addition, several gaps were identified, indicating the occurrence of insertions and deletions, and several regions with nucleotide differences between taxa were observed.

This condition suggests the low potential of this gene as a barcode because it shows less conservative properties, as found in the study by Risah dan Janah (2025). According to Rohimah et al., (2018), the rbcL gene exhibits a low mutation rate, allowing it to produce a high level of homology among species within a genus. This statement is also supported by Aulia (2022) and Alshehri et al (2019), which found that the ability of the rbcL gene to distinguish species levels is weak and can only distinguish up to the genus level. Based on these results, the use of rbcL or matK alone is insufficient to distinguish local taro varieties, mainly due to the limited variation in the plastid genome. Therefore, a combination of markers (rbcL-matK) or additional markers that evolve more rapidly, such as ITS/ITS2/trn-psbA, is needed for intraspecific resolution.

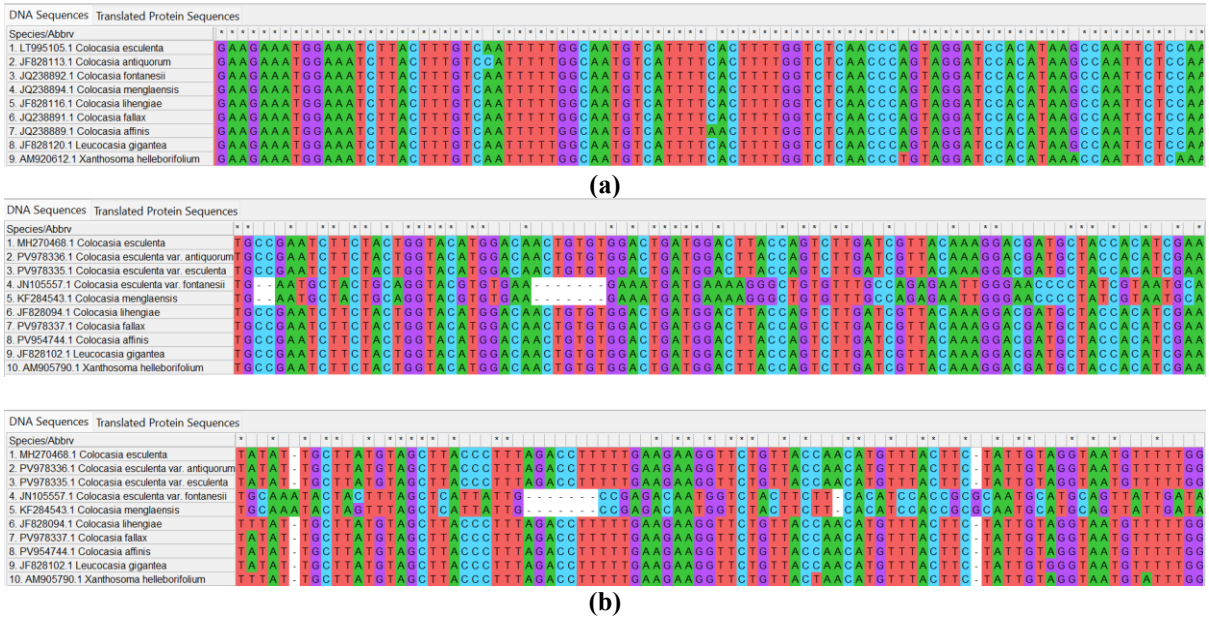
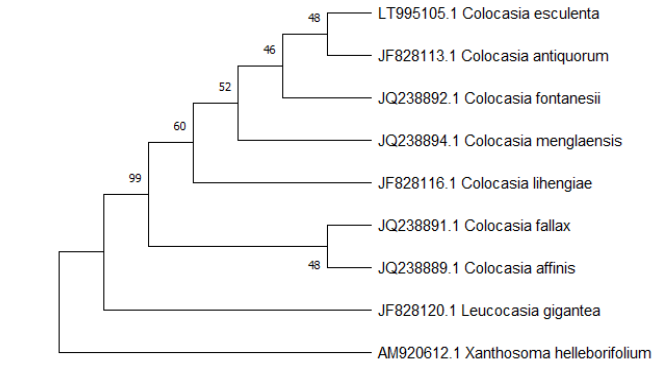


Figure 1. Sequence alignment results using ClustalW, a) matK, b) rbcL
The asterisk (*) indicates homology, while the gap symbol (-) indicates the presence of insertions or deletions

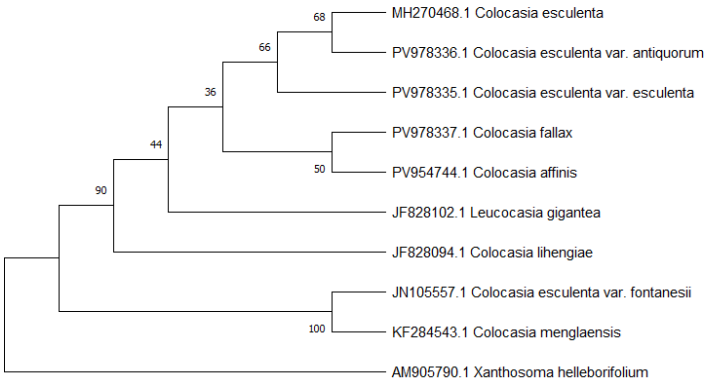
Phylogenetic Analysis

A phylogenetic tree is a graphical representation used to reconstruct the relationships between taxa based on their evolutionary history. The closer the relationship between species in a tree, the smaller the genetic distance and the higher the level of DNA sequence similarity between them. Therefore, individuals or species that evolved from the same ancestor in a relatively close time frame will be clustered in one clade (Sindiya et al., 2018; Anzani et al., 2021). The accuracy of phylogenetic tree reconstruction is usually reinforced by bootstrap values, which are numbers located on the branches of phylogenetic trees. In this study, 1000 replications were used to increase the reliability of the branching. Therefore, the higher the bootstrap value, the higher the accuracy of the reconstruction results (Oktafia dan Badruzsaufari, 2021).

The results of the phylogenetic tree reconstruction for the target matK gene are presented in Figure 2a, which illustrates the formation of four main clades, comprising three ingroup clades and one outgroup clade. Clade 1 includes five species: *C. esculenta, C. antiquorum, C. fontanesii, C. menglaensis*, and *C. lihengiae*. This finding is due to the large number of similar sequence orders among these species. This is because the matK gene exhibits a high level of conservation among species within a genus, often resulting in tight clades within taxonomic groups with short evolutionary divergence (Amandita et al., 2019). The species *C. fallax* and *C. affinis* appear to form a separate clade, with a bootstrap value of 48. Xanthosoma helleborifolium serves as an outgroup and appears to root the tree successfully. Outgroups are essential in phylogenetic tree construction because they can be used to identify primitive (plesiomorphic) and derived (apomorphic) characters of ingroup taxa and to determine the starting point for constructing a phylogenetic tree (Subari et al., 2021).



a



b

Gambar 2. Phylogenetic tree of a) matK gene b) rbcL gene, using MEGA X with the Neighbour Joining method and 1000 bootstraps

Overall, the cluster pattern that formed confirmed that matK is a good marker for grouping species at the interspecific level, but is insufficient for distinguishing varieties or local populations of the same species. These finding align with those Dong et al. (2012), who found that the level of matK variability is low at the intraspecific level, particularly in plant genera. Several studies have also demonstrated that high genetic homology in matK can hinder the separation of closely related species in terms of evolution (Anzani et al., 2021; Aulia, 2022). Other studies have revealed inconsistencies or discrepancies between the results obtained from using DNA genes in chloroplasts and those from using DNA genes in plant cell nuclei, leading to misleading phylogenetic tree construction patterns (Fahey et al, 2021: Perez-Escobar et al, 2021). Nucleotide sequences in the cell nucleus are more informative than most genes in plastids, including chloroplasts, so the use of DNA in the cell nucleus can provide better results in phylogenetic tree construction than phylogenetic tree construction using DNA sequences found in chloroplasts (Perez-Escobar et al, 2021).

The results of the phylogenetic tree reconstruction of the target rbcL gene are shown in Figure 2b, which indicates that *C. esculenta*, along with its varieties *C. esculenta var. antiquoru*m and C. *esculenta var. esculenta*, form a single main clade marked with a bootstrap value >60. Meanwhile, *C. fallax* and *C. affinis* form their own clade, as do C. esculenta var. fontanesii and C. *menglaensis*, which also form their own clade with a high bootstrap value of 100. The bootstrap value that appears is statistical support information for the branches formed. The rbcL gene itself is known to have a low mutation rate, indicating minimal variation at the intraspecific level (Basith, A 2015). Rohimah et al. (2018) lso reported that rbcL produces high levels of homology among species within a genus, especially among species originating from neighboring habitats or geographical areas. Therefore, although effective in phylogenetic analyses at the genus and interspecies levels, rbcL is generally less informative in distinguishing varieties or local populations.

Genetic distance analysis using the pairwise distance method

Based on the results of genetic distance analysis using pairwise distance, it can be seen that the highest genetic distance at the matK locus is between the species C. antiquorum and L. gigantea, which is 0.012 (1.2%). Meanwhile, the closest genetic distances were found between *C. esculenta* and *C. fontanesii, C. esculenta* and *C. menglaensis, C. fontanesii* and C. *menglaensis*, and *C. lihengiae and C. esculenta*, *C. fontanesii*, and *C. menglaensis*, with a value of 0.000 or 0%. A genetic distance value of 0.00 (0%) indicates that species with this genetic distance originate from the same population (Zamroni et al, 2014). The similarity of these sequences indicates a close evolutionary relationship, as stated Bramasta et al. (2021) the higher the genetic distance value, the more distant the relationship between taxa, and vice versa.

Table 3. Pairwise genetic distance in the matK gene

	1	2	3	4	5	6	7	8	9
Colocasia esculenta									
Colocasia antiquorum	0,001								
Colocasia fontanesii	0,000	0,001							
Colocasia menglaensis	0,000	0,001	0,000						
Colocasia lihengiae	0,000	0,001	0,000	0,000					
Colocasia fallax	0,001	0,003	0,001	0,001	0,001				
Colocasia affinis	0,004	0,006	0,004	0,004	0,004	0,003			
Leucocasia gigantea	0,010	0,012	0,010	0,010	0,010	0,012	0,012		
Xanthosoma helleborifolium	0,045	0,046	0,045	0,045	0,045	0,046	0,046	0,040	

Table 4. Pairwise genetic distance in the matK gene

Spesies	1	2	3	4	5	6	7	8	9	10
Colocasia esculenta										
Colocasia esculenta var antiquorum	0,000									
Colocasia esculenta var esculenta	0,000	0,000								
Colocasia esculenta var fontanesii	1,054	1,054	1,054							
Colocasia menglaensis	1,058	1,058	1,058	0,002						
Colocasia lihengiae	0,002	0,002	0,002	1,050	1,054					
Colocasia fallax	0,000	0,000	0,000	1,054	1,058	0,002				
Colocasia affinis	0,000	0,000	0,000	1,054	1,058	0,002	0,000			
Leucocasia gigantea	0,005	0,005	0,005	1,054	1,058	0,007	0,005	0,005		
Xanthosoma helleborifolium	0,027	0,027	0,027	1,027	1,031	0,025	0,027	0,027	0,027	

The results of genetic distance calculations using pairwise distance at the rbcL locus show that the highest genetic distance is between the species *C. menglaensis* and *C. esculenta, C. esculenta var fontanesii,* and *C. esculenta var esculenta,* as well as *between C. menglaensis* and *C. fallax, C. affinis*, and *L. gigantea,* which is 1.058 (105.8%). Meanwhile, the closest genetic distance was found between C. *esculenta* and *C. esculenta var antiquorum* and *C. esculenta var esculenta*, with a value of 0.000 or 0%. This condition indicates a very close kinship relationship. According to Dharmayanti (2011), a low pairwise distance value indicates a closer phylogenetic relationship between taxa.

Conclusion

This study demonstrated that phylogenetic analysis based on the chloroplast markers rbcL and matK is capable of revealing basic patterns of relatedness among the Colocasia esculenta species and varieties used; however, both have limitations when applied at the intraspecific variation level. The results of sequence alignment and phylogenetic tree reconstruction indicate that matK exhibits a high level of conservation, making it effective for grouping species at the interspecific level; however, it is less sensitive in distinguishing local varieties with low divergence. Meanwhile, rbcL exhibits greater nucleotide variation than matK, yet retains relatively conservative evolutionary characteristics, which limit its discriminatory ability in C. esculenta varieties.

Genetic distance analysis, conducted through pairwise distance, confirmed these findings, whereby several pairs of species or varieties showed a genetic distance value of 0%, indicating complete sequence similarity. In contrast, the highest divergence values appeared in species that were taxonomically more distant. This pattern reinforces the notion that the plastid genome does not provide sufficient variation for resolution at the variety level, particularly in taxonomic groups that are thought to have undergone relatively recent evolutionary divergence.

Overall, the results of this study confirm that the use of rbcL or matK alone is insufficient to distinguish between local taro varieties. A multilocus combination, such as rbcL–matK, and the addition of markers with faster evolutionary rates, such as ITS, ITS2, or trnH–psbA, are recommended to obtain better genetic resolution at the intraspecific level. This approach is expected to provide a more comprehensive picture of the genetic diversity and kinship structure of local taro varieties, as well as support future taro conservation and breeding programmes.

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