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Genetic diversity analysis among *Philodendron* spp. by Random Amplified Polymorphic DNA

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ABSTRACT. Variety development in ornamental plants increased rapidly due to the high demand during the COVID-19 outbreak. Therefore, analyzing the genetic diversity of ornamental plants is essential to assist breeders in improving and developing new cultivars with desirable traits. In this study, the genetic diversity of *Philodendron* species was analyzed. *P. erubescens*, *P. 69686*, *P. burle-marx*, *P. lacerum*, and *P. micans* were selected as the most popular species cultivated in Indonesian nurseries. These cultivars were assessed both genetically using Random Amplified Polymorphic DNA (RAPD) markers and morphologically. The genetic relationship among *Philodendron* spp. species was analyzed using DARwin 6.0 software, and a dendrogram was constructed based on the UPGMA algorithm. Based on the two selected RAPD primers, the five samples showed no clear genetic relatedness to each other, and the primers were insufficient to construct a single consensus dendrogram.

Keywords: biodiversity; intra-species; molecular marker; *Philodendron*; RAPD

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INTRODUCTION

The ornamental plant market is highly dynamic, with demand changing rapidly within a short period. The COVID-19 pandemic further accelerated this trend, as many people sought new hobbies to cope with prolonged quarantine. Recent studies have reported a significant surge in the global demand for aroid ornamental plants, especially *Philodendron*, due to their aesthetic value, air-purifying abilities, and adaptability to indoor environments (Oboni & Hossain, 2025). Therefore, the development of new varieties with distinct characteristics has become essential to meet the ever-changing market demands. Additionally, social media platforms have played a key role in enhancing consumer interest and driving market expansion in the post-pandemic era (Mubarok *et al*., 2023). *Philodendron* has gained popularity both as cut foliage and potted plants, not only in the domestic market but also internationally, attracting breeders to develop new cultivars of this genus. In this study, five different species were used as samples: *P. burle-marx, P. micans, P. lacerum, P. erubescens*, and *P.* 69686.

Philodendron spp. belongs to the Araceae family and comprises genus of ornamental plants consisting of 447 identified species native to the Americas and the West Indies region. These plants commonly propagated due to their heart-like shaped leaves, diverse color patterns, and tolerance of indoor environment (Nainwal, 2019). The popularity of *Philodendron* has increased with the emergence of variegated types. Variegation, which can arise from irradiation, *in vitro* mutagenesis, or natural mutation (Karunananda *et al*., 2021) has led to the development of diverse genotyping. Despite their widespread occurrence in nature, relatively few variegations have been characterized at the molecular level (Yu *et al*., 2007). The normal varieties of *Philodendron* are also widely distributed in the ornamental plant market (Barbosa *et al*., 2019) including in Indonesia; however, genetic analyses remain limited. Recent studies have successfully established in vitro propagation protocols and sequenced the chloroplast genomes of *Philodendron* species, providing valuable insights into their genetic diversity and potential for large-scale cultivation (Klanrit *et al*., 2023).

Information on plant genetic diversity resources can assist plant breeders in improving and developing new cultivars with desirable characteristics such as pest resistance, vibrant color, or

photosensitivity by streamlining the selection process for favorable traits. Consequently, genetic analysis has led to significant advancements and numerous developments. However, analysis in the molecular level never been done on Philodendron cultivar in Indonesia yet, given the mutation rate in the variegation types. PCR-based marker became popular in plant genetics and breeding especially for gene mapping, tagging, segregation analysis, genetic diagnosis, forensic examination, phylogenetic analysis, and other useful application (Atienzar & Jha, 2006; He *et al*., 2014). One of the genetic markers widely used is the PCR-RAPD (Random Amplified Polymorphic DNA) marker, which is utilized for genotyping, phylogenetic analysis, and molecular selection due to its rapidity, low cost, and lack of requirement for prior nucleotide sequence information (Atak *et al*., 2011). RAPD markers have previously been applied to *Philodendron* (Devaraja *et al*., 2014). In that study, 60 RAPD primers were screened across 20 *Philodendron* varieties, with 21 primers producing clear amplification and identifying 348 polymorphic bands out of 354 scored loci, demonstrating 98 % polymorphism and effective differentiation among cultivars (Devaraja *et al*., 2014). Unlike that study, the present research focused on five Philodendron varieties commonly cultivated as ornamental plants in Indonesia, which have not been previously characterized at the molecular level. Furthermore, instead of conducting broad-scale primer screening, this study employed nine pre-selected RAPD primers previously reported for their reliability and reproducibility, enabling a more targeted and efficient approach. In addition, this research incorporated a genetic similarity analysis using DARwin 6.0 software to construct a dendrogram, providing insight into the genetic relationships among the selected cultivars—an aspect that was not addressed in (Devaraja *et al*., 2014).

This highlights the importance of continuing molecular characterization efforts, as such studies aim to investigate morphological and molecular diversity among *Philodendron* cultivars in Larch Studio Nursery. These efforts are essential for developing new hybrids and conserving genetic resources.

MATERIALS AND METHODS

This research was conducted at the Plant Tissue Culture Laboratory, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, from December 2021 to December 2022. *P. burle-marx*, *P. micans*, *P. lacerum*, *P. erubescens*, and *P.* 69686 became the primary materials for this research, DNA extraction was performed using the Plant Genomic DNA Kit (Tiangen®, Cat. number GDP305-02). A total of nine primers were used in this study, including OPA-1, OPA-3, OPA-7, OPA-16, OPC-9 (Proligo, Japan), OPD-13, OPE-19, and OPN-16, OPN-19 (IDT, USA). The research comprised several stages, including plant preparation, morphological observation, DNA extraction, primer amplification, visualization by electrophoresis, analysis of electrophoresis results, and construction a dendrogram by DARwin 6.0 software (Perrier & Jacquemoud-Collet 2006).

Plant preparation. Five different *Philodendron* varieties were collected from Tanema Nursery by Larch Studio (Kemang, Jakarta). All plant samples subsequently maintained in the Atma Jaya Catholic University of Indonesia greenhouse and conditioned by periodic application of 2% Bactocyn solution.

Morphological observation. Morphological observations were conducted on each *Philodendron* variety prior to DNA extraction. The observed traits included leaf shape, leaf color, leaf size (length and width), and overall plant height. Measurements were taken using a 30 cm ruler for plant height and leaf dimensions, while leaf shape and color were documented through visual inspection and photography under natural lighting. Each plant was labeled and photographed to ensure accurate comparison.

DNA extraction & PCR amplification. For individual plant analysis, DNA for each sample was extracted from three to four leaves following the protocol of the Plant Genomic DNA Kit (Tiangen®, Cat. number GDP305-02). Each *Philodendron* variety (*P. erubescens*, *P. 69686*, *P. burle-marx*, *P. lacerum*, and *P. micans*) was represented by a single plant, with no biological replication performed. The study focused on assessing the capacity of selected RAPD primers to reveal genetic differences

among the varieties. *Coleus* sp. was used as an outgroup. The concentration of extracted DNA was measured using a NanoDrop™ 2000 to determine Polymerase Chain Reaction (PCR) mix. A total of nine 10-mer oligonucleotides primers were used in the RAPD analysis (Table 1). The PCR reaction mixture consisted of 5µL GoTaq® master mix, 0.5 µL primer, 3 µL DNA template, and 1.5 µL nuclease-free water (NFW). The PCR thermal profile, modified from Devaraja *et al*. (2014), included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minutes, annealing at 37°C for 1 minutes, and extension at 72°C for 2 minutes, with a final extension at 72°C for 8 minutes. PCR-RAPD products were visualized on 2% agarose gels in 1× TAE buffer, electrophoresed at 80 volts for 90 minutes, and DNA fragments were measured using a 1-kb DNA ladder marker (Geneaid, Cat. Number DL006).

PCR result visualization & molecular weight measurement. Visualization of PCR products was performed by staining the agarose gel with ethidium bromide, followed by transillumination under short-wavelength UV light. Nine 10-mer primers, namely OPA-1, OPA-3, OPA-7, OPA-16, OPC-9, OPD-13, OPE-19, OPN-16, and OPN-19 (Table 1) were initially screened to assess their reproducibility. Primers that produced weak bands and failed detect clear polymorphisms patterns were excluded from further analysis (Ram *et al*., 2008). The specific-species bands were analyzed and measured by following protocols by (Matsumoto *et al*., 2019).

Data analysis. The amplified bands were scored as 1 (present) or 0 (absent) to generate a binary data matrix, with each band considered as a distinct locus. The genetic dissimilarities were calculated according to dice as follows:

$$Dij = \frac{b+c}{2a+(b+c)},$$

Notes:
Dij = Dissimilarity between units i and j
a = Number of variables where xi = presence, and xj = presence
b = Number of variables where xi = presence, and xj = absence
c = Number of variables where xi = absence, and xj = presence

The binary data werethen analyzed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to construct a dendrogram based on hierarchical clustering, utilizing DARwin 6.0 software (Perrier & Jacquemoud-Collet, 2006).

RESULTS AND DISCUSSION

Based on observations, the samples variant exhibited significant differences in morphological appearance. Therefore, it can be concluded that all five *Philodendron* species under study displayed notable variation in their morphological characteristic (Figure 1). *Philodendron lacerum* had the largest leaf size and the greatest vegetative plant height. The leaf shape of each species also differed from one another: *P.69686* (1) had a Y-like leaf, *P. burle-marx* (2) and *P. lacerum* (5) had hastate leaves, *P. micans* (3) had a cordate form, and *P. erubescens* (4) had a lanceolate leaf shape. Leaf coloration also varied among the samples: *P. lacerum* and *P. micans* displayed light green, *P. burle-marx* showed dark green, *P.*69686 exhibited semi-dark green, and *P. erubescens* presented varigated leaves, which may happen due to mutation (Figure 1). A summary of the morphological traits is presented in Table 2. However, no consistent relationship was observed between morphological traits and the genetic groupings generated by RAPD analysis. Although each variety exhibited distinct differences in leaf shape, size, and color, morphological features were not reflected in the clusters formed by OPD-13 and OPN-16 primers.

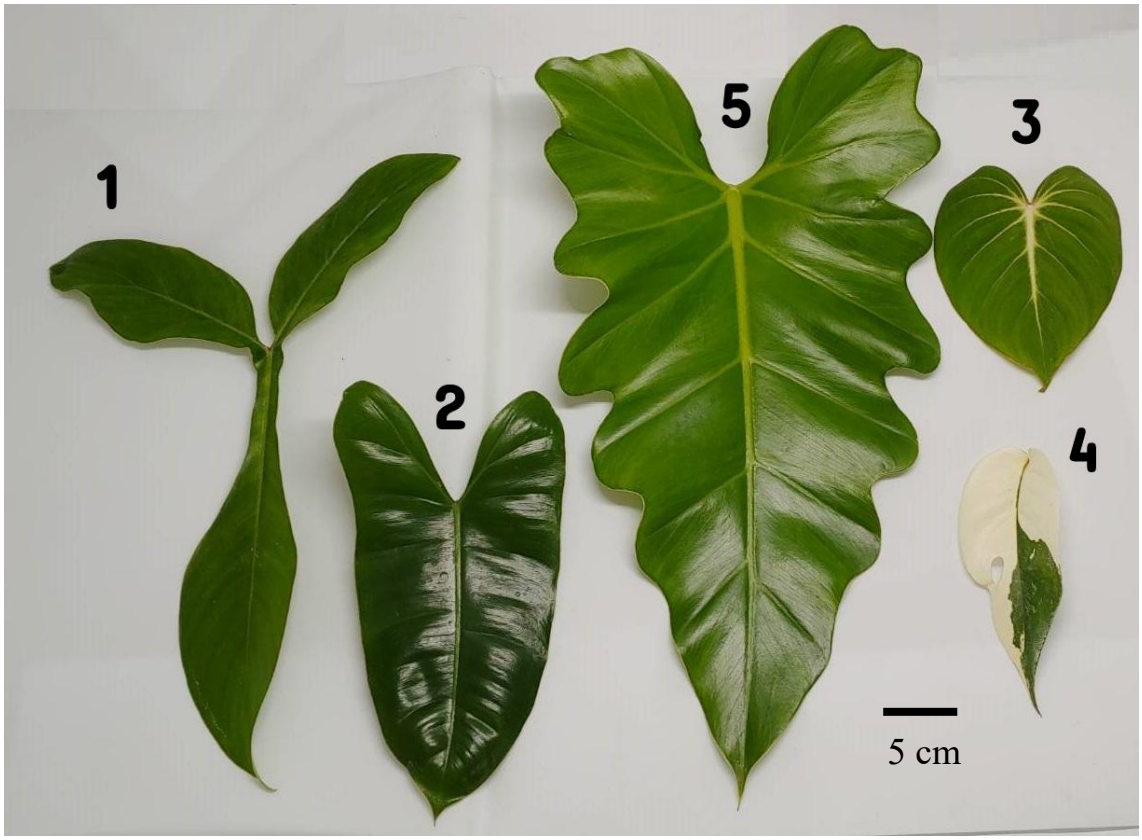

Fig. 1. Leaf morphology. 1: *P.*69686, 2: *P. burle-marx*, 3: *P. micans*, 4: *P. erubescencs*, 5: *P. lacerum*

Out of the nine primers screened in this study (Table 1, Figure 2), two primers OPD-13 and OPN-16—were considered effective in distinguishing between different *Philodendron* species. These primers produced clear, distinct polymorphic bands and generated a greater number of bands compared to the other primers (Figure 3). A total of 22 bands were detected from primer OPD-13, and 19 bands were detected using primer OPN-16 (Table 1). These two primers produced the clearest and most distinct polymorphic bands among all primers tested. Amplification with OPD-13 in species-specific bands at 2250 bp in *P. erubescens* and 314 bp *P. lacerum,* while amplification with OPN-16 produced bands in 1741 bp in *P. erubescens*, 680 bp in *P. burle-marx*, and 300 bp in *P. lacerum*, indicating the presence of species-specific diagnostic markers capable of distinguishing among the five *Philodendron* species. Both primers generated 100% polymorphic bands (Table 1). The size of the amplified products ranged from 250 to 2500 base pairs.

Table 1. Primer for RAPD analysis

Primer	Sequence (5'-3')	Total amplified bands	Percentage polymorphic bands (%)
OPA-1	CAGGCCCTTC	10	100
OPA-3	AGTCAGCCAC	11	100
OPA-7	GAAACGGGTG	7	100
OPA-16	AGCCAGCGAA	8	100
OPC-9	CTCACCGTCC	4	100
OPD-13	GGGGTGACGA	22	100
OPE-19	ACGGCGTATG	11	100
OPN-16	AAGCGACCTG	19	100
OPN-19	GTCCGTACTG	7	86

The genetic relationship among the *Philodendron* variants were analyzed using UPGMA cluster analysis, which revealed that all five varieties could be differentiated using RAPD markers. Although the dendrograms constructed from primers OPD-13 and OPN-16 showed different clustering patterns,

both revealed relatively low genetic relatedness among the genotypes, suggesting a high level of divergence. The dendrogram generated using primer OPD-13 formed two major clusters. Cluster 1 comprised *P. burle-marx* (2) and *P.* 69686 (1), while Cluster 2 included *P. lacerum* (5) and *P. erubescens* (4). In contrast, the dendrogram generated using OPN-16 formed a single cluster consisting of *P. micans* (3) and *P.69686* (1) (Figure 2). These dendrograms were constructed independently to evaluate the discriminatory power and consistency of each individual primer in resolving genetic variation. Since this study serves as a preliminary assessment, primer-specific analysis was prioritized to identify those producing the clearest and most informative banding patterns. Although combining data from both primers could potentially improve genetic similarity resolution, it was beyond the scope of this initial investigation and may be explored in future research involving a greater number of markers and samples.

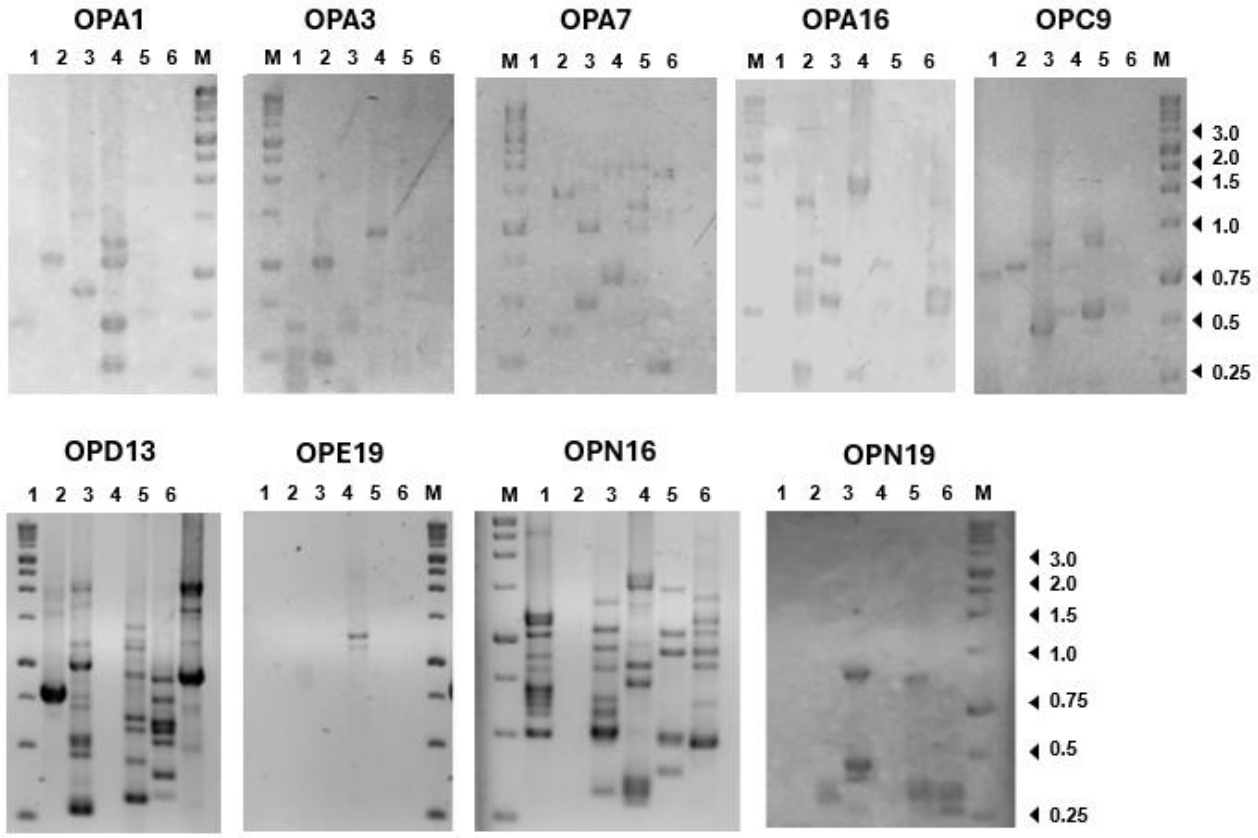

Fig. 2. Electropherogram of nine RAPD primers: OPA-1, OPA-3, OPA-7, OPA-16, OPC-9, OPD-13, OPE-19, OPN-16, dan OPN-19

Table 2. Morphological characteristics of five *Philodendron* varieties observed in the greenhouse prior to DNA extraction

Philodendron variety	Leaf shape	Leaf color	Leaf size	Plant height	Notes
P. 69686	Y-like	Semi-dark green	Moderate	Moderate	–
P. burle-marx	Hastate	Dark green	Moderate	Moderate	–
P. micans	Cordate	Light green	Moderate	Moderate	–
P. erubescens	Lanceolate	Variegated	Moderate	Moderate	Color variation may be mutation
P. lacerum	Hastate	Light green	Largest	Tallest	–

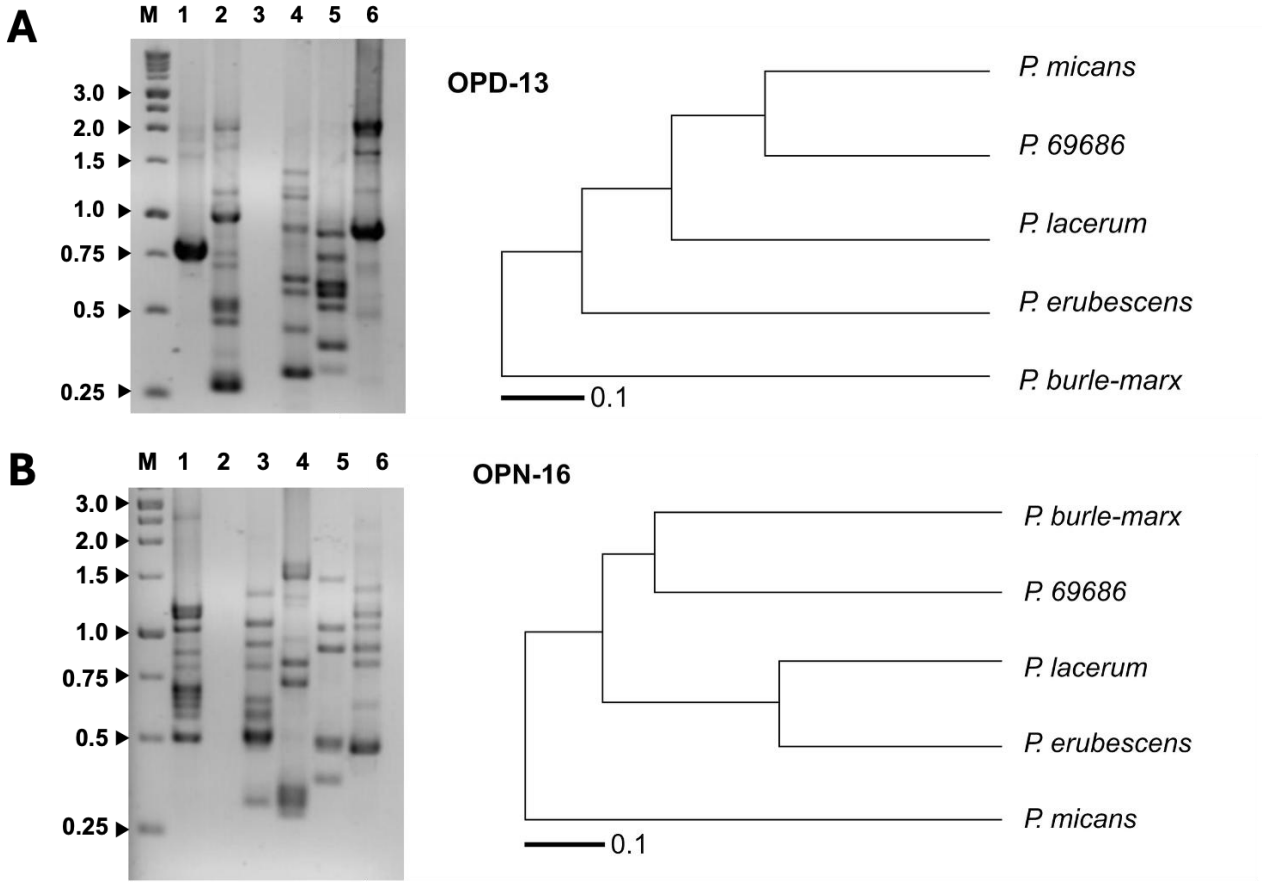

Fig. 3. RAPD electropherogram (left) and their respective dendrogram (right) using selected primer OPD-13 (A) and OPN-16 (B); M: 1 kb ladder, 1: *P*.69686, 2: *P. burle-marx*, 3: *P. micans*, 4: *P. erubescencs*, 5: *P. lacerum,* 6: *Coleus* sp. (outgroup)

Based on the morphological characteristics observed, genetic differences among the species were anticipated and subsequently confirmed through RAPD analysis. The high level of polymorphism detected suggests that the studied Philodendron species are highly heterozygous. This may be attributed to an open pollination system, which promotes genetic recombination, or to extensive propagation through tissue culture, which can result in soma clonal variation. Furthermore, *Philodendron* is widely recognized for its inherent morphological variability, with individuals of the same species often displaying considerable differences leaf shapes, sizes, and color. This high phenotypic enables *Philodendron* species to a wide range of climatic conditions (Croat *et al*., 2002).

P. micans did not generate any bands with primer OPN-16, and *P. burle-marx* showed similarly failed to amplify with primer OPD-13. This absence of amplification could have been caused by several factors. One likely explanation is the lack of homology between the primers and their respective binding sites on the DNA template, which would prevent proper annealing. This is supported by the fact that using the same template with a different primer (OPN-16) successfully generated RAPD amplicons. These possibilities are supported by Probojati *et al*. (2019). Probojati et al. (2019), who emphasized the importance of primer-template compatibility in successful amplification. Furthermore, Tuwo *et al*. (2021) reported that the performance of primer OPN-16 varies significantly across plant species and PCR conditions, reinforcing the need for species-specific optimization (Tuwo *et al*., 2021). Therefore, further investigations, including optimization of PCR protocols, redesigning primers targeting conserved regions, or sequencing the target loci, are recommended to comprehensively understand and overcome amplification failures observed in *P. micans* and P. *burle-marx.*

The dendrograms constructed from RAPD markers revealed distinct clustering patterns among the *Philodendron* varieties. However, no consistent relationship was observed between these genetic groupings and the morphological traits of the samples. Although each variety exhibited clear differences in leaf shape, size, and coloration, such morphological characteristics were not reflected in the dendrogram clusters formed by primers OPD-13 and OPN-16. For example, *P. micans* and *P. 69686* clustered together using primer OPN-16, despite displaying markedly different leaf morphologies. This suggests that morphological differentiation may not reliably indicate underlying genetic similarity in *Philodendron*, possibly due to phenotypic plasticity or convergent evolution, where similar traits arise independently. Environmental factors might also influence morphological traits without altering the genetic markers detected by RAPD. Therefore, while morphological observation provides valuable phenotypic information, it may not align precisely with molecular-based classification, highlighting the importance of combining both approaches in diversity studies.

Recent studies have highlighted that combining RAPD with other marker systems such as ISSR and SSR can enhance the accuracy of genetic diversity assessments, particularly ornamental plants (Mei *et al*., 2015). While RAPD markers are widely used due to their simplicity and cost-effectiveness, they are known to have low reproducibility and are prone to generate spurious bands. To address these limitations, it is recommended to perform repeatability tests and to standardize PCR amplification protocols. Furthermore, RAPD markers can be further improved into Sequence Characterized Amplified Region (SCAR) markers, which utilize longer and more specific primers, thereby improving marker reliability (Rajesh *et al*., 2014). To further increase the credibility of results, additional molecular markers such as Inter Simple Sequence Repeats (ISSR), Amplified Fragment Length Polymorphism (AFLP) may be incorporated to complement RAPD analysis (Donahoo *et al*., 2013), For instance, (Debajit *et al*., 2015), demonstrated that the combined use of RAPD and ISSR markers provided more effective resolution in determining the genetic relationships among *Cymbopogon flexuous* cultivars compared to the use of either marker alone.

In the initial step of pre-PCR processing DNA extraction plays a critical role in the success of the overall PCR process. At the end of the extraction process, DNA concentrations ranged from 8.6 to 33.9 ng/μL with A260/280 ranged from 1.80 to 2.34. However, the A260/230 ratios were low, ranging from 0.21 to 0.63, indicating substantial contamination by organic compounds such as polysaccharides and polyphenols. These impurities can act as PCR inhibitors and negatively affect amplification efficiency and product quality (Rådström *et al*., 2004). The inhibitory effects of such compounds may result from DNA precipitation, denaturation, or interference with the ability of the DNA polymerase enzyme to bind magnesium ion (Demeke & Jenkins, 2010). The type of sample material used for DNA extraction also influences the yield and purity of the resulting DNA. High-quality, intact DNA is typically obtained from finely ground grain and oilseeds samples. In contrast, highly sheared DNA may provide an insufficient template for PCR, leading to reduced detection sensitivity and impaired quantification (Kumar *et al*., 2016).

RAPD primers are randomized and do not require prior information for use. Therefore, preliminary primer screening before RAPD analysis is recommended to minimize resources waste (Samal *et al*., 2003). In this study, the limited number of RAPD primers tested reduced the likelihood of identifying the most suitable primers. Recent research has demonstrated that combining RAPD with other marker systems, such as ISSR, can enhance the resolution and reproducibility of genetic diversity analyses, particularly in plant species with complex or narrow genetic backgrounds (Abdelhamid *et al*., 2024). Primers, which are suitable for RAPD analysis, should produce clear, amplified PCR products and generate as many polymorphic markers as possible. Annealing temperature significantly influences band quality: lower temperature may cause primer to anneal to non-specific sites, whereas higher temperatures can lead to smearing and DNA denaturation (Larekeng *et al*., 2019). To further validate and strengthen the findings obtained through RAPD, it is recommended to incorporate additional molecular approaches such as quantitative PCR (qPCR), DNA sequencing, or more robust genetic markers like SSRs or SNPs (Gineikiene *et al*., 2009). These

methods can confirm the polymorphisms observed and provide more reliable insights into genetic relationships.

CONCLUSION

In the present study, all tested samples exhibited high genetic diversity and showed no significant relatedness. From a molecular perspective, nearly all amplified bands were polymorphic, indicating that the samples were genetically distinct from one another. Morphologically, the *Philodendron* did not share a common trait or significant characteristic. The dendrogram generated primers OPD-13 and OPN-16 revealed different clustering patterns, and the distinct banding profiles prevented the construction of a consensus tree. In addition, the primers were too few, decreasing the similarity rate of one primer with the others. Furthermore, the limited number of primers used likely contributed to the low similarity rates observed between primer profiles. Therefore, concludes that the two selected primers were insufficient for comprehensive molecular analysis. Future research should consider employing more sensitive molecular markers, such as AFLP or RFLP, to enhance genetic diversity assessment. Such approaches could facilitate more accurate genetic profiling and support the strategic combination of traits for the development of potential hybrid lines.

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