BIODIVERSITAS
Volume 24, Number 9, September 2023
Pages: 5015-5021

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240945

Genetic similarity among *Dendrobium* species from Indonesia using RAPD markers

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Manuscript received: 12 July 2023. Revision accepted: 27 September 2023.

Abstract. *Hartati S, Samanhudi, Cahyono O. 2023. Genetic similarity among* Dendrobium *species from Indonesia using RAPD markers. Biodiversitas 24: 5015-5021. Dendrobium* genus orchids are one of the most popular commodities in the world due to their diverse range of flower sizes, shapes, and colors. To enhance plant breeding programs and genetic resources, it is necessary to obtain information on genetic similarity between orchids of the *Dendrobium* genus through molecular analysis techniques. Therefore, this study aimed to assess the genetic similarity among five *Dendrobium* species using molecular markers, specifically RAPD. The plant material used was obtained from five species of the *Dendrobium* spp, namely (i) *Dendrobium mirbelianum*; (ii) *Dendrobium lamellatum,* (iii) *Dendrobium secundum,* (iv) *Dendrobium bracteosum,* and (v) *Dendrobium purpureum*. The analysis was carried out to determine the genetic diversity of the *Dendrobium* orchids using RAPD markers. A total of five RAPD primers were used for amplification, namely OPD 8, OPA 7, OPA 13, OPB 12, and OPB 18. The scoring data were analyzed using NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) version 2.02 which produced data in the form of cluster dendrograms. The dendrogram that was constructed by Unweighted Pair Group Method Using Arithmetic Average (UPGMA) classified the five *Dendrobium* species into two main clusters. The results showed that there were two clusters, namely Cluster A consisting of *D. mirbelianum*, *D. lamellatum*, and *D. secundum* while Cluster B consisted of *D. bracteosum* and *D. purpureum*. Furthermore, the polymorphism of the five RAPD primers was very high, ranging from 91.6 to 100%, measuring 250-1900 bp. The coefficient of genetic similarity analyzed using the five RAPD primers ranged from 0.24 to 0.77. The species *D. mirbelianum* and *D. lamellatum* had a high coefficient of genetic similarity. Which can be discovered through parental selection in breeding program.

Keywords: Dendrogram, flowers, primer, similarity

INTRODUCTION

Dendrobium genus orchids are highly popular commodities worldwide, both as potted plants and cut flowers, due to their diverse range of flower sizes, shapes, and colors. These flowers flourish throughout the year or seasonal as per the breeding season and have significant potential in the cut flower industry. This is in accordance with the opinion of De et al. (2013) who stated that *Dendrobium* is a popular potted plant and cut flower around the world due to its floriferousness and long vase life. Another opinion was also explained by Tang et al. (2020) that although *Dendrobium* species originate from China, Japan, the Philippines, Thailand and other countries, they are mainly distributed in tropical and subtropical regions in Asia and Oceania.

Apart from the ornamental value, *Dendrobium* orchids can also be used as medicinal plants. Moreover, the use of orchids as traditional medicine, specifically the genus *Dendrobium* (Orchidaceae), has been practiced in China for more than 2300 years. In traditional Chinese medicine, the dried stems of this species are recognized as a superior tonic. The *Dendrobium* orchids also nourish the kidneys, produce lost body fluids, and reduce stomach disease (Cheng et al. 2019).

Genetic diversity in a population of a species is the variation necessary for the selection of plant improvements through crossbreeding. This genetic diversity must be maintained because it is important to obtain new traits. Recently, there has been increased awareness of the importance of biodiversity, including agricultural biodiversity, conservation for sustainable use and development.

According to Hartati et al. (2019), crossbreeding must be carried out to increase the genetic diversity of orchids due to the decline in their natural habitats. Crosses of *Dendrobium* orchids which have potential as ornamental and medical flowers are needed to create superior hybrids with desired properties. Orchids can easily cross with different genera because they have a weak reproductive barrier. In 2015, the Royal Horticulture Society registered over 250,000 cultivars of orchids and more than 110,000 hybrids with significant phenotypes. Approximately 3,000 new hybrid orchids are added every year and become the most diverse commodity from other plants. Although most of the orchid hybrids are derived from intergeneric crosses, *Dendrobium* hybrids have mainly been obtained from

intrageneric crosses produced by species differences and similarities in the genus (Cheng et al. 2019).

Information on genetic diversity and relationships between orchids of the *Dendrobium* genus through molecular analysis techniques is needed in plant breeding programs and genetic resources. This is because the genetic diversity in *Dendrobium* orchids population, resulting from plant breeding programs can lead to increased adaptability in new areas. However, orchid breeders face the challenge of increasing genetic diversity to improve productivity or stimulate economic growth. The combination of rising demand and increased genetic diversity will increase income while maintaining the sustainability of *Dendrobium* orchids (Lopes et al. 2015).

Molecular analysis is needed to obtain more accurate information about genetic diversity than morphological analysis, which can be influenced by environmental conditions and plant growth. Genetic diversity is an importantstep in the improvement of hybrid crosses. In this study, the molecular marker used is RAPD (Random Amplified Polymorphic DNA). RAPD uses a primer with a short sequence of 10 nucleotides that is used to amplify the target DNA sequence randomly, thereby generating high levels of polymorphism (Khor et al. 2020).

Determination of the parentage of crosses in orchids is carried out by assessing the genetic diversity of a species through genetic analysis. The RAPD marker was used to analyze genetic similarity between species. The random primer approach in RAPD allows the assessment of the relationship between plant species. One of the advantages of the RAPD molecular analysis is its fast implementation. Furthermore, RAPD is considered a reliable technique due to its easy usability, affordability, cost-effectiveness, and the requirement of only a small amount of DNA template (Duangrath and Sumontip 2020). However, there are significant issues with the reproducibility of RAPD profiles. Therefore, it is essential to maintain highly tight and constant PCR reaction conditions in order to replicate RAPD profiles. Basically, meticulous attention to RAPD protocols is essential since the creation of RAPD profiles is extremely sensitive to the circumstances of the PCR reaction (Vekariya et al. 2017). Another significant flaw is that RAPD is a dominant marker, which limits the quality of the data (Amiteye 2021). However, recent improvements have made the RAPD method more effective marker methods like SCAR, SRAP, and CAPS (Babu et al. 2021).

Plant propagation and use of genetic resources require knowledge of genetic diversity and relationships between species. The analysis can be carried out using molecular markers, which involves the assessment of genetic distance, genetic diversity, genome analysis, and developing molecular. maps. Identification of molecular markers is also needed to achieve the desired plant breeding results. In this study, RAPD were used as markers due to their cost-effectiveness, simplicity, and ability to be performed in most modern laboratories. RAPD technique is useful in plant breeding because it has a simple and fast procedure, requires a low amount of DNA, and is effective for hybrid characterization without using complicated processes (Choopeng et al. 2019). Compared to traditional methods, DNA-based molecular marker approaches are considered more advanced due to their high authenticity and consistency. However, the selection of suitable techniques and markers should be based on the nature of the genetic structure of the species (Borah et al. 2021). According to several publications, the RAPD method has been widely used to determine the genetic relationships in different orchid species, such as *Vanda coerulea* (Manners et al. 2013), *Rhynchostylis retusa* L. (Permatasari et al. 2018), *D. chrysotoxum* Lindl (Tikendra et al. 2019b), *Rhynchostylis gigantea* (Duangrath and Sumontip 2020), *Spathoglottis plicata* (Auvira et al. 2021), *Coelogyne* (Hon et al. 2021), and *Phalaenopsis amabilis* (Mursyidin et al. 2022). Therefore, this study aimed to examine the genetic similarity of *Dendrobium* orchids to provide basic information on plant breeding. The novelty of this research is that parents who have a large genetic similarity are found to be crossed, which will eventually produce new hybrid orchids.

MATERIALS AND METHODS

Plant materials

This study was conducted at the Laboratory of Plant Breeding, Faculty of Agriculture, Sebelas Maret University, Surakarta, located in Surakarta, Central Java, Indonesia. Five species are used from the genus *Dendrobium* spp., namely (i) *D. mirbelianum*, (ii) *D. lamellatum*, (iii) *D. secundum*, (iv) *D. bracteosum*, and (v) *D. purpureum*. RAPD primers: OPD 8, OPA 7, OPA 13, OPB 12, and OPB 18

Procedures

Orchid DNA was extracted using a method in which CTAB was modified by the addition of RNase treatment until a final concentration of 250 µg mL^{-1} was obtained (Poerba and Ahmad 2013).

In this study we used five RAPD primers from the 20 primers (Operon Technology Ltd) that were selected previously. The five selected primers, namely OPD-8 (*Dendrobium* (Choopeng et al. 2019); OPA-7 (*Dendrobium* (Tikendra et al. 2019a), *Coelogyne* (Hartati 2020), *Phaius* (Hartati et al. 2021), *Rhynchostylis retusa* (Oliya et al. 2021), *Dendrobium* (Joshi et al. 2023)); OPB-12, OPA-13 and OPB-18 (Coelogyne (Hartati 2020), Phaius (Hartati and Samanhudi 2022) have been shown to produce polymorphic bands in orchids.

Table 1. *Dendrobium* spp. orchids and origin area

Code	Species name	Origin area
1	*D. mirbelianum*	Java
2	*D. lamellatum*	Kalimantan
3	*D. secundum*	Sumatera
4	*D. bracteosum*	Papua
5	*D. purpureum*	Sulawesi

PCR reaction was performed with a total volume of 15 µL containing 0.2 n MdNTP; 1 x reaction buffer; 2mM $MgCl_2$; DNA sample 25 ng; 1 pmol; and 1 unit of Taq DNA polymerase (Promega) using a Thermocycler (Takara) for 45 cycles. Denaturation at 94°C for 2 min, followed by 45 cycles consisting of 1 min of denaturation at 94°C, 1 min of incubation at 60°C and 2 min of extension at 72°C. After 45 cycles, followed by 5 min of DNA extension at 72°C and cooling at 25°C. PCR amplification results were shown on a 1.5% agarose gel in Tris Acetic Acid EDTA buffer by electrophoresis using Mupid Mini Cell for 50 min at 50 volts. Then soak in ethidium bromide solution with final concentration of 1 µL/100 mL for 10 minutes. The resulting DNA fragmentation is detected by a UV generator and then photographed with a camera. Standard size marker of 100 bp plus DNA scale (Fermentas) is used to determine the band size of DNA amplification results.

Data analysis

Genetic similarity was assessed by scanning DNA bands, which were translated into binary data. A value of 1 indicated the presence of a band while a value of 0 signified an empty band. The analysis was carried out in clusters using NTSYSpc (Numerical Taxonomy System) version 2.02 with UPGMA (Unweight Pair Group Method Arithmetic). The clustering method used the DICE SIMQUAL (Similarity for Coefficients Qualitative Data) and SAHN (Sequential Agglomerative Hierarchical and Nested). In this study, the genetic similarity was shown using a dendrogram.

RESULTS AND DISCUSSION

DNA amplification

The results of the amplification were electrophoresed and visualized as shown in Figure 2. A total of five RAPD primers were used for amplification, namely OPD 8, OPA 7, OPA 13, OPB 12, and OPB 18. Subsequently, the results were electrophoresed to separate DNA bands based on size. Assessing Genetic similarity through morphological characters was very difficult, due to the variability caused by plant growth conditions. According to The et al. (2020), the estimation of genetic similarity by RAPD markers was influenced by the specific genome of the accession selected and the specific RAPD primers examined.

Amplification using RAPD OPD 8 primers produced the same bands on *D. mirbelianum* and *D. lamellatum* with a DNA band size of 450 bp, 550 bp, and 850 bp. The DNA bands produced by OPD 8 measuring 650 bp, 750 bp, 800 bp, 1000 bp, and 1100 bp specifically amplify the orchid *D. bracteosum*. Amplification using RAPD OPA 7 primers produced the same bands on *D. mirbelianum, D. lamellatum* and *D. bracteosum* with a DNA band size of 650 bp, 800 bp. At 1650 bp amplifying DNA of orchids *D. secundum* and *D. purpureum.*

Amplification using RAPD OPA 13 primers showed that at sizes 400, 650, and 1150 bp, the orchids *D. secundum, D. bracteosum,* and *D. purpureum* had DNA bands of the same size. Furthermore, DNA bands at sizes 450, 750, 900, and 1100 bp in orchids *D. bracteosum* and *D. purpureum* had the same band size. The *D. mirbelianum* and *D. lamellatum* had the same band size, measuring 800 bp. Monomorphic bands were found at the size of 500 bp, indicating that the species *D. secundum, D. bracteosum, D. purpureum, D. mirbelianum*, and *D. lamellatum* amplified DNA bands at the same size and locus due to their genetic similarities. There were also specific alleles at sizes 475, 550, and 1000 bp which only amplified 1 genotype at a certain size. Amplification using RAPD OPB 12 primers primers produced the same bands on *D. mirbelianum* and *D. lamellatum* with a DNA band size 550 bp, and 750 bp. The orchids *D. secundum, D. bracteosum* had DNA bands of the same size at 450 bp. *D. mirbelianum, D. lamellatum* and *D. bracteosum* amplified DNA bands at the same size of 750 bp. Amplification using RAPD OPB 18 primers produced the same bands on *D. lamellatun, D. secundum* and *D. purpureum* with a DNA band size of 400 bp. *D. bracteosum, D. purpureum* the same size of 1100 bp.

The presence of the same DNA band at a certain size in *D. secundum, D. bracteosum,* and *D. purpureum* indicated that these orchids had higher genetic similarity to other orchids and tended to be in the same cluster, as presented in Figure 2. The similarities of size and loci in the amplified DNA bands in the samples were high for each species, indicating that the genetic similarity between these species was low. All species in the RAPD OPA 13 primer could be amplified, as highlighted by Zakiyah et al. (2019), where a high number of amplified bands increased the percentage of polymorphism, indicating the effectiveness of the marker used to amplify each genotype. The similarity in DNA band sizes and the presence of DNA bands at the same locus indicated the similarity between the analyzer genotypes.

Amplification using RAPD OPB 12 primer showed that at sizes 250, 550, and 750 bp the *D. mirbelianum* and *D. lamellatum orchids* had the same size. DNA bands with a size of 700 bp in orchids *D. bracteosum* and *D. purpureum* had the same band size. Furthermore, DNA bands measuring 450 bp in *D. secundum,* and *D. bracteosum* had the same band size. Kurniawati et al. (2019) used RAPD as a genetic marker to characterize several *Dendrobium* orchids. Based on RAPD results using RAPD OPA10 and OPA18, *D. anosmum gigantea alba* and *D. anosmum gigantea* showed different genetic characteristics but some DNA fragments had the same size (918 bp and 492 bp). Interspecies orchids *D. fimbriatum* and *D. crumenatum* showed the same number of 4 fragments, with one identical size of 1424 bp. Oliya et al. (2021) also used RAPD markers to evaluate the genetic stability of micro individuals of the epiphytic orchids species *Rhyncostylis retusa*, with 10 random primers used to amplify RAPD markers in six samples, consisting of five in vitro regenerants and their parent plants which produced 23 RAPD bands ranging in size from 275 bp to 1,100 bp were produced yielding PIC values ranging from 0.28 in OPC-11 to 0.50 in OPA-3, OPA-6, OPA-07, and OPB-08, respectively.

Table 2. RAPD markers used for DNA amplification

Primers	Nucleotide sequence (5'- 3')
OPD 8	GTGTGCCCCA
OPA 7	GAAACGGGTG
OPA 13	CAGCACCCAC
OPB 12	CCTTGACGCA
OPB 18	CCACAGCAGT

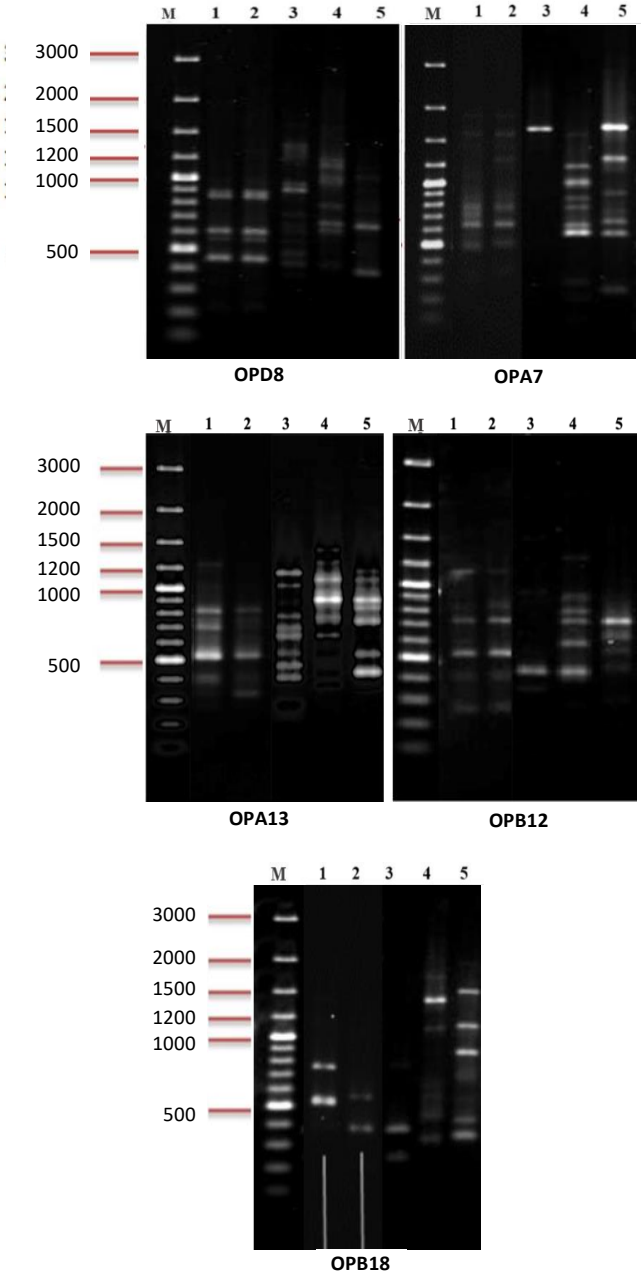

Figure 1. Results of DNA amplification with primers OPD 8, OPA 7, OPA 13, OPB 12, OPB 18. 1: *D. mirbelianum*, 2: *D. lamellatum*, 3: *D. secundum*, 4: *D. bracteosum*, 5: *D. purpureum*

The low polymorphism indicated a fairly high genetic stability of the micro-propagated *R. retusa* individuals. Mursyidin et al. (2022) discovered that each primer produced a different number and size of DNA fragments. The *Phalaenopsis amabilis* that was analyzed using RAPD OPA-10. and OPB-05 produced the least number of DNA fragments, 7 bands, while OPB-06 yielded 32 bands, the largest number of DNA fragments. According to Aljanabi and Alhasnawi (2021), the total number of fragments generated by the six primers used (OPE A-10, OPE G-14, U-17, OPE K-02, OPA-18, and M 32) by molecular markers among five apricot genotypes was 80 with an average of 13.33 primer fragments.

There were DNA bands with different thicknesses in each primer due to differences in the concentration and purity of the DNA sample. RAPD primers bind to complementary DNA base sequences, allowing for DNA amplification. The results of DNA band amplification were visualized usinggel electrophoresis. Larekeng et al. (2019) reported that annealing temperature during the amplification process in PCR affected the quality of the resulting band. Furthermore, it was discovered that excessively high or low annealing temperatures can hinder primers attachment to the target DNA sequence. In this study, the DNA bands produced in each sample were different in number because the primer had a distinct sequence and amplified the sample DNA in different amounts. Primers that had various annealing temperatures increased the production of the resulting DNA bands. In addition to polymorphism, the quality of the amplified bands was also an important factor in primer selection. This was because incorrect primers resulted in less clear DNA bands and reduced the accuracy of the data when analyzed. Tikendra et al. (2019a) employed RAPD and ISSR markers to test genetic homogeneity in *D. moschatum* orchids. Out of 18 arbitrary RAPD primers screened, 10 primers produced 48 bands with an average of 4.8 bands per primer and band sizes ranging from 250 to 2500 bp. The RAPD analysis showed a low polymorphism level of 4.8% among regenerated orchids and parent plants.

The results of the electrophoresis that had been visualized were analyzed by scoring, where a score of 1 was assigned to the visible band and 0 to the invisible band. This scoring must be carried out carefully to ensure the results obtained are in line with expectations. Smear under the DNA band reduced the accuracy of the data used for analysis because the DNA produced from isolation had a low concentration and purity. Meanwhile, the drawback of the leaf extraction method included the high accumulation of phenolics and polysaccharides. This was because the combination of polysaccharides with genomic DNA yielded a thick and adhesive DNA, posing difficulties in amplification. According to Inglis et al. (2018), yellow or brown DNA samples indicated contamination with polyphenols. The extraction method using CTAB as a lysis buffer and PVPP reduced the polyphenol content in the sample. The percentage of polymorphism for the five primers in *Dendrobium* spp. was presented in Table 3.

Table 3. Percent polymorphism of 5 primers on *Dendrobium* spp. in RAPD PCR reactions

Primer	Sequence 5'3 to 3'	Size (bp)	Amplified band	Polymorphic band	Monomorphic band	% Polymorphic	% Monomorphic
OPD 8	GTGTGCC CCA	400-1300	12	12	0	100	0
OPA 7	GAAACGG GTG	250-1900	15	15	0	100	0
OPB 12	CCTTGAC GCA	250-1200	13	12	1	92.3	7.7
OPA 13	CAGCACC CAC	300-1200	16	15	1	93.75	6.25
OPB 18	CCACAGC AGT	250-1750	12	12	0	100	0

DNA bands ranging from 250 to 1900 bp were successfully amplified by five primers, exhibiting a high polymorphism of 91.6-100%. The OPD 8 primer generated amplified 12 bands with a size of 400-1300 bp, resulting in 100% polymorphism. Similarly, the OPA 7 primer produced 15 bands with a size of 250-1900 bp, achieving 100% polymorphism. The OPB 12 primer generated 13 amplified bands with a size of 250-1200 bp, yielding a 92.3% polymorphism and 7.7% monomorphism. The OPA 13 primer produced 16 amplified bands with a size of 300-1200 bp, resulting in a 93.75% polymorphism and 6.25% monomorphism. The OPB 18 primer yielded 12 amplified bands with a size of 250-1750 bp and a 100% polymorphism. These results showed that the OPA 13 primer had the highest number of amplified bands due to its complementary to the target DNA. Aloysius et al. (2020) reported that high polymorphism reaching 90-100% was also produced by the *S. plicata* orchids variant. Based on the RAPD analysis, genomic DNA samples from *S. plicata* orchids variants produced 3-12 DNA bands with a 90-100% polymorphism. This indicated that primers showed strong DNA polymorphism in *S. plicata* orchids variants. Tikendra et al. (2019) found that *D. chrysotoxum* Lindl. exhibited RAPD profile of 11 primers (OPA-01, 03, 04, 05, 10, OPC-07, 08, OPD-01, 08, OPF-14, and OPG-15), indicating 98.81% monomorphism and low degree polymorphism of 1.19% among regenerated orchids and parent plants. The striking monomorphic banding patterns show 100% genetic homogeneity between the in vitro clones and the parent plant. According to (Alkhayri et al. 2022), RAPD markers are more effective in identifying genetic polymorphisms based on their polymorphic banding characteristics than ISSR-PCR which produces low polymorphism rates. Characterization using RAPD markers was carried out on *P. tankervillae*, *P. montanus*, *P. collasus*, and *P. amboinensis* using two random primers. The OPA-02 amplification produced 11 PCR bands, while OPA-16 amplification yielded 9 PCR bands (Hartati et al. 2021).

Setting the annealing temperature is an important step in PCR amplification. According to (Noflindawati et al. 2021), the primer attaches to the target DNA sequence at annealing temperature. The calculated temperature refers to the melting temperature value of each primer. Inappropriate annealing temperature prevents the primer from attaching to the target DNA, leading to failed amplification. RAPD has been widely used for polymorphism analysis in orchids, such as *Coelogyne pandurata*. Kurniawati et al. (2019) define polymorphism as the difference in DNA bands observed in agarose gel. The RAPD primers used produced 95.83% polymorphic bands in the amplified orchids *Coelogyne pandurata* with 43 bands, measuring 200-2100 bp. According to Khor et al. (2020), it is very important to demonstrate the obtained RAPD profile and can be achieved using different molecular marker systems. Indeed, RAPD profiling represents only the screening of a small fraction of the entire plant genome.

Cluster analysis

Clusters were grouped based on the similarity matrix, where species with greater genetic similarity were classified in the same cluster. Genetic similarity is the inverse of genetic similarity which is calculated by the formula 1-value of similarity (Table 4). Genetic similarity is described by the value of the similarity matrix. The closer the value to 1, thehigher the similarity between species and the lower the genetic similarity. Matrix the lowest similaritywas found in *D. lamellatum* and *D. bracteosum* orchids with a value of 0.10, while the highest similarity was found inorchids *D. mirbelianum* and *D. lamellatum* with a value of 0.77.

In this study, *D. mirbelianum* and *D. lamellatum* were shown to have a high coefficient of genetic similarity, indicated by a straight line on the dendrogram. Therefore, the species were grouped into the same cluster, as shown in Figure 2. Species in the same cluster tended to have a high similarity of phenotypes or originate from the same geography. Based on the results, *D. mirbelianum* and *D. lamellatum* were in the same cluster but in different geographical areas of Java and Kalimantan. According to (Nasution et al. 2021), geographical area has no impact on the genetic diversity of a species. Although different environmental conditions such as variations in soil, temperature, and rainfall cause distinct clusters of genotypes, populations from the same geographic area tended to cluster together. Figure 2 shows a dendrogram compiled by UPGMA analysis, representing the clustering of genetic similarity coefficient grouped by cluster.

Table 4. Coeficient similarity of five *Dendrobium* species using RAPD markers

	1	2	3	4	5
1	1.00				
2	0.77	1.00			
3	0.29	0.25	1.00		
4	0.19	0.10	0.18	1.00	
5	0.31	0.33	0.35	0.38	1.00

Note: 1. *D. mirbelianum*; 2. *D. lamellatum*; 3. *D. secundum*; 4. *D. bracteosum*; 5. *D. purpureum*

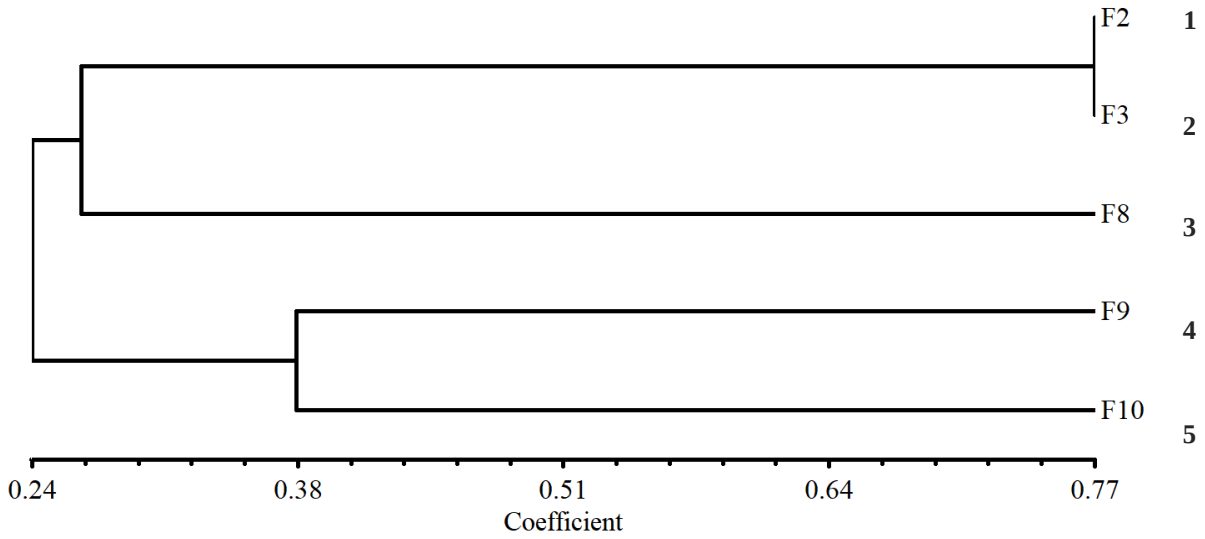

Figure 2. Grouping of five *Dendrobium* spp. based on five RAPD primers. 1. *D. mirbelianum,* 2. *D. lamellatum,* 3. *D.secundum,* 4. *D. bracteosum,* 5. *D. purpureum*

Dendrobium spp. had a genetic similarity with a value of 0.24-0.77. The dendrogram analysis divided the cluster into two main groups based on the similarity coefficient of 0.24. Cluster A contained *D. mirbelianum, D. lamellatum,* and *D. secundum* with a genetic similarity coefficient of 0.77. Cluster B consisted of *D. bracteosum* and *D. purpureum*. The genetic similarity coefficient for this cluster was 0.26. The selection of parents with different clusters increased genetic similarity in hybrid yields. Basavaraj et al. (2020) investigated *Dendrobium* orchids and found two main clusters with a similarity of 0.25-0.36. Based on the results, cluster A was divided into 3 sub-clusters, namely sub-cluster 1 which included *D. aqueum* and *D. crepidatum* with a similarity value of 0.32, and sub-cluster 2 containing *D. macrostachyum* and *D. ovatum* with a similarity value of 0.36. Meanwhile, Cluster B included *D. herbaceum* and *D. jerdonianum* with a similarity value of 0.36. According to Basavaraj et al. (2020), the use of RAPD to determine phylogenetic relationships between species was more relevant, as it grouped species into parents and sub-clusters with the same morphological descriptive characters.

Based on the results, the highest similarity coefficient was observed in *D. mirbelianum* and *D. lamellatum* with a value of 0.77, indicating a high potential for successful hybridization. According to Yuhanna et al. (2021), Dendrogram with RAPD analysis was useful for detecting genetic differences in orchid plants that were difficult to identify based on morphological characters. Generally, typical orchid plants had high and complex diversity patterns. This indicated that individuals with high diversity and heterotic value increased hybrid yield. Meanwhile, the selection of a parent plant with low genetic similarity will complicate the breeding process and reduce the variability of the resulting hybrid. To ensure sustainable agriculture, genetic similarity must be maintained within a population is maintained and prevent its decline. Plant breeding programs also required populations with high genetic similarity as the basis for effective parent selection. Therefore, monitoring the genetic similarity of each species in a population is an important strategy for the agricultural industry.

In conclusion, the analysis of *Dendrobium* spp. with five RAPD primers showed a genetic similarity ranging from 0.24 to 0.77. The high genetic similarity between *D. mirbelianum* and *D. lamellatum* can contribute to the high opportunity of successful inter-specific hybridization between both species provided that other compatibilities exist.

ACKNOWLEDGEMENTS

This study is supported by RISTEK-BRIN with the Basic Research scheme for the 2022 fiscal year with contract numbers 054/E5/PG.02.00.PT/2022 and 469.1/UN27.22/PT.01.03/2022.

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