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Genetic Variability Analysis of Phaius spp Orchid based on RAPD Markers

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***Abstract*—*Phaius spp* live in tropical forests with high humidity due to deforestation, which has resulted in the extinction of many endemic orchid species, especially orchids *Phaius spp,* so it needs to be conserved through a plant breeding program. There are not many research reports on the genetic diversity of orchids *Phaius spp,* even though the study of genetic diversity plays an important role in plant breeding programs and genetic conversation. This study aims to determine the genetic diversity of *Phaius spp. This* research was carried out using molecular markers RAPD (*Random Amplified Polymorphic DNA*). DNA from *Phaius spp* was extracted and then amplified by PCR using 6 RAPD primers. The results of this study found that four species of orchids of the genus *Phaius spp* (*Phaius tankervillae* (1); *Phaius montanus* (2); *Phaius collasus* (3); and *Phaius amboinensis* (4)) amplified using 6 RAPD primers to produce 37 DNA bands with a size of 250-1600 bp, and produce 100% polymorphic bands The genetic similarity coefficient of the 4 orchid species ranges from 0.19-0.53 and the genetic variability ranges from 47%-81%.**

***Keywords*— Genetic diversity; orchid; *Phaius spp*; polymorphic; RAPD.**

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I. INTRODUCTION

Indonesia is a tropical country with environmental conditions that qualify for the life of orchid plants. Diversity of orchid species is found in tropical forests with altitudes between 500 masl and 1500 masl [1]. Orchids are one of the most sought-after flowering plants, making them much sought after both in their natural habitat and those that have been cultivated. One of the most popular orchids is the *Phaius* spp. Compared to other plant families, many species of the family *Orchidaceae* are threatened with extinction due to rampant deforestation, which has resulted in the extinction of many endemic orchid species. Thus, there is an urgent need for broad and targeted studies on *Orchidaceae* [2]. The genus *Phaius* consists of 30 species in tropical parts of Asia, Australia, Africa, and various islands in the Pacific Ocean and the Indies. The *Phaius* orchid has a high selling value because it has large flower sizes and various colors [3]. *Phaius* spp needs a habitat with certain characteristics to live. This orchid is often found in highly humid forest areas, widespread in peninsular Malaysia, Sumatra, Java, Kalimantan, and Sulawesi [4]. To increase the genetic diversity of orchids *Phaius spp* and protect orchids from extinction due to poaching required crosses.

The diversity of Indonesian orchids scattered in various regions of the archipelago is an untapped potential fully excavated. Studying wild orchids is very important to gain knowledge about the diversity of flora in Indonesia. Excessive extraction of orchids from natural habitats for economic purposes must be avoided because it causes population decline. There needs to be a balance between utilization and orchid conservation. The basis of the cross is the need for genetic information.

Knowledge of genetic diversity is basic capital for breeding experts and population geneticists in crop development and improvement, especially as an initial step for plant selection and for the identification of genes that have the potential to carry prominent characters [5].

Analysis of plant diversity can use morphological analysis, but morphological analysis produces inaccurate data because morphological characterization takes longer. Besides, morphological analysis data is very influential on the environment in which the plant lives. Morphological characterization is only effective at certain stages of development, for example, during flowering and fruit

emergence, so the tools used are more numerous and less effective in diversity analysis. The most efficient way to assess genetic diversity is to use molecular markers. Genetically based markers use polymorphic DNA and restriction fragment length polymorphism. DNA in plants describes the physiological character of a plant regardless of environmental factors. DNA is also spread in all parts of the plant so that it is easier to analyze, and the data obtained is also more accurate. Genetic diversity is used for organisms' potential development and extinction prevention. RAPD is an appropriate marker to analyze genetic diversity because RAPD can show an organism's fingerprint patterns [6].

Although morphological information about orchid species has been widely spread, genetic differences are always available, making genetic information indispensable for the conservation and improvement of plant species [7]. RAPD (*Random Amplified Polymorphic DNA*) is a method used to analyze genetic diversity between species. It was successfully used to analyze the genetic diversity of *Coelogyne pandurata* and *Coelogyne rumphii*, so it is hoped that it can be used to analyse the genetic diversity of *Phaius* spp. RAPD is widely used because it is cheap, fast, and easy to work with high polymorphism. To amplify random regions of DNA in the sample, a short random primer (10 nucleotides) was utilized [8]. This study aimed to analyze the genetic link of four orchid species (*Phaius spp*) using RAPD molecular marker. Information on genetic links is crucial for successful plant breeding and cultivar classification.

II. Materials and Method

This study was carried out at the LIPI (Indonesian Institute of Sciences) Center for Plant Conservation Botanical Garden, located at Jl. Ir. H. Juanda No. 13 Bogor, West Java, Indonesia and was carried out in. The samples used in this study were four species of the genus Phaius spp, namely *Phaius tankervillae* (1); *Phaius montanus* (2); *Phaius collasus* (3); and *Phaius amboinensis* (4). The sample was taken from the Center for Plant Conservation of the LIPI Botanical Gardens, Bogor. CTAB, Taq DNA polymerase, PVPP, Tris-HCL, PCR buffer reaction, EDTA, NaCl, MgCl2, chloroform, isoamyl, mercaptoethanol, sterile distilled water, absolute ethanol, quartz sands, 70% alcohol, dNTP mix, and primer were among the chemicals used for DNA analysis utilizing the RAPD method.

One gram of young leaves was rinsed and mixed with 0.1g of PVPP 40g and liquid nitrogen, 5 ml of extraction buffer containing 2% (100 Mm Tris HCl, CTAB, NaCl 1.4 M, 20 Mm EDTA), and 1 percent mercaptoethanol was placed in a 2 mL Eppendorf tube, plus After that, it was incubated for 5 minutes at 65^{o}C, the supernatant was separated by centrifugation for 10 minutes at 4^{o}C, 11,000 rpm. CIAA was used to purify the supernatant DNA in a ratio of 24:1, centrifuged for 10 minutes at 4^{o}C, 11,000 rpm. CIAA was used to purify the supernatant DNA in a ratio of 24:1, centrifuged and transferred to a fresh tube and incubated for 30 minutes in 5 mL of cold isopropanol, centrifuged for 10 minutes at 11,000 rpm. The mixture was discarded, and the DNA was cleaned with 70% ethanol. As much as 1/10 of the total volume of the DNA precipitate was dissolved in 1 mL of TE buffer plus 3M sodium acetate with Ph 5.2. The mixture was frozen for 30 minutes before being centrifuged at 14,000 rpm for 10 minutes. The DNA palette was washed and dried.

Agarose gel was placed in a mold containing TAE buffer; the DNA sample was mixed with loading dye and then electrophoresed for 57 minutes at 50 volts. The electrophoresis finding was examined using UV transilluminator and then photographed with a camera. The mixture was dissolved in 500 mL TE buffer and stored at 20^{o}C. DNA amplification was performed for 3 hours. By mixing DNA, Primer mastermix (tap polymerase, dNTPs, $MgCl_2$, and water) into the PCR machine

A total of 10 RAPD primers (OPA2, OPA7, OPA 9, OPA 13, OPA 16, OPB 12, OPB 17, OPB 18, OPD 8, OPD 11) were selected into 6 RAPD primers based on the ability of the primers to produce DNA amplification by PCR (T w8able 1). Scanning DNA bands reveals genetic variety, and Binary data is converted from DNA bands. A value of 1 indicates the band exists, whereas a value of 0 indicates that the band is empty. The analysis was carried out in clusters using NTSYSpc (*Numerical Taxonomy System*) with UPGMA (Unweight Pair Group Method Arithmatic). The clustering method uses the DICE SIMQUAL (Similarity for Qualitative Data) and SAHN (Sequential Agglomerative Hierarchical and Nested) coefficients. Genetic diversity is shown using a dendogram.

TABLE I
PRIMARY SEQUENCE OF RAPD TO BE SELECTED

Primer	Sequence of Nucleotides (5'-3')
OPA 2	TGCCGAGCTG
OPA 7	GAAACGGGTG
OPA 9	GGGTAACGCC
OPA 13	CAGCACCCAC
OPA 16	AGCCAGCGAA
OPB 12	CCTTGACGCA
OPB 17	AGGGAACGAG
OPB 18	CCACAGCAGT
OPD 8	GTGTGCCCCA
OPD 11	AGCGCCATTG

TABLE II
RAPD PRIMER SEQUENCES USED FOR DNA AMPLIFICATION OF *PHAIUS* SPP.

Primer	Sequence of Nucleotides (5'-3')
OPA 7	GAAACGGGTG
OPA 9	GGGTAACGCC
OPA 13	CAGCACCCAC
OPB 12	CAGCACCCAC
OPB 17	AGGGAACGAG
OPB 18	CCACAGCAGT

III. Results and Discussion

A population composed of individuals with variations in genetics is called the diversity within the population. One species in one population with other populations that have genetic variation is called inter-population diversity. Technological advances can positively influence biotechnology in the agricultural sector, so that superior seeds can be produced from genetic engineering. Genetic engineering can improve plant traits. Assessment of plant genetic diversity using DNA, isozyme, and morphological markers. DNA markers as the most accurate genetic information. This is because DNA can be found in almost all cells in both living and dead tissues

Genetic markers that are used for genetic variation knowledge of a species are necessary because they are unaffected by the environment and perfectly stable. The

RAPD PCR technique is a method used to analyze genetic diversity using a primer genome that targets random sequences so that the resulting polymorphism is higher than other methods.

The analysis of genetic variation and patterns of population/species genetic diversity enables us to develop specific breeding, fast adaptation, and conservation methodologies. Molecular markers are not affected by plant growth and environmental conditions, so molecular markers are widely used to overcome the shortcomings of morphological and biochemical analysis. Several marker-based techniques are available for identifying and characterizing genetic variation. Each molecular marker has its uniqueness and must be adapted to the genetic analysis needs [9].

A. RAPD (Random Amplified Polymorphic DNA) Amplification

The results of electrophoresis in the form of DNA bands were translated by means of qualitative scoring on the appearance of the bands assessed without differentiating the thickness of each fragment. Scoring is done by aligning the band arrangement at a certain distance. The data is processed in the form of binary data by coding for the presence of a band with the number 1 and the number 0 for the code that no band is found in one fragment.10 primers were selected first with 4 samples of *Phaius spp*. orchids, then selected 6 primers with DNA banding patterns with high polymorphism to be used in the analysis of DNA samples.

Selection is done by selecting the amplification of 10 primers with UV transilluminator photos that produce clear and firm DNA bands. Of the 10 primers used, only 6 primers produced legible DNA band photos. While the 4 primers that were not used could not read the DNA bands. RAPD primers capable of amplifying many polymorphic bands are good primers used to detect polymorphisms in a population through RAPD analysis. This study uses RAPD markers, so that homozygous dominant and heterozygous genes will both produce DNA bands, which cannot be distinguished which are homozygous dominant and which are heterozygous. The recessive homozygous gene does not produce DNA bands [10].

(Figure 1) shows that the DNA bands amplified by the RAPD primers OPB 12 and OPB 17 have different light intensities, and there are *smears* on the DNA bands. It can be seen from the sample DNA band (1) *Phaius tankervillae,* which was amplified by OPB 12 had the least number of bands, namely 1 DNA band, this was because the OPB 12 primer had a different sequence from *Phaius tankervillae,* so it was not complementary and affected the amplification results. Smears were in the DNA bands of samples (1) *Phaius tankervillae* and (2) *Phaius montanus* which were amplified by OPB 12 primer and sample DNA bands (3) *Phaius collasus* and (4) *Phaius amboinensis* amplified by OPB 17 primer.

Isolation of DNA from plants requires the process of destroying cell walls and membranes. Then the extracted DNA is purified. The destruction of cell walls and membranes using chemical and thermal mechanisms. DNA must be purified from contaminants in the form of secondary metabolites, polyphenols, and polysaccharides present in plant parts because these contaminants can reduce the quality of isolated DNA. Proper DNA isolation protocol is very important to obtain high-quality DNA, and DNA quality is very influential in accurate and consistent DNA amplification results. In addition to paying attention to the extraction protocol, other things that need to be done to improve DNA quality results are the need for modification of incubation time and temperature, the addition of organic solvents during extraction, and the washing steps of DNA pellets [11].

Smear indicated that the isolated genomic DNA was not intact because it was cut during the isolation process. Many factors cause DNA to be cut during isolation, one of which is the presence of endonuclease enzymes and contaminants in the form of polysaccharides. The presence of polysaccharides in DNA samples will inhibit enzymatic activity because it can inhibit activity *Taq polymerase*. NaCl is used to remove polysaccharide contamination from DNA. Additional CTAB and NaCl use helps break down and degrade polyphenols in the leaves [12].

Ten primers were first selected with 4 samples of *Phaius spp*. orchids, then 6 primers with DNA banding patterns with high polymorphism were selected to be used in DNA sample analysis. The 6 RAPD primers used in this study, namely OPA7, OPA 9, OPA13, OPB12, OPB17, OPB18 succeeded in amplifying 37 loci with the percentage of polymorphism in the sample of *Phaius* spp reaching 100% (Table 2, Fig. 1). The ability to analyze polymorphisms is the basis for selecting molecular markers, if a marker is not able to detect the genetic differences that exist in a group of individual, then its use becomes ineffective [13]. The number of DNA bands found in the 6 RAPD primers ranges from 4 loci (was in the OPB primer 18) to 8 loci (was in the OPA primer 9). The amount of polymorphic in all primers reached 100%, and the magnitude of monomorphic was 0%. With 6 loci being the average number of DNA bands observed per primer (Table 3).

TABLE III
DNA BAND SIZE AND PERCENTAGE OF POLYMORPHISM

No	Primer	Size (bp)	Number of Bands	Polymorphic Bands	Monomorphic Bands	% polymorphic	% monomorphic
1	OPA 7	300-1000	7	7	0	100	0
2	OPA 9	250-1600	8	8	0	100	0
3	OPA 13	400-1400	6	6	0	100	0
4	OPB 12	600-1600	5	5	0	100	0
5	OPB 17	300-1500	7	7	0	100	0
6	OPB 18	500-1000	4	4	0	100	0

Note: *% Polymorphic = (Number of DNA Bands / Pita Polymorphic Bands) x 100%.*

One of the most frequently used DNA isolation methods and has good isolation results is the CTAB method. In addition to the extraction of flowering plants, CTAB buffer is widely used to extract aquatic plants, medicinal plants to woody plants. Cetyl trimethyl Ammonium Bromide is a detergent buffer contained in CTAB that purifies DNA from plant tissue samples. The factor that causes the inhibition of the attachment reaction of the polymerase enzyme to the DNA chain during PCR is the high content of phenolic compounds in the DNA. High phenolic compounds resulted from a poor DNA isolation protocol, so there were still many contaminants in the DNA.

The success of DNA isolation also depends on the source of the sample to be extracted. Tissue in the form of fresh or frozen young leaves is a common example of a DNA extraction source, but if fresh tissue is unavailable, dry samples can be used, although artificial drying can degrade the DNA [14].

DNA bands with different thicknesses in each primer are due to differences in the concentration and purity of the sample DNA [15] RAPD primers bind to complementary DNA base sequences so that DNA can be amplified. The results of DNA band amplification were visualized using gel electrophoresis [16].

According to Choi et al. [17], incubation time affects agarose gel electrophoresis amplification results. The more DNA bands that are amplified, the higher the intensity of DNA band brightness. For RAPD amplification results, a high percentage of polymorphism is expected, as in the Auvira et al. [18] study on the percent polymorphism in orchids *S. plicata* was 100% using RAPD OPA 9 primers. OPA 13 and OPA 18 with an average number of DNA bands per primer was 4.8. The extensive polymorphism in RAPD markers shows that this method successfully analyzed genetic diversity in *Phaius* spp [19].

The 6 RAPD primers used in this study, namely OPA7, OPA 9, OPA13, OPB12, OPB 17, OPB 18 succeeded in amplifying 37 loci with the percentage of polymorphism in the sample *Phaius* spp reaching 100% (Table 2, Figure 1). OPB 12 primer produced a band at 600 bp in sample (1) *Phaius tankervillae* and (4) *Phaius amboinensi,* 700 bp in sample (2) *Phaius montanus* and (3) *Phaius collasus,* 1400 bp in sample (2) *Phaius montanus* , size 1500 bp in sample (2) *Phaius montanus,* (3) *Phaius collasus* and (4) *Phaius amboinensis,* size 1600 bp in sample (4) *Phaius amboinensis.*

OPB 17 primer produced a band with a size of 300 bp in sample (2) *Phaius montanus,* size 400 bp in sample (4) *Phaius amboinensis,* size 600 bp in sample (1) *Phaius tankerville* and (3) *Phaius collasus,* size 700 bp in sample (1) *Phaius tankerville* and (4) *Phaius amboinensis,* size 800 bp in sample (1) *Phaius tankerville,* (2) *Phaius montana,* and (4) *Phaius amboinensis,* size 1000 bp in sample (1) *Phaius tankerville,* and (4) *Phaius amboinensis,* size 1500 bp in samples (2) *Phaius montanus* and (3) *Phaius collasus.* orchid *PhaiusMontanus* and *Phaius colossus* has the highest band of the same size on the primary OPB 12, while orchids *Phaius tankervillae* and *Phaius amboinensis* has the largest number of the same size on the OPB 17. This indicates that more tape that is of the same size will increase the similarities between the species (Figure 1).

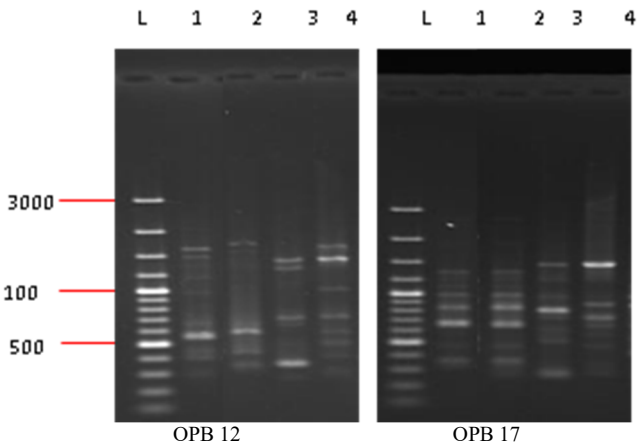


Fig. 1 DNA bands generated by RAPD OPB 12 and OPB 17: (1) *Phaius tankervillae,* (2) *Phaius montanus*, (3) *Phaius collasus*, (4) *Phaius amboinensis*.

The polymorphism results (Table 3) showed that the OPA 7 primer had a DNA band size of 300-1000 bp with a total of 7 DNA band fragments. The resulting polymorphic band is 7 bands, and no monomorphic band in the OPA7 primer OPA 9 primer has a DNA band size of 250-1600 bp with a total of 8 DNA band fragments. The resulting polymorphic band is 8 bands, and there is no monomorphic band in the OPA8 primer. OPA 13 primer has a DNA band size of 400-1400 bp with a total of 6 DNA band fragments. The resulting polymorphic band is 6 bands, and there is no monomorphic band in the OPA13 primer. OPA 12 primer has a DNA band size of 600-1600 bp with a total of 5 DNA band fragments.

The resulting polymorphic band is 5 bands and there is no monomorphic band in the OPA12 primer. OPA 17 primer has a DNA band size of 300-1500 bp with a total of 7 DNA band fragments. The resulting polymorphic band is 7 bands and there is no monomorphic band in the OPA12 primer. OPA 18 primer has a DNA band size of 500-1000 bp with a total of 4 DNA band fragments. The resulting polymorphic band is 4 bands and there is no monomorphic band in the OPA18 primer.

The selection of primers in genetic analysis affects the polymorphic bands produced because each primer has its attachment site polymorphism is strongly influenced by primer selection because each primer has a specific sequence attachment site. Primer selection is used to determine primers capable of amplifying DNA bands with high light intensity so that they are easily visible and have high polymorphism. Variations in amplified DNA fragments (DNA polymorphisms) are caused by the distribution of bases in the genome to which the primers attach. Differences between amplified DNA profiles (both in number and size) greatly affect the population's genetic diversity level. Setting the annealing temperature in the PCR amplification procedure is an important step. A change of 1 degree in the annealing temperature can cause failure in DNA amplification [20].

The high level of polymorphism in the population indicates the effectiveness of individuals in the selection process (random mating) and reproduction in their habitat. In contrast, low levels of population polymorphism are thought to be related to a constraint on gene flow by environmental factors or limited population size, leading to interpopulation, thereby reducing the population's genetic diversity. [21].

The value of a marker for detecting polymorphism within a population is referred to as information. It provides an estimate of the marker's discriminating power based on the number of detectable alleles and their frequency distribution. More complete and reliable descriptions of genotypes and genetic diversity patterns can aid in developing breeding programs and incorporating diverse germplasm into the genetic base [22].

B. Cluster analysis of Phaius spp.

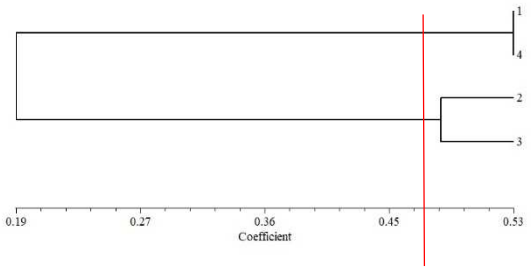
In this research, dendrogram analysis of genetic diversity was carried out using the UPGMA (*Unweighted Pair Group Method with Arithmetic Mean*) on the NTSYS (*Numerical Taxonomy System*) pc. The similarity coefficient values based on 6 RAPD primers ranged from 0.19-0.53, with genetic diversity ranging from 47%-81%. The closer to 1, the more similar the species, while the closer to 0 the more genetically diverse species (Fig 2). The small genetic distance between cluster 1 and cluster 2 reflects the low genetic barrier within the species [23]. The further distance of species in a dendrogram cluster will increase genetic diversity. As in previous studies, genetic diversity can be used as basic information for the conservation of a population [24].

TABLE IV
SIMILARITY MATRIX *PHAIUS SPP.* BASED ON 6 PRIMERS RAPD

	1	2	3	4
1	1.00			
2	0.19	1.00		
3	0.29	0.48	1.00	
4	0.53	0.13	0.14	1.00

Note: (1) *Phaius tankervillae,* (2) *Phaius montanus*, (3) *Phaius collasus*, (4) *Phaius amboinensis*

Differences that exist in a species in the population are a picture of diversity. The basis of the plant breeding program is the existence of intraspecific and interspecific differences in a species in the population. If a species in a population is high, then hybridization aimed at increasing a gene that is beneficial to plants will be difficult. In addition to the basis of plant breeding, genetic diversity in a species can be used as a carrier for new physiological traits that can tolerate pathogens or diseases in plants and are resistant to biotic or abiotic stresses that can damage plants [25].



Note: (1) *Phaius tankervillae,* (2) *Phaius montanus*, (3) *Phaius collasus*, (4) *Phaius amboinensis*.

Fig. 2 Dendrogram of grouping of 4 samples of *Phaius spp* based on 6 primers

There are two main ways to analyze the results of the similarity matrix, namely PCA (*Principal Coordinate* Analysis) and dendrogram in the form of grouping using a tree diagram. Dendrogram is a grouping of samples with genetic resemblance to each other. Samples with genetic differences will be grouped into clusters in the dendrogram [26]. In (Fig. 2), the 4 samples of orchids *Phaius* spp divided into 2 clusters at a coefficient similarity of 0.19-0.53 are cluster 1 containing orchids (1) *Phaius tankervillae* and (4) *Phaius amboinensis* and cluster 2 containing orchids (2) *Phaius montanus*, and (3) *Phaius collasus.* The genetic diversity obtained from this analysis can be used to select cultivars as parents for breeding purposes [27].

The similarity coefficient of *Phaius tankervillae* and *Phaius collasus is* 0.29. The similarity coefficient of *Phaius tankervillae* and *Phaius amboinensis is* 0.53. The similarity coefficient of *Phaius montanus* and *Phaius collasus is* 0.48. The similarity coefficient of *Phaius montanus* and *Phaius amboinensis is* 0.13. The similarity coefficient between *Phaius collasus* and *Phaius amboinensis is* 0.4 (Tabel 4).

The higher the similarity coefficient value, the more similar the plants will be. The dendogram diagram shows the genetic diversity divided into two clusters. The first cluster consists of (1) *Phaius tankervillae* and (4) *Phaius amboinensis* which have closer genetic closeness. The second cluster consists of (2) *Phaius montanus* and (3) *Phaius collasus*. The coefficient similarity of (1) *Phaius tankervillae* and (4) *Phaius amboinensis* are 0.53. The coefficient similarity of (2) *Phaius montanus* and (3) *Phaius collasus* are 0.48.

This is evident from Hartati's research (2020) that morphologically the *Phaius amboinensis* orchid and the orchid *Phaius tankerville* have a similarity of 80% and are divided into 2 clusters [28]. The more similar the morphology of the orchid, the higher the possibility of genetic similarity. This study shows that molecular markers can be used to compare genetic relationships in orchid species with the most similar morphological descriptions, so it is difficult to rely solely on morphological analysis [29].

Previous research on the endemic orchids Papua *Grammatophyllum scriptum, Dendrobium spp, Collagen asperata, Bulbophylum phalaenopsis, Phalaenopsis ambilis,* and *Ascoglossum calepsum* have diversity High genetic values ranged from 10-94% analysed with 10 RAPD primers [30]. Amplification of Paphiopedilum orchid cultivars using 22 RAPD primers resulted in 94% polymorphism with 32-60% genetic diversity [31]. Besides being used as a basis for plant breeding, knowledge of the genetic diversity of a species provides important insights into aspects of population dynamics and becomes an important prerequisite for designing conservation strategies [32].

Genetic information is very important because the loss of genetic diversity reduces a species' resilience and speeds up extinction. Genetic information is also useful for detecting 'genetic erosion', namely the loss or threat of varieties due to *inbreeding* [33]. As in (Fig. 2), the orchids (1) *Phaius tankervillae* and (4) *Phaius amboinensis* should be crossed with cluster orchids to avoid *inbreeding*.

The parents in the hybridization program were selected from different clusters, depending on the purpose of the hybridization and based on genetic distance. High genetic diversity depends on the selection of parents for plant breeding programs. In addition to conservation, genetic diversity also affects the economic value of a plant.

Inbreeding depression and *hybrid vigor* are factors that decrease genetic diversity in a population caused by crosses between species with close genetic distances [34].

The decrease in mean performance caused by mating between relatives is known as inbreeding depression. Increased genetic homozygosity is the impact of inbreeding depression so that it can increase adverse genotypes. The result of an artificial or natural crossing of parents with close genetic distance causes an increase in the deleterious allele in the next generation. Adverse alleles Some are recessive alleles and increase homozygosity so that genetic diversity decreases [35].

IV. Conclusion

This study provides information on the genetic variability 4 species *Phaius* spp *(Phaius tankervillae, Phaius montanus*, *Phaius collasus*, and *Phaius amboinensis*.) were analyzed by 6 RAPD (OPA7, OPA 9, OPA13, OPB12, OPB 17, OPB 18) ranged from 47%-81% and genetic similarity ranged from 019-0.53 with a polymorphism rate of 100%. This study also concluded that the *Phaius tankervillae* and *Phaius amboinensis* orchids were crossed with orchids in other clusters to increase genetic diversity in *Phaius spp*.

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