The appearance of DNA bands pattern based on the result of primary selection of RAPD orchid Phaius spp.

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Abstract. This study aimed to examine the appearance of the DNA band pattern resulted from the selection of RAPD primers on Phaius spp. namely Phaius tankervillae, Phaius montanus, Phaius collasus and Phaius amboinensis. The research material was performed in the Center for Plant Conservation of the LIPI Bogor Botanical Gardens. Molecular analysis was carried out at the Laboratory of the Center for Horticultural and Tropical Studies IPB using RAPD. The study showed that the 2 primers RAPD OPA 02 and OPA 16 can be used for DNA amplification of orchids Phaius spp (Phaius tankervillae, Phaius montanus Phaius collasus and Phaius amboinensis) because they produce clear DNA bands. The result of PCR amplification on Phaius tankervillae, Phaius montanus, Phaius collasus, and Phaius amboinensis using OPA 02 and OPA 16 primers produced 11 and 9 DNA bands, respectively, with an average of 5 DNA bands per primer. In the band pattern at 800 bp on OPA 02 primers resulting sharp and clear band pattern quality.

1. Introduction
Orchidaceous is one of largest flowering plant families of the angiosperms. Indonesia is a country that has the largest orchid germplasm in the world. Of the approximately 26,000 species of orchids in the world, Indonesia has 6,000 species of orchids because Indonesia is a tropical country that has a suitable environment for orchid growth [1]. Many orchids are terrestrial plants but also many species of orchids are epiphytes, i.e. growing hanging on the trunks, branches and twigs of tropical rainforest trees without being parasitic and relying on nutrients from fungi for early seed germination [2].

Development by destroying the forest will damage the orchid host plants, thus threatening the orchid population in the wild [3]. Orchids Phaius spp have flowers with large sizes and varied colors so that the demand and popularity of orchids increases and has the potential to become a promising business, especially as potted plants and cut flower stalks [4]. The method used to increase genetic diversity and increase the population of orchids is by plant breeding. Genetic diversity is the basis of plant breeding, one of the main factors in genetic analysis is the selection of primers with the right DNA sequence for high amplification results [5]. Morphological analysis has limitations because it affects environmental factors and the stage of plant development. In contrast to morphological markers, molecular markers based on DNA polymorphisms are more informative, and are not bound to
environmental conditions [6]. Comparative studies using DNA-based marker systems are needed to gather information on the genetic diversity of natural orchids, which is an important step to facilitate conservation. PCR test is used to see the DNA bands used for genetic analysis [7]. RAPD (Random Amplification of Polymorphic) is a fast, simple, and efficient method for evaluating genetics of plant material. The RAPD technique has been used to identify the genetic diversity of many plants. Although RAPD has some limitations such as allele dominance and sometimes has low reproducibility but there are many advantages to this RAPD technique. RAPD has the potential to provide a high level of polymorphism compared to other analytical techniques [8]. For that this study aims to examine the primary types of RAPD and the genetic closeness of 4 types of Phaius Orchids, namely Phaius tankervillae, Phaius collasus and Phaius amboinensis.

2. Methods
The current study used four species of Phaius spp., namely: Phaius tankervillae; Phaius montanus; Phaius collasus and Phaius amboinensis. The sample was taken from the Center for Plant Conservation of the LIPI Botanical Gardens, Bogor.
DNA isolation of plant samples was carried out with the following procedure. One gram of young leaves was washed and added with 0.1 g of PVPP 40 g and liquid nitrogen, put into an Ependorf tube with a volume of 2 ml, plus 5 ml of extraction buffer containing 2% CTAB, 100 Mm Tris HCl pH 8, NaCl 1.4 M, 20 Mm EDTA) and 1% mercapto ethanol. After that, it was incubated for 5 minutes at 65°C, the supernatant was separated by centrifugation at 11,000 rpm for 10 minutes at 4°C. DNA in the supernatant was purified with CIAA in a ratio of 24:1, centrifuged at 11,000 rpm for 10 minutes at 4°C. The DNA in the supernatant was purified with CIAA in a ratio of 24:1, centrifuged and transferred to a new tube and added with 5 ml of cold isopropanol, incubated for 30 minutes, centrifuged for 10 minutes at 11,000 rpm. The liquid was discarded, and the DNA was cleaned with 70% alcohol. The DNA precipitate was dissolved in 1 ml of TE buffer, added with 3M sodium acetate with a pH of 5.2 as much as 1/10 of the total volume. The liquid was stored in the freezer for 30 minutes and then centrifuged for 10 minutes at 14,000 rpm. The DNA palette was washed and dried.
The DNA quantity test was done as follows. 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 a voltage of 50 volts. The results of the electrophoresis were observed under a UV transiluminator and then photographed using a camera. Then dissolved with 500 mL of TE buffer and stored at 20°C. DNA amplification by mixing DNA, Primer master mix (MgCl, tap polymerase, dNTPs, water) was inserted into the PCR machine for 3 hours.

3. Results and discussion
RAPD analysis was carried out using 2 primers to match randomly between DNA fragments of Phaius tankervillae, Phaius montanus, Phaius collasus and Phaius amboinensis. RAPD analysis resulted in amplification of loci where DNA bands would be present or absent, thus affecting the intensity of the brightness of the resulting DNA bands, in addition, the brightness of DNA bands was also influenced by the number of copies of the amplified sequence. The faint band in the RAPD analysis indicates that there is a primer mismatch in the DNA [9].
The primer used must be able to present a firm banding pattern so that it can be used to detect polymorphisms and genetic diversity [10]. The results of electrophoresis show that the brightness of the DNA bands depends on the difference in DNA concentration. The difference in DNA concentration may be due to DNA degradation after storage, so to get DNA with high concentrations, a good quality sample is needed and taken while it is still fresh [11].
High genetic similarity is caused by high gene flow due to random crossing in populations with a small number of species [12]. The polymorphism percentage produced is 100%. The highest number of bands is 7 bands on OPA 7 primer and at least 4 bands on OPA 18 primer. RAPD (Random Amplified Polymorphic DNA) technique has been widely used to analyze the genetic diversity of orchid groups.
for example in the genus Catasetum orchid with polymorphism 83%-100% [13]. RAPD is a useful technique for plant breeding programs, and is a simple, fast procedure, requiring a low amount of DNA samples [14].

![Image](image1.png)

**Figure 1.** The DNA band patterns in the samples of *Phaius tankervillae* (1), *Phaius montanus* (2), *Phaius collasus* (3), and *Phaius amboinensis* (4) were analyzed using RAPD OPA 02 primers.

![Image](image2.png)

**Figure 2.** DNA band patterns in the samples of *Phaius tankervillae* (line 1), *Phaius montanus* (line 2), *Phaius collasus* (line 3), and *Phaius amboinensis* (line 4) were analyzed using RAPD OPA 16.
Amplification PCR results of *Phaius tankervillae, Phaius montanus, Phaius collasus, Phaius amboinensis* with OPA 02 and OPA 16 primers produced 11 and 9 DNA bands, respectively, with an average of 5 DNA bands per primer. Band resolution is not clearly visible, this is due to differences in amplified DNA fragments. The more amplified DNA fragments, the stronger the DNA band resolution (Figures 1 and 2). Among the 4 species of orchids *Phaius* spp that succeeded in amplifying DNA band patterns the most that appeared was in the band pattern at 800 bp on OPA 02 primers with sharp and clear band pattern quality.

The same band pattern indicates closeness or kinship. Figures 1 and 2) show two species of *Phaius* spp that have close genetic diversity, namely *Phaius tankervillae, and Phaius amboinensis*. This is indicated by the number of bands and the same arrangement and located in the same base pairs, namely in the OPA 02 primer at 800 bp and OPA 16 at 700 bp. From the results of the amplification of 4 species of *Phaius* spp, *Phaius montanus and Phaius collasus*, each band was independent, there was no similarity in the pattern of the band with other cultivars in the photo running results. This shows that the species *Phaius* spp above have distant kinship relationships. Genetically distant species will have different morphological appearances [15].

The DNA banding pattern in the sample *Phaius montanus* analyzed using RAPD OPA 02 and OPA 16 primers showed different patterns. The OPA 02 primer produced more DNA bands, the OPA 16 primer only showed 2 DNA fragments. The results produced by OPA 16 primers were more assertive than those of OPA 02 primers.

DNA banding patterns in samples *Phaius collasus* analyzed using RAPD OPA 02 and OPA 16 primers showed different patterns. The OPA 02 primer produces more and firmer DNA bands. The OPA 16 primer produced 9 DNA fragments that were clearly visible. Both primers showed a clear and firm DNA banding pattern.

DNA banding pattern insamples *Phaius collasus* analyzed using RAPD OPA 02 and OPA 16 primers showed different patterns. In OPA 02 primers, 11 fragments of DNA bands were more abundant and firmer. OPA 16 primer produced 9 distinct DNA fragments. Both primers showed a clear and firm DNA banding pattern.

4. Conclusion
The result of PCR amplification on *Phaius tankervillae, Phaius montanus, Phaius collasus, Phaius amboinensis* with OPA 02 and OPA 16 primers produced 11 and 9 DNA bands, respectively, with an average of 5 DNA bands per primer. In the band pattern at 800 bp on OPA 02 primers shows sharp and clear band pattern quality.

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