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# Orchids Cytogenetics and Karyotype of *Phaius callosus* and *Phaius montanus*

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**ABSTRACT**

The *Phaius callosus* and *Phaius montanus* orchids are ornamental orchids with high ornamental value and are classified as endangered plants. Genetic information from *Phaius callosus* and *Phaius montanus* orchids will facilitate plant breeding as it is essential to improve ornamental plant properties. Chromosome analysis aims to determine chromosomes' shape, number, and size. The research was conducted at the Cytology Laboratory of the LIPI Biology Research Center, Bogor, West Java. Analysis of research results based on observations of shooting results and data on the size and shape of chromosomes was carried out descriptively. The chromosome number of *Phaius callosus* and *Phaius montanus* had a similarity of 2n = 46, metacentric in shape. The chromosome size of *Phaius callosus* was 1.30 ± 0.29, while that *of Phaius montanus* was 2.32 ± 1.01. The karyotype formula of *Phaius callosus* and *Phaius montanus* had a similarity of 2n=46=23m which was dominantly metacentric.

**KEYWORD**
breeding, chromosome, orchid, shape, size

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## 1. INTRODUCTION

*Phaius* is a terrestrial plant from the *Epidendroideae* subfamily that acts as an ornamental plant and a high-value ethnobotanical plant (Ackerman, 2012; Kanwal, 2014). Currently, the availability of *Phaius* plants in nature is classified as rare and on the verge of extinction due to over-harvesting, such as in Australia, Japan, and India (Hirano et al., 2009; ShuFen et al., 2012; Simmons et al., 2018). *Phaius* has a characteristic that is the presence of *bulbs*, and the shape of the leaves is *lanceolate* with a pointed tip (Kurniawan et al., 2020). The results of the molecular analysis showed that the genus *Phaius* was grouped into one *cluster*, which confirmed morphologically that there were similarities in both stems, leaves, and flowers (Yuhanna et al., 2021). The differences in the characteristics of the *Phaius callosus* and *Phaius montanus* plants are found in the location of the flowers, the dorsal sepal color pattern, the

presence of spurs, the cross-section of the lips, and the number of polynias (Hartati et al., 2021).

The relationship between plants is not only seen based on morphology but can also be seen based on genetic relationships. Cytology is a branch of science that deals with cells, including chromosomes. Chromosomal studies play a role in determining plant genetic diversity, as indicated by the number of chromosomes and karyotype analysis (Daviña et al., 2009; Than et al., 2011).

Knowledge of the cytological characteristics of orchids is beneficial in developing orchid plant breeding, especially in forming new varieties and improving plant properties. There are several known cytological characteristics of orchids, including *Bulbophyllum auricomum* Lindl. (Than et al., 2011), *Catasetum* (De Oliveira et al., 2014), *Coelogyne pandurata* Lindley (Hartati et al., 2017), *Gastrodia javanica* (Blume) Lindl. (Aoyama & Yokota, 2012), *Cattleya* (Da Silva et al., 2017), *Chloraea* and *Gavilea* (Aoyama et al., 2013). Studies on *Phaius callosus* and *Phaius montanus* have never been carried out before, so this research is in the form of a chromosome analysis study that aims to determine the number, size, shape, and karyotype pattern of the two species of *Phaius callosus* and *Phaius montanus*.

## 2. METHODOLOGY

The research was carried out in March-June 2019 at the Cytology Laboratory of the LIPI Biology Research Center, Bogor, West Java. The *Phaius callosus* and *Phaius montanus* orchids are from the LIPI Bogor collection. Materials for chromosome observation preparations using meristematic root tips, HCl, Orcein, 45% glacial acetic acid, alcohol, and Acetocarmin.

The chromosome preparations were taken from the root meristem, cleaned, then soaked in distilled water and stored in the refrigerator for 24 hours. The root pieces were washed three times using distilled water, then soaked in 45% acetic acid and placed in the refrigerator for one hour. The material was washed using distilled water three times, then soaked in a 1 N HCl solution and heated in an oven at 60°C for five minutes. It was stained preparations by soaking root pieces in 2% aceto orcein solution and storing them in the refrigerator for 24 hours. Preparation of preparations is done by pressing the preparation material placed on a glass preparation and giving a drop of 45% acetic acid.

Observations were carried out descriptively by observing and measuring the shape of the chromosomes from the photographed chromosome images. Furthermore, the arrangement of the karyotype and ideogram of the two orchids was carried out. Karyotype asymmetry analysis was performed by calculating the intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2).

Intrachromosomal asymmetry index:

$$A1 = 1 - \left[\frac{\sum_{i=1}^{n} \frac{bi}{Bi}}{n}\right]$$

$bi$ = average short arm length of each pair of homologous chromosomes
$Bi$ = average arm length of each pair of homologous chromosomes
$n$ = the number of pairs of homologous chromosomes

Interchromosomal asymmetry index:

$$A2 = SD/\overline{X}$$

SD = standard deviation of chromosome length in a karyotype
$\overline{X}$ = average length of chromosomes in one karyotype

## 3. RESULTS AND DISCUSSION

### 3.1. Chromosome number

Each plant species has different characteristics, which are determined by the nature of genetic information contained in the chromosomes. The number of chromosomes is a distinguishing factor for plant identification at the cellular level (George & Mathew, 2016). Information on the number of chromosomes is also needed in crosses, such as the crosses that have been carried out by Hartati et al. (2017) between Coelogyne pandurate (2n = 36) and Coelogyne rumphii (2n = 72) to obtain orchid offspring with a chromosome number of 2n = 54. The number of chromosomes of the two orchids, Phaius callosus and Phaius montanus, is shown in Table 1.

**Table 1.** Chromosome number of Phaius callosus and Phaius montanus

| Orchid Species | Chromosome Number |
|---|---|
| *Phaius callosus* | 2n = 46 |
| *Phaius montanus* | 2n = 46 |

Table 1 shows that *Phaius callosus* has a chromosome number of 2n = 46, and the chromosome number of *Phaius montanus* is also 2n = 46. These results are in line with Daviña et al. (2009), which state that the number of chromosomes of *Orchidoideae* is 2n = 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 56, 64. And the number of chromosomes in *Epidendroideae* is 2n = 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58. The results of other chromosomal analysis studies show the number of chromosomes as *Coelogyne pandurate* 2n = 2x = 36 and *Coelogyne rumphii* 2n = 4x = 72 (Hartati et al., 2017).

### 3.2. Chromosome size

Chromosomes are divided into two parts, the centromere and the chromosomal arms. The centromere is the head of the chromosome, with the two arms generally different in size. The measurement of the arm length of the chromosome is carried out to determine the size of the chromosome. The length of the two arms determines the distinctness of one chromosome from another. Each individual of a species has the same number of chromosomes but may have different chromosome sizes. Orchids from the subfamily *Epidendroidae* have a small chromosome size of 0.5–2.5 m (Daviña et al., 2009). Chromosome sizes of *Phaius callosus* and *Phaius montanus* orchids are presented in Table 2.

**Table 2.** Chromosome size of Phaius callosus and Phaius montanus

| Orchid Species | Long Arm (q)(µm) | Short Arm (p) (µm) | Total Arm (q+p) (µm) |
|---|---|---|---|
| *Phaius callosus* | 1.27 ± 0.46 | 1.03 ± 0.42 | 1.30 ± 0.29 |
| *Phaius montanus* | 1.27 ± 0.53 | 1.05 ± 0.48 | 2.32 ± 1.01 |

The measurement results showed that the average long arm and the short arm of *Phaius callosus* were 1.27 ± 0.46 m and 1.03 ± 0.42 m. The long and short arms of *Phaius montanus* were 1.27 ± 0.53 m and 1.05 ± 0.48 m, respectively. Chromosomal size can be affected by various factors. Differences in chromosome size can be influenced by various treatments during chromosomal preparation, causing changes in chromosome size (Simmons et al., 2018). In addition, differences in condensation levels, gene, and protein content can also affect chromosome size (Apriyanti et al., 2013).

### 3.3. Chromosome shape

The chromosome structure consists of a long arm and a short arm bounded by a centromere. The ratio between the long and short arms determines chromosomal shape. According to

Ramadhani et al. (2012), the classification of chromosomes based on length and shape plays a role in analyzing chromosome images, such as making karyograms and ideograms.

The chromosomes' shapes based on the centromere's location are grouped according to the metacentric, submetacentric, acrocentric, and telocentric forms (Felix and Guerra, 2010). The arm ratio and chromosome shape of *Phaius callosus* and *Phaius montanus* are shown in Table 3.

**Table 3.** Chromosomes of *Phaius callosus* and *Phaius montanus*

| Orchid Species | Chromosome Shape |
|---|---|
| *Phaius callosus* | 2n = 46 = 23m |
| *Phaius montanus* | 2n = 46 = 23m |

*Note : m = metacentric*

The *Phaius callosus* and *Phaius montanus* orchids have metacentric chromosomes. The shape of the chromosome can be determined based on the location of the centromere (Setiawan et al., 2018). The metacentric shape generally shows that the long and short arms are approximately the same size, so the centromere is located in the middle of the chromosome. Research related to the shape of chromosomes was also carried out on orchids of the *Cattleya* genus by Da Silva et al. in 2017, and the metacentric chromosome shape was also obtained.

### 3.4. Karyotype patterns and ideograms

Classifying chromosomes based on length and shape can form a karyotype pattern (Mut Hukumar et al., 2013). Orchid cells in the metaphase phase are used in taking cell images because the chromosomes are very clearly visible. The karyogram is a pair of homologous chromosomes arranged from the largest to the smallest chromosome pairs. The ideogram is also a diagram arranged by chromosome size, from the chromosome with the most extended arm to the chromosome with the shortest arm. Research on the karyotype pattern of the hybrid orchid *C. pandurata* X *C. rumphii* conducted by Hartati et al. (2017) showed the results of the same karyotype pattern, and the orchid has a metacentric shape. The *Phaius callosus* and *Phaius montanus* orchids have the same karyotype pattern: metacentric dominant. The result of the ratio of the sizes of the two arms of the chromosome is not much different, indicating that the size deviation between the arms of the chromosome is slight. The karyogram and ideogram of the *Phaius callosus* and *Phaius montanus* orchids are as follows:


**Figure 1.** *Phaius callosus* karyogram

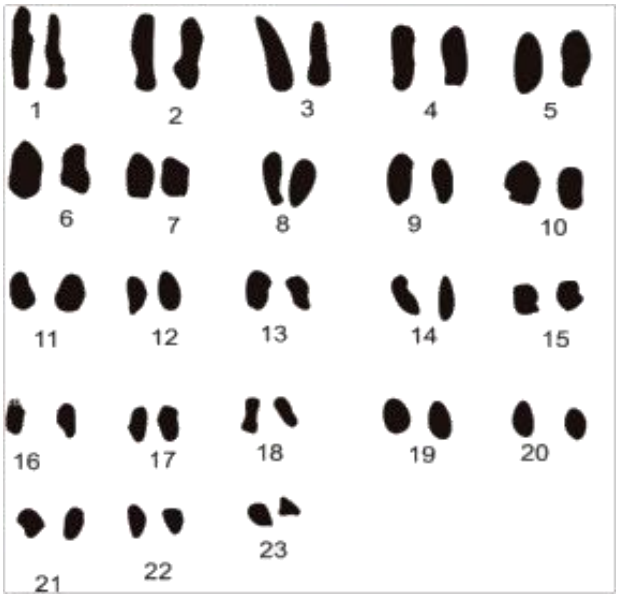


**Figure 2.** *Phaius montanus* karyogram

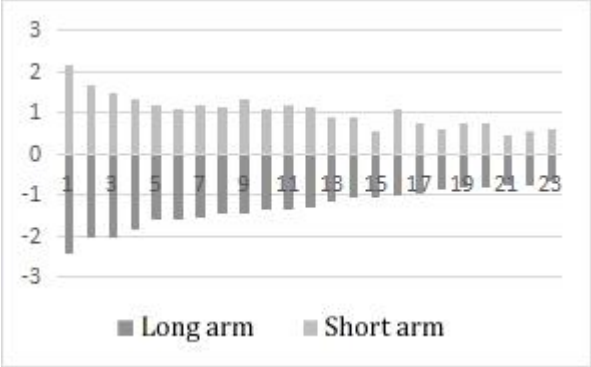


**Figure 3.** *Phaius callosus* ideogram

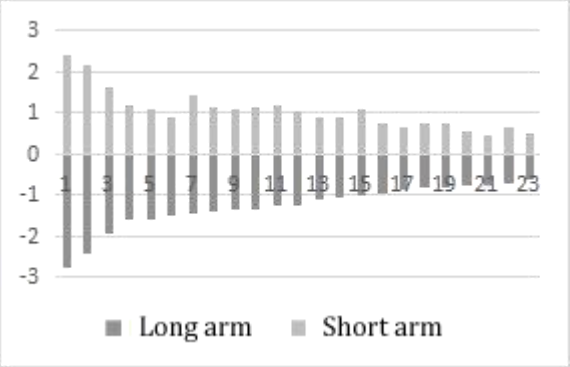


**Figure 4.** *Phaius montanus* ideogram

### 3.5. Chromosomal asymmetry index

The intrachromosomal asymmetry index (A1) has a value range of 0–1 and serves to identify variations in the shape of chromosomes in one karyotype. The results of the calculations for A1 and A2 for the Phaius callosus and Phaius montanus orchids are shown in Table 4.

**Table 4.** Phaius callosus and Phaius montanus chromosome asymmetry index values

| Orchid Species | Intrachromosomal Asymmetry Index (A1) | Interchromosomal Asymmetry Index (A2) |
|---|---|---|
| *Phaius callosus* | 0.20 | 0.35 |
| *Phaius montanus* | 0.17 | 0.41 |

Intrachromosomal asymmetry index values (A1) in Phaius callosus and Phaius montanus were 0.20 and 0.17, respectively. The A1 index of both orchids showed low values, indicating that the chromosomes of the two orchids were metacentric. Based on the two small A2 index values, it can be seen that the size dispersion of the orchid chromosomes is not significant. Interchromosomal asymmetry in Epidendroideae is known to have a bimodal karyotype, which means a symmetrical karyotype (Daviña et al., 2009).

## 4. CONCLUSION

Based on the research, it can be concluded that the number of chromosomes in Phaius callosus and Phaius montanus is 2n = 2x = 46. The chromosomes of the two orchids were all metacentric, with the average long and short arms of Phaius callosus 1.29 ± 0.49 m and 1.04 ± 0.44 m and Phaius montanus 1.29 ± 0.52 m and 1.29 ± 0.52 m, respectively. 1.06 ± 0.46 m. Phaius callosus has a karyotype arrangement, and Phaius montanus tends to be the same. The A1 value of Phaius callosus orchids is 0.20, and Phaius montanus is 0.17.

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