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Chromosome and Karyotype on Hybrid *Dendrobium bigibbum* and *D. lineale*

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Abstract. *Dendrobium* is the genus with the highest species diversity in Indonesia. The aesthetic value and unique structure of flower shape and color make the demand for this genus the highest compared to other genus in the Orchidaceae family. Cytological information plays an important role in the success of plant breeding programs. This research aims to study the cytology of chromosomes and karyotype in *D. bigibbum* and *D. lineale*, as well as the hybrid. Cytological observations were observed through chromosome preparations made using the squashing method. The karyotype of the hybrid chromosomes of *D. bigibbum* as parent male x *D. lineale* as parent female and *D. bigibbum* as parent female x *D. lineale* as parent male is $2n=2x=38=19m$. The chromosome size of *D. bigibbum* was $1.84 \pm 0.30 \mu m$, and *D. lineale* was $1.77 \pm 0.31 \mu m$, while the hybrid of *D. bigibbum* x *D. lineale* had a shorter size ($1.18 \pm 0.21 \mu m$) than the hybrid of *D. lineale* x *D. bigibbum* ($1.37 \pm 0.21 \mu m$). Analysis of the A1 (≤ 0.15) and A2 (≤ 0.18) asymmetry index of these *Dendrobium* had small or near zero values, so heard had symmetrical karyotype pattern in the form of metacentric and low size dispersion.

1. Introduction

The development of ornamental plants, especially orchids in Indonesia has increased. The development of orchid harvest area during the period from 1997 to 2015 showed an average increase of 3.95% [7] with the growth of production capacity for the period 1997 to 2019 increasing by an average of 8.28% [30]. Orchid production in 2022 will reach 10.2 million with around 35% in the form of potted orchids and 65% in the form of cut orchids [8]. According to [10] orchids are the most diverse plants and have beautiful flowers. The most popular orchids are the genus *Dendrobium* with a proportion of 34%, followed by *Ocindium* 26%, *Cattleya* 20%, *Vanda* 17%, and others at 3% [19]. *Dendrobium* has the property of flowers that remain fresh for a long time (perennial flowers), have diverse shapes and colors [30, 3].

The richness of *Dendrobium*'s genetic diversity is enhanced through a plant breeding program to develop hybrid orchids. The genus *Dendrobium* has been found as many as 1190 species [10], and 275 species have been found in Indonesia [12]. The diversity of orchids in Indonesia, according to [2] is relatively slow in the development of production due to several factors, including inaccurate cultivation, lack of innovation and appropriate technology, dependence on imported seeds, and capital. Breeding is beneficial for producing superior



varieties and providing a positive domino effect in plant cultivation [5]. One of the breeding programs that often carried out is crossing and plant selection to produce superior varieties.

Plant cytology information improves the success of orchid breeding. According to [14] the genetic compatibility of the crossed species is the key to the success of crossing. Plant cytological characters can be obtained by observing chromosomal characteristics. The characteristics of the observed chromosomes include the number, size, shape, and location of the centromere [6]. This research is important to find out the cytological information on *Dendrobium* hybrids from elders *D. bigibbum* and *D. lineale*.

2. Material and Methods

2.1 Materials

The material used was the meristematic root tips of the orchid plant which were then used as preparation, the orchid species observed were *D. bigibbum*, *D. lineale*, and *hybrid from D. bigibbum x D. lineale*, *D. lineale x D. bigibbum*. The research was carried at the Genetics Laboratory and the Cytology Laboratory of the Indonesian Institute of Sciences (LIPI) Biology Research Center, Bogor. The materials used in this study are aquades, 45% acetic acid, HCl 1N, 2% aceto orcein solution, immersion oil, clear nail polish, aluminum foil and hydroxyquinoline. The tools used in this study were droppers, tweezers, razor blades, IS1153Pli Euromex iScope Trinocular Microscope, cover glass, object glass, water bath, petridish, needle, bunsen lamp, refrigerator, vial bottle, measuring flask, optilab camera, and laptop (Microsoft Excel, Microsoft Word, and Corel Draw X7 (64 bit) software were installed).

2.2 Making preparation

The preparation was made by cutting the root tip ± 5 mm long from a healthy plant at 06.00-08.00 WIB as a sample. Samples were soaked in a 0.002M solution of hydroxyquinoline for 3 h at 180C as a pre-treatment. Following the fixation process, the sample was soaked in 45% acetic acid for 10 minutes at room temperature. The hydrolysis process of the sample was soaked in a mixture of 45% acetic acetate and HCL 1N in a ratio of 1:3 and incubated in a waterbath at 600C for 5 minutes. The samples were stained with a 2% aceto orcein solution method on the prepared glass. Finally, squasing using the thumb and preparation can be observed under a microscope with a magnification of 100x10.

2.3 Observations chromosome

Chromosome observations were carried out under a microscope with a magnification of 100x10 and documented using optilab. Chromosomal images were observed using corel draw X7 (64bit) software and Microsoft Excel. The observation results were analyzed descriptively to explain the cytological information. The observed variables were in the form of number, shape, size, karyotype, and chromosomal asymmetry index of orchid chromosomes. The number of chromosomes is calculated directly on the chromosome image, the size of the chromosomes is calculated by calculating the length of the long arm (q), the length of the short arm (p), and the total length (q+p) based on the results of size calibration from microscopes and micrometers. The shape of the chromosomes is determined based on the ratio of the chromosomal arm ratio. Karyotypes are arranged based on the similarity of size and shape between homologous chromosomes and are presented in the form of a karyogram. The chromosomal asymmetry index was calculated using the formula suggested by [25]. The intrachromosomal asymmetry index (A1) is calculated by summing the ratio of the average short arm length and long arm length of each

homologous chromosome pair divided by the number of homologous chromosome pairs, while the interchromosomal asymmetry index (A2) is calculated by dividing the standard deviation by the average total length of the chromosomes.

3. Results and Discussion

3.1 Chromosome number

Each species of orchid has a variable number of chromosomes. This is due to the evolutionary process that takes place from time to time. The difference in the number of chromosomes in each orchid species is influenced by evolutionary processes such as environmental adaptation and genetic mutations. The environment (physics and chemistry) has an influence on the process of cell division, so the gene composition of one chromosome can undergo changes [20]. Genetic mutation is a process of change in the gene arrangement to form a new species [23]. According to [35], observations regarding the number of chromosomes are useful as cytogenetic markers for chromosomal differentiation.

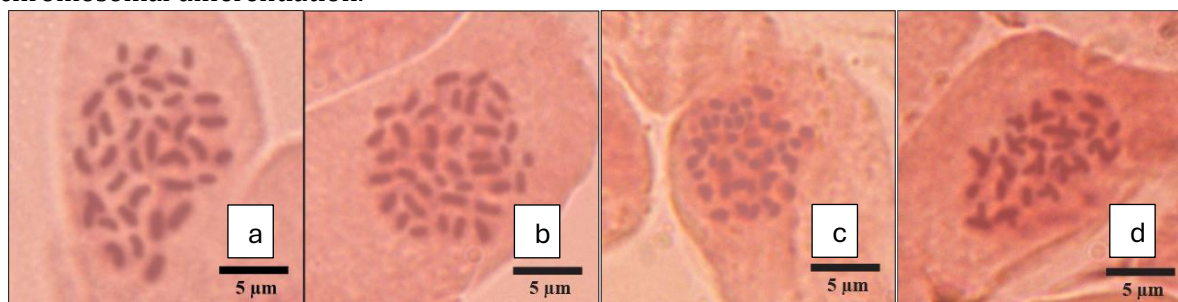


Figure 1. Number of *Dendrobium* chromosomes $2n=2x=38$. (a) *D. bigibbum*, (b) *D. lineale*, (c) *D. bigibbum* x *D. lineale*, (d) *D. lineale* x *D. bigibbum*

D. bigibbum, *D. lineale*, and their hybrids had same number of chromosomes, namely $2n=2x=38$ (Figure 1). The majority of species from *Dendrobium* have a chromosome count of $2n=2x=38$ [4]. Some other species of *Dendrobium* that have a chromosome count of $2n=2x=38$ such as *D. Stardust* 'Fire Bird' [21], and *D. cariniferum* [37]. Most species in a genus have the same number of chromosomes. The number of chromosomes of the *Dendrobium* hybrid and its elders has a uniform number of chromosomes. These results can be concluded that the process of crossing until fertilization does not occur deviations or deviations. According to [16] chromosomal deviations can occur due to differences in the number of elder chromosomes or the treatment of certain chemicals.

The observation results in Figure 1 show that *Dendrobium* orchid plants have diploid chromosomes with basic chromosome 19. The number of *Dendrobium* chromosomes is generally composed of (x) 19 with $2n=38$ and only a small percentage have (x) 20 with $2n=40$ [38]. Research on the genus *Vanda* conducted by [29] found that *Vanda* orchids generally have basic chromosomes 19, 20, and 21. The genus *Phalaenopsis* has a base chromosome of 19 [16, 17, 26]. The genera *Cymbidium* and *Cattleya* have a chromosome count of $2n=40$ [34].

3.2 Chromosome size

The size of the *Dendrobium* chromosomes has a wide variety of sizes. *D. bigibbum* had an average q of $0.97 \pm 0.16 \mu\text{m}$ and p $0.87 \pm 0.14 \mu\text{m}$ so it has a total arm of $1.84 \pm 0.3 \mu\text{m}$. *D. lineale* had an average q of $0.93 \pm 0.17 \mu\text{m}$ and p $0.83 \pm 0.15 \mu\text{m}$ so it has a total arm of $1.77 \pm 0.31 \mu\text{m}$. Hybrid *D. bigibbum* x *D. lineale* had an average q of $0.64 \pm 0.13 \mu\text{m}$ and p $0.54 \pm 0.09 \mu\text{m}$ so it has a total arm of $1.18 \pm 0.21 \mu\text{m}$. Hybrid *D. lineale* x *D. bigibbum* had an average q of $0.73 \pm 0.12 \mu\text{m}$ and p 0.64 ± 0.10

μm so it has a total arm of $1.37 \pm 0.21 \mu\text{m}$. (Table 1). This result is in accordance with the research of [34] the Orchidaceae family has a chromosome size between 0.5–5 μm .

Table 1. Size and shape of chromosomes *D. bigibbum*, *D. lineale*, and their hybrids

Species name	Long arm (q) (μm)	Short arm (p) (μm)	Total arm (q+p) (μm)	Arm ratio (q/p $\pm$ SD) (μm)	Chromosome shape
<i>D. bigibbum</i>	0.97 ± 0.16	0.87 ± 0.14	1.84 ± 0.30	1.27 ± 0.31	38m
<i>D. lineale</i>	0.93 ± 0.17	0.83 ± 0.15	1.77 ± 0.31	1.22 ± 0.04	38m
<i>D. bigibbum</i> x <i>D. lineale</i>	0.64 ± 0.13	0.54 ± 0.09	1.18 ± 0.21	1.18 ± 0.06	38m
<i>D. lineale</i> x <i>D.</i> <i>bigibbum</i>	0.73 ± 0.12	0.64 ± 0.10	1.37 ± 0.21	1.14 ± 0.07	38m

The size of chromosomes is further grouped descriptively, namely long, medium, and short chromosomes. According to [28], long chromosomes have a size of $>5 \mu\text{m}$, medium chromosomes have a size of 3.0–4.9 μm , and short chromosomes have a size of 0.1–2.9 μm . Based on Table 1, *D. bigibbum*, *D. lineale*, hybrid *D. bigibbum* x *D. lineale*, and hybrid *D. lineale* x *D. bigibbum* are included in the group of short chromosomes, with average total lengths of $1.84 \pm 0.3 \mu\text{m}$, $1.77 \pm 0.31 \mu\text{m}$, $1.18 \pm 0.21 \mu\text{m}$, and $1.37 \pm 0.21 \mu\text{m}$ respectively. The results of size observation showed that chromosomes between species had differences in size, although the difference in size was small.

The observed length of chromosomes shows the difference in size between each cell. The difference in the size of the length of chromosomes in each cell is due to chromosomal morphological factors, proteins bound within the DNA and chromatin condensation patterns. Genetic material in the form of DNA sequences will appear when condensed [15, 22]. The condensation rate occurs due to treatment in chromosome preparation [17]. The process of hydrolysis increases the spread of chromosomes. Hydrochloric acid and hydrolase enzymes contained during the hydrolysis process will dissolve the middle lamella and unsolidified meristem tissue.

3.3 Chromosome shape

The determination of the shape of chromosomes is determined based on the ratio of the arms of each chromosome to its centromere ($r=q/p$). The shape of chromosomes is based on the opinion of [9], which categorizes chromosomes with an arm ratio of $1.0 < r \leq 1.67 \mu\text{m}$ is metacentric (m), $1.68 < r \leq 00 \mu\text{m}$ is submetacentric (cm), $3.01 < r \leq 7.00 \mu\text{m}$ is acrocentric (ak), and $r > 7.00 \mu\text{m}$ is telocentric (T).

Based on Table 1 the chromosomal forms of *D. bigibbum*, *D. lineale*, and their hybrids are 38 metacentrics (m). The average ratio of Dendrobium chromosome arms is between 1.0–1.67 μm so that it has a metacentric chromosomal shape. The majority of Dendrobium chromosomal forms are metacentric [29, 34]. The Orchidaceae family generally has metacentric chromosomal forms [33], such as in the genera Phaius [18], Cattleya [31], Coelogyne [16] and Dendrobium [15]. The similarity of chromosomal shapes between hybrids and their elders found suggests a peculiarity of the Dendrobium species, that crosses of species that have the same number and shape of chromosomes produce similar offspring. This peculiarity is useful by plant breeders for further development studies. Complete plant genetic information will support breeding so that it is able to produce plants with good phenotypics [13].

3.4 Karyotype

Karyotypes are arranged based on homologous chromosome pairs from largest to smallest size [11, 24]. Karyotype feeding is carried out by paying attention to the length of chromosomes, the number of chromosomes, the size and position of satellites, and the comparison of long arms and short arms [32]. According to [1], chromosomal karyotypes function to determine abnormalities in the structure and number of chromosomes. This deviation can occur due to aberration (damage) due to exposure to radiation or certain substances.

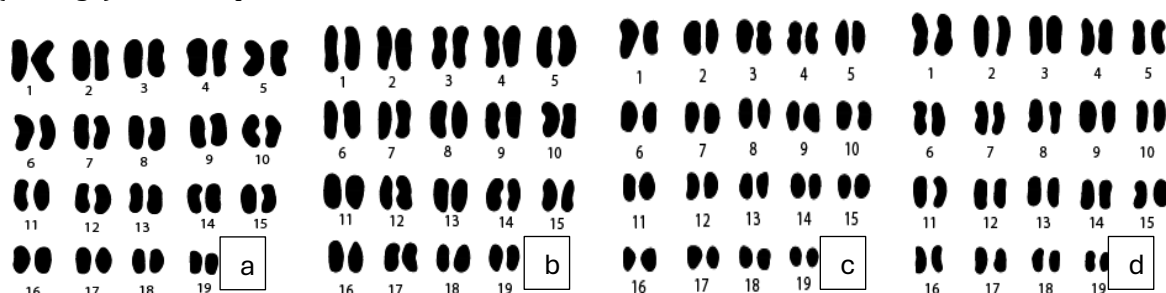


Figure 2. Karyogram of *Dendrobium*. (a) *D. bigibbum*, (b) *D. lineale*, (c) *D. bigibbum* x *D. lineale*, (d) *D. lineale* x *D. bigibbum*.

Each pair of chromosomes has 2 homologous chromosomes which means that the ploid pattern is diploid and no satellites of each of the observed chromosomes are found (Figure 2). Based on the observations that have been made, it can be determined that the karyotype formula for *D. bigibbum*, *D. lineale*, and their hybrid is $2n=2x=38=19m$. This shows that the hybrid and its elders are composed of 19 pairs of metacentric diploid chromosomes. The size of all the observed species is medium chromosomes.

D. bigibbum, *D. lineale*, and its hybrids are composed of metacentric chromosomes. The whole chromosome is metacentric so the karyotype is symmetrical. Symmetrical karyotype is when metacentric and submetacentric chromosomes have similar sizes [31].

3.5 Index asymmetry chromosomal

The chromosomal asymmetry index that is calculated is generally the intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2). The A1 index is used to determine the variation in chromosome shape in a karyotype and the A2 index is used to determine the variation in chromosome size [27]. The value of the Asymmetric Index has a value ranging from 0 to 1 and has a positive correlation with its variation [27], which means that the index value close to 0 has low variation. According to [33], the smaller the value of the A1 index, the more symmetrical the karyotype pattern will be, because the majority is composed of metacentric chromosomes.

Table 2. Chromosome asymmetry index *D. bigibbum*, *D. lineale*, and their hybrids

Species name	Intrachromosome (A1)	Interchromosome (A2)
<i>D. bigibbum</i>	0.1	0.16
<i>D. lineale</i>	0.11	0.18
<i>D. bigibbum</i> x <i>D. lineale</i>	0.15	0.18
<i>D. lineale</i> x <i>D. bigibbum</i>	0.12	0.15

Based on Table 2, the index value of A1 *D. bigibbum* is 0.1, *D. lineale* is 0.11, and hybrid *D. bigibbum* x *D. lineale* is 0.15, and hybrid *D. lineale* x *D. bigibbum* is 0.12. The A1 index value of the

hybrid *Dendrobium* and its elders shows small or close to zero values, so it has a chromosomal shape that tends to be metacentric. The results of this calculation are in accordance with the previous discussion that the observed species have the majority of metacentric chromosomal forms. The A2 index values of *D. bigibbum*, *D. lineale*, hybrid *D. bigibbum* x *D. lineale*, and hybrid *D. lineale* x *D. bigibbum* were 0.16, 0.18, 0.18, and 0.15, respectively. These results show that *Dendrobium* hybrids and their elders have low chromosomal size aberrations or dispersions because they have small or near-zero A2 index values. If the value of the A1 and A2 asymmetric indices between chromosomes is small or close to 0, it means that the shape and size of the chromosomes have the same similarity [36]. These results show that the three observed species have a high degree of similarity, so that the hybridization process can be carried out to produce a new superior species.

4. Conclusion

Based on the results of the research and discussion on Chromosome and Karyotype on Hybrid *Dendrobium bigibbum* and *D. lineale*, it can be concluded that:

- D. bigibbum*, *D. lineale*, hybrid *D. bigibbum* x *D. lineale*, and hybrid *D. lineale* x *D. bigibbum* had a chromosome number of $2n=2x=38$ with metacentric chromosomal shapes.
- The size of the chromosomes of *D. bigibbum* is $1.84 \pm 0.3 \mu\text{m}$, *D. lineale* is $1.77 \pm 0.31 \mu\text{m}$, hybrid *D. bigibbum* x *D. lineale* is $1.18 \pm 0.21 \mu\text{m}$, and hybrid *D. lineale* x *D. bigibbum* is $1.37 \pm 0.21 \mu\text{m}$. Hybrid *Dendrobium* is smaller than its elder.
- The karyotype pattern formula of *Dendrobium* hybrid and its elder is the same, i.e. $2n=2x=38=19m$.
- Hybrid and elder *dendrobium* have a small variation in shape and size because it has an index value of $A1 \leq 0.15$ and an A2 index ≤ 0.18 .

References

- [1] Annah A Z dan Aziz I R 2019 *Prosiding Seminar Nasional Biodiversitas Indonesia*
- [2] Anggraeni N 2022 *J Pemikiran Masyarakat Ilmiah Berwawasan Agribisnis* **8**(2) 639-648
- [3] Apriliyana R, Wahidah B F 2021 *J Mahasiswa Biologi* **1**(2) 33-46
- [4] Atoche J A, Iiyama C M, and Cardoso J C 2022 *Plants* **11**(469) 1-21
- [5] Azhar J R, Farhanah, Firmansyah et al 2023 *Haura Utama*
- [6] Aziz I R 2019 *J Teknosains* **13**(2) 126-129
- [7] Badan Pusat Statistik [BPS] 2018 *Badan Pusat Statistik Republik Indonesia*
- [8] Badan Pusat Statistik [BPS] 2022 *Badan Pusat Statistik Republik Indonesia*
- [9] Baltisberger M and Hörandl E 2016 *Perspectives in Plant Ecology, Evolution and Systematics* **18** 1-14
- [10] Basavaraj B, Nagesha N, and Jadeyegowda M 2020 *Intl J Curr Microbiol Appl Sci* **9**(1) 2157-2169
- [11] Chao Y T, Chen W C, Chen C Y, Ho H Y, yeh C H, Kuo Y T, Su C L, Yen S H, Hsueh H Y, Yeh J H, and Hsu H L 2018 *Plant Biotech J* **16** 2027-2041
- [12] Darmawati I D A, Rai I N, Dwiyan R, and Astarini I A 2018 *Biodiversitas* **19**(3) 1110-1116
- [13] Fauziah S, Sulmartiwi L, and Wiidjiati 2020 *J of Marine and Coastal Sci* **9**(2) 76-85
- [14] Firdauzi N F 2014 *J Biology Science and Education* **3**(2) 197-204
- [15] Hartati S, Darsana L, and Cahyono O 2014 *Caraka Tani J of Sustainable Agriculture* **29**(1) 25-30
- [16] Hartati S, Nandariyah, Yunus A, and Djoar D W 2017 *Biodiversity* **18**(2) 555-559
- [17] Hartati S, Muliawati E S, Yuniastuti E, Manurung I R, dan Purmiyoto C W W 2022 *IOP Conference Series: Earth and Environmental Science* **1114**(1) 012105
- [18] Hartati S, Samanhudi, Cahyono O, and Wibowo A 2023 *Int Conf Modern and Sustainable Agric (ICOMSA)* **1133**(1) 012064
- [19] Kementerian Pertanian 2007 *Badan Penelitian dan Pengembangan Pertanian, Kementerian Pertanian*
- [20] Khairunisa K, Razak A, Satria R 2023 *J Pendidikan Biologi* **6**(1) 21-28

- [21] Kondo H, Phlaetita W, Mii M, Kikuchi S, Deguchi A, and Miyoshi K 2020 *Biotech* **56** 738-749
- [22] Kurnianingsih R, Rosidah S, Ayu D S, Eka S, Prasedya, and Astuti SP 2021 *J Biologi Tropis* **21**(3) 1096-1105
- [23] Lestari E G 2021 *Berita Biologi* **20**(3) 335-344
- [24] Liu Y, Duan S D, Jia Y, Hao L H, Xiang D Y, Chen D F, and Niu S C 2023 *Horticulturae* **9** 329
- [25] Medeiros-Neto E, Nollet F, Moraes A P, and Felix L P 2017 *Genetics and Molecular Biology* **40**(3) 610-619
- [26] Mohammadi M, Kaviani B, and Sedaghathoor S 2021 *Ornamental Horticulture* **27**(2) 204-212
- [27] Muliawati E S, Hartati S, Parjanto, Sukaya, Nandariyah, Yuniastuti E, Manurung I R, Purmiyoto C W W 2023 *E3S Web of Conferences* **373** 1-6
- [28] Ramesh T, Kumari K G, Muruganantham P, Praveena R, Vanithamani J, and Koperuncholan M 2022 *Plant Archives* **22**(1) 130-135
- [29] Ramesh T and Renganathan P 2013 *International J Curent Trends Res* **2**(1) 126-131
- [30] Riniarsih D 2020 *Pusat Data dan Sistem Informasi Pertanian Sekretariat Jenderal Kementerian Pertanian*
- [31] Silva G M D, Varella T L, and Karsburg I V 2017 *The Japan Mendel Society* **82**(2) 137-140
- [32] Tuapttinaya P M J 2018 *J Biology Science dan Education* **7**(1) 56-68
- [33] Turco A, Albano A, Medagli P, Wagensommer R P, and D'Emerico S 2021 *CompCytogen* **15**(4) 507-525
- [34] Vilcherrez-Atoche J A, Liyama C M, and Cardoso J C 2022 *Plants* **11**(4) 469-490
- [35] Wang Y, Chen Y, Wei Q, Chen X, Wan H, and Sun C 2022 *Gene* **846** 1-1.2
- [36] Yulianto F K dan Parjanto. 2010 *Agrosains* **12**(2) 60-65
- [37] Zhang X and Gao J 2021 *Plant Cell Tissue Organ Culture* **144**(2) 409-420
- [38] Zheng S G, Hu Y D, Zhao R X, Yan S, Zhang X Q, Zhao T M, and Chun Z 2018 *Springer link Planta* **248** 769-784