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Chromosome and karyotype analysis of *Dendrobium* spp.

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Abstract Cytogenetics is a crucial aspect in determining the success of breeding programs for orchid plants. Chromosome contains DNA, which is the genetic information for each species. The characterized orchid chromosomes were Dendrobium *lamelatum*, *Dendrobium macrophyllum*, *Dendrobium mirbelianum*, *Dendrobium purpureum*, and *Dendrobium secundum*. The results showed that all Dendrobium orchids had a metacentric shape with 2n = 38 chromosomes, except for *D. purpureum* with 2n = 40. The size of each arm of *D. lamellatum*, *D. macrophyllum*, *D. mirbelianum*, *D. purpureum*, and *D. secundum* is 2.51 ± 0.55 µm to 2.67 ± 0.54 µm, 1.54 ± 0.41 µm to 1.86 ± 0.37 µm, 5.28 ± 0.16 µm to 6.31 ± 0.17 µm, 2.45 ± 0.60 µm to 2.62 ± 0.64 µm, and 2.70 ± 0. 49 µm to 2.89 ± 0.51 µm. The index value (A1) in all species showed a metacentric shape, while the index value (A2) in all species showed a deviation in chromosome size is small.

Keywords: Breeding, Cytogenetic, Orchids

Introduction

Orchids belongs to Orchidaceae, which comprise approximately 4.000 to 5.000 species in Indonesia (Lalla and Sudiarta, 2022). The plants possess a high economic value due to their unique shape, color, and relatively long durability (Apriliyania and Wahidah, 2021). Moreover, the uniqueness of each orchid can be determined through variations in size, composition, number of flowers, stems, and length. These species are often used as cut flowers, cosmetic ingredients, perfumes, and other industrial materials.

One of the largest genera in the Orchidaceae family is Dendrobium, with approximately 1,600 species in Indonesia (De *et al*., 2015). The genus is characterized by its yellowish-green stems, round or lanceolate leaf shape, as well as various flower shapes and colors. Furthermore, Dendrobium exhibits substantial characteristic variation across its different species (Yuan *et al*., 2018).

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Each plant within *Dendrobium* species possesses a distinct genetic makeup. Cytogenetics plays an important role in plant breeding programs by providing genetic information about the relationship between species in certain groups (Sharma and Mukai, 2015). The understanding of chromosome number, variation in chromosome length, karyotyping, and symmetry also provides deeper insights. Chromosome contains DNA, which is the genetic information for each species. This genetic material consists of a chromosome center or centromere and chromosome arms. According to a previous study, information about chromosome is useful in creating superior varieties, protecting germplasm, and refining taxonomy at the genetic level (Hartati *et al*., 2022a).

Orchids exhibit a fascinating diversity of chromosomes, with each species having its distinct set, ranging from 2n = 20 to 2n = 62. The basic chromosome that is often found in orchids is multiples of x = 19, 20, 21, and 22 (Sharma and Bhattacharyya, 2023). Several chromosome studies have been carried out on various types of orchids, including Phaius (Hartati *et al*., 2022a), Phalaenopsis (Lee *et al*. 2020, Chen *et al*. 2013), Paphiopedilum (Lee *et al*., 2011, Huy *et al*. 2019), Dendrobium (Wang *et al*. 2022, Zheng *et al*. 2018, Niu *et al*. 2021), Cattleya (Silva *et al*. 2017), Calanthe (Ramesh *et al*., 2022a), Vanda (Ramesh *et al*., 2022b, Hartati *et al*., 2022b), Cypripedium (Choi *et al*., 2021), Peristylus (Kurniawan and Riyadi 2021), and *Aerides odorata* (Nugraheni *et al*., 2022). Therefore, this study aimed to characterize the chromosomes of *Dendrobium lamelatum*, *Dendrobium macrophyllum*, *Dendrobium mirbelianum*, *Dendrobium purpureum*, and *Dendrobium secundum*.

Materials and methods

Material

The materials used in this study included the root tips of *D. lamelatum*, *macrophyllum*, *D. mirbelianum*, *D. purpureum*, and *D. secundum*. Meanwhile, the used chemicals were distilled water, 45% acetic acid, 1N HCl, 2% acetoorcein solution, immersion oil, and hydroxyquinoline. This study was conducted at the Plant Breeding Laboratory, Faculty of Agriculture, Sebelas Maret University. The observed parameters include the number, size, shape, and chromosome karyotype of each plant.

Preparation of preparations

Root tip cutting into sizes of 1-2 cm was carried out at 06.00 – 07.00 WIB, followed by soaking in hydroxyquinoline solution for 3 hours at ± 20°C. To

remove the residual solution, the samples were washed with distilled water. Furthermore, the fixation process was performed by immersing root tips in a 45% acetic acid solution for 10 min. The samples were then immersed in a mixture of 1N HCl and 45% acetic acid with a 3:1 proportion at ± 60°C for 1-5 minutes using a water bath. They were cut or sliced into sizes of ± 0.5 mm and dripped with 2% acetoorcein solution. The preparations were covered with a cover glass and pressed (*squashing*). Observation on a microscope was carried out with 100 x 10 magnification (Hartati *et al*., 2022b).

Chromosome observation

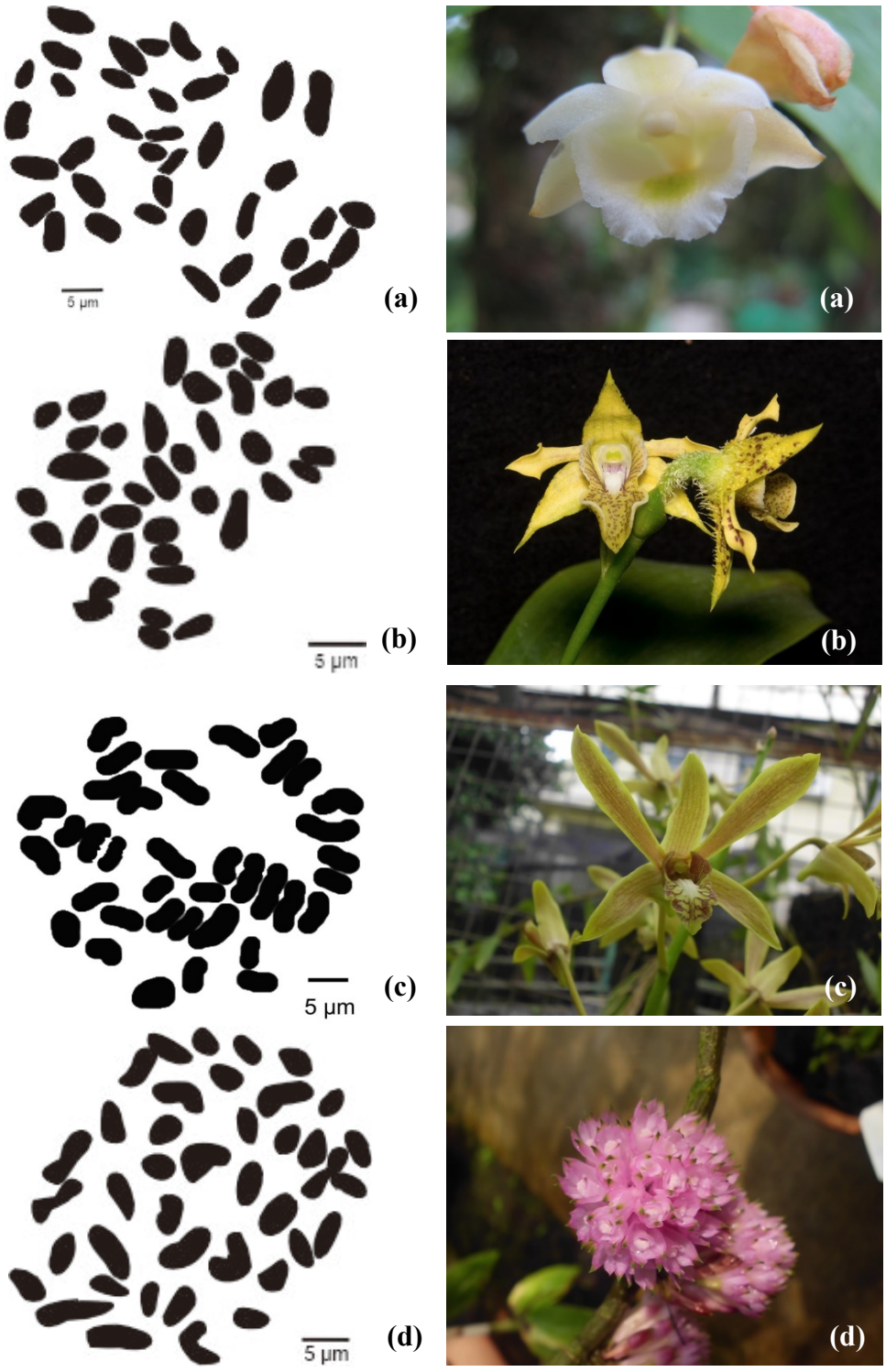
Observations using a microscope when the chromosomes were in the prometaphase and metaphase. Chromosome observations include number, long arm length (q), short arm length (p), shape, and karyotype. The shape of the chromosome is obtained from the ratio of the long arm and the short arm of chromosome (r=q/p). The shape of the chromosomes was determined by Hartati *et al*. (2022a): a) metacentric if $1.0 < r < 1.7$; b) submetacentric if $1.7 < r < 3.0$; c) acrocentric if $3.0 < r < 7.0$; and d) telocentric if the ratio > 7.0. Karyotyping is performed through pairing of homologous chromosomes based on similarity in chromosome size and shape.

Analysis of karyotype asymmetry index included intrachromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2). The intrachromosomal asymmetry index (A1) is calculated according to Neto *et al*. (2017), compared the mean short arm of each homologous chromosome pair (bi) and the average length of each homologous chromosome pair (Bi) was divided by the number of chromosome pairs that homologous (n). While the interchromosomal asymmetry index (A2) is calculated by dividing the standard deviation of the chromosome arm length by the average chromosome arm length in one karyotype.

Results

Number of chromosomes

One of the important parameters was the number of chromosomes, which could be clearly observed during prometaphase or metaphase when the samples were spread out. This study took root tips at 06.00-07.00 WIB where chromosome was actively carrying out the division process.


5 µm
(a)
(a)
5 µm
(b)
(b)
5 µm
(c)
(c)
5 µm
(d)
(d)

(e)

Figure 1. Chromosomes from (a) *Dendrobium lamelatum*, (b) *Dendrobium macrophyllum*, (c) *Dendrobium mirbelianum*, (d) *Dendrobium purpureum*, and (e) *Dendrobium secundum*.

Figure 2. Flowers from (a) *Dendrobium lamelatum*, (b) *Dendrobium macrophyllum*, (c) *Dendrobium mirbelianum*, (d) *Dendrobium purpureum*, and (e) *Dendrobium secundum*.

Table 1. Number of dendrobium orchid chromosomes

Orchid Species	Number of Chromosomes
Dendrobium lamelatum	2n = 38
Dendrobium macrophyllum	2n = 38
Dendrobium mirbelianum	2n = 38
Dendrobium purpureum	2n = 40
Dendrobium secundum	2n = 38

Chromosome size

The internal part of chromosome consisted of arms and centromere. Furthermore, centromere was the central indentation, which served as a spindle attachment point and played a role in cell division. It also divided the chromosome into two arms, namely long and short. The long and short arms had different sizes, and their length was often used to determine the total chromosome size.

Table 2. Dendrobium orchid chromosome size

Orchid Species	Long Sleeve (q)(µm)	Short Sleeve (p)(µm)	Total Arm (q + p)(µm)
Dendrobium lamelatum	2.67 ± 0.54	2.51 ± 0.55	5.18 ± 1.09
Dendrobium macrophyllum	1.86 ± 0.37	1.54 ± 0.41	3.40 ± 0.78
Dendrobium mirbelianum	6.31 ± 0.17	5.28 ± 0.16	11.59 ± 0.30
Dendrobium purpureum	2.62 ± 0.64	2.45 ± 0.60	5.07 ± 1.24
Dendrobium secundum	2.89 ± 0.51	2.70 ± 0.49	5.59 ± 0.10

Chromosome shape

Based on centromere position, chromosome shape was classified into metacentric, submetacentric, acrocentric, and telocentric. The sample was considered metacentric when the centromere was in the middle, thereby equally dividing the arms. Submetacentric was characterized by the length of one arm being shorter than the other because centromere was at the center nor the end. Acrocentric was indicated by centromere position, which was near the point of chromosome. Telocentric was characterized by the centromere being at the end, leading to the presence of one arm.

Table 3. Form of Dendrobium orchid chromosomes

Orchid Species	Arm Ratio (r=q/p)	Chromosome shape
Dendrobium lamelatum	1.07 ± 0.06	m = 19
Dendrobium macrophyllum	1.23 ± 0.16	m = 19
Dendrobium mirbelianum	1.20 ± 0.03	m = 19
Dendrobium purpureum	1.07 ± 0.09	m = 20
Dendrobium secundum	1.08 ± 0.08	m = 19

Karyotype

Karyotype was a structural description of chromosomes, such as number, shape, centromere position, distribution of euchromatin and heterochromatin, and satellite size. The analysis played a role in identifying abnormal chromosome to facilitate its association with genetic disorders.

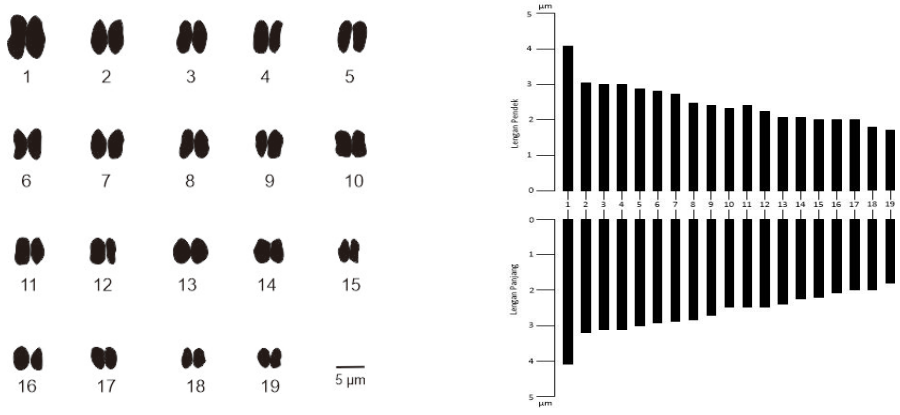


Figure 3. Karyotype and ideogram of *Dendrobium lamelatum*

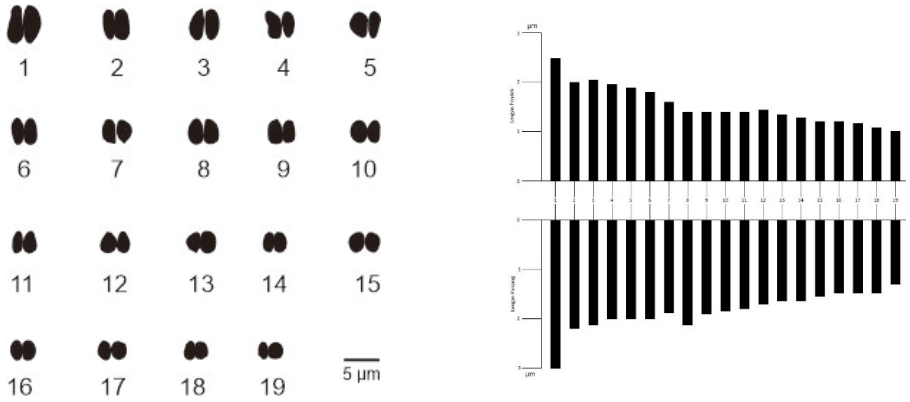


Figure 4. Karyotype and ideogram of *Dendrobium macrophyllum*

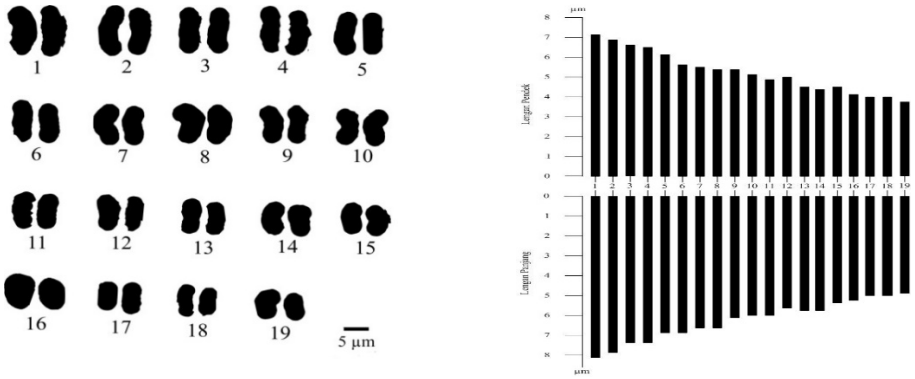


Figure 5. Karyotype and ideogram of *Dendrobium mirbelianum*

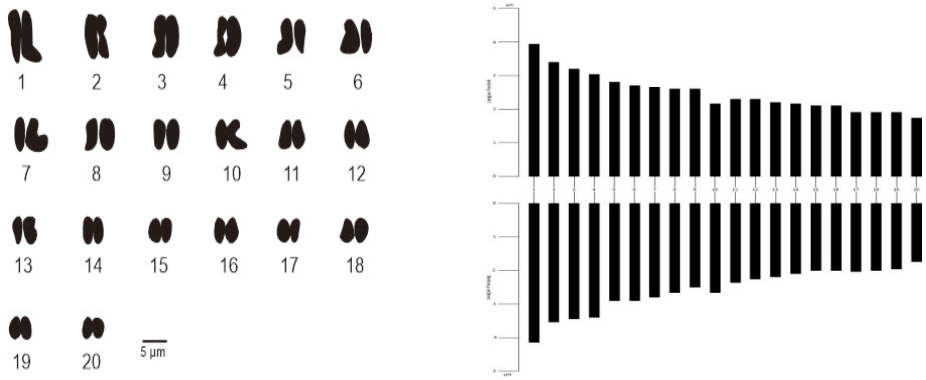


Figure 6. Karyotype and ideogram of *Dendrobium purpureum*

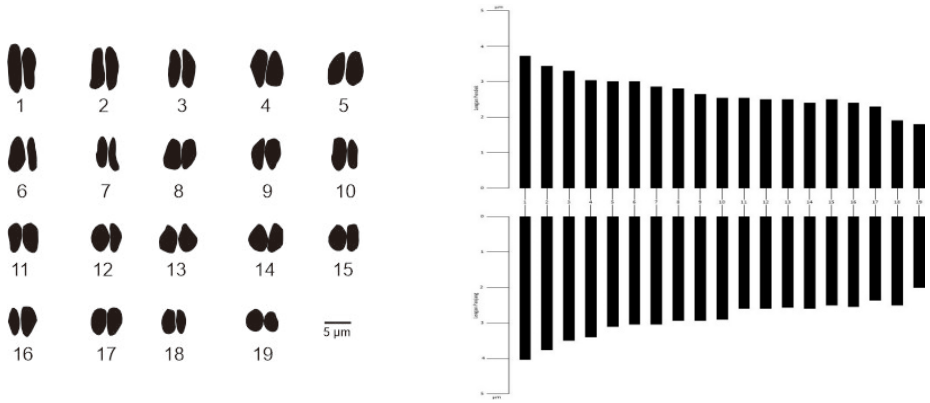


Figure 7. Karyotype and ideogram of *Dendrobium secundum*

Karyotype asymmetry index

It is important to determine chromosome morphology as an index of karyotype asymmetry. The most commonly used karyotype asymmetry indexes are the intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2). In a karyotype pattern, there are various forms of chromosomes that can be identified using the intrachromosomal asymmetry index (A1). The interchromosomal asymmetry index (A2) is used for identifying chromosomal size deviations in a karyotype pattern.

Table 4. Intrachromosomal Asymmetry Index (A1) and Interchromosomal Asymmetry Index (A2) of Dendrobium Orchids

Orchid Species	**A1**	**A2**
Dendrobium lamelatum	0,951	0,21
Dendrobium macrophyllum	0,957	0,22
Dendrobium mirbelianum	0,955	0,025
Dendrobium purpureum	0,953	0,24
Dendrobium secundum	0,951	0,017

Discussion

Orchids had a wide variety of chromosomes with the number ranging from 2n = 2x = 12 to 2n = 2x = 240 (Moraes *et al*., 2017). Previous studies on Orchidaceae chromosomes majorly focused on species with commercial purposes, such as Cattleya, Dendrobium, Phalaenopsis, and Cymbidium. The samples found in Cattleya and Cymbidium were 2n = 40, while 2n = 38 was

obtained in Dendrobium and Phalaenopsis (Atoche *et al*., 2012). The results showed that *D. lamelatum*, *D. macrophyllum*, *D. mirbelianum*, and *D. secundum* had 2n = 38 chromosomes. Furthermore, some Dendrobium species had 2n = 38 chromosomes, such as *D. chrysotoxum* (Zhang *et al*., 2021), *D. stardust* (Kondo *et al*., 2020), *D. draconis* (Bunnag and Hongthongkham, 2015), and *D. formosum* (Yenchon and Te-chato, 2014), etc.

A previous study revealed that *Dendrobium purpureum* had 2n = 40 chromosomes. According to Zheng *et al*. (2018), the number of chromosomes for Dendrobium orchids was 2n = 38, but some species had 2n = 40. Several types of orchids had more basic samples numbering 19 than 20, with 280 species having 19 basic chromosomes. Meanwhile, 274 species of orchids have 20 basic chromosomes (Hartati *et al*., 2014). In this study, Dendrobium has 19 and 20 basic chromosomes.

D. lamelatum had long, short, and total arms of 2.67 ± 0.54 µm, 2.51 ± 0.55 µm, and 5.18 ± 1.09 µm, respectively, followed by *D. macrophyllum* (1.86 ± 0.37 µm, 1.54 ± 0.41 µm, 3.40 ± 0.78 µm), *D. mirbelianum* (6.31 ± 0.17 µm, 5.28 ± 0.16 µm, 11.59 ± 0.30 µm), *D. purpureum* (2.62 ± 0.64 µm, 2.45 ± 0.60 µm, 5.07 ± 1.24 µm), and *D. secundum* (2.89 ± 0.51 µm, 2.70 ± 0.49 µm, 5.59 ± 0.10 µm).

Chromosome size was divided into 3 categories, including long (>5.0 µm), medium (3.0 to 4.9 µm), and short (3.0 to 4.9 µm) (Ramesh *et al*., 2022b). According to Grosso *et al*. (2018), The chromosome size of Dendrobium was small, with the smallest being less than 1 µm. The results also showed that was only a little difference in size among species within the same genus. However, there was a large difference between the species within a genus in this study. Chromosome with a larger size was caused by a larger heterochromatin content compared to others (Hsu *et al*., 2020).

Each plant had a different chromosome shape, which was affected by the position of the centromere. Centromere was an indentation area characterized by the presence of a kinetochore and functioned in regulating the movement of chromosomes (Kursel and Malik, 2016). Furthermore, it divided the chromosomes into long and short arms. According to Aoyama *et al*. (2013), its location could be divided into various categories, including median, submedian, and subterminal.

According to Hartati *et al*. (2022a), chromosome shape was determined by arm proportions. Ratios between 1.0 and 1.7, 1.7 and 3.0, 3.0 and 7.0, and ≥ 7.0 were considered to be metacentric (m), submetacentric (sm), acrocentric, and telocentric, respectively. The results showed that the arm ratio of *D. lamelatum,*

D. macrophyllum, D. mirbelianum, D. purpureum, and *D. secundum* was 1.07 ± 0.06, 1.23 ± 0.16, 1.20 ± 0.03, 1.07 ± 0.09, and 1.08 ± 0.08, respectively. All observed Dendrobium species had 19 pairs of metacentric chromosomes, but *Dendrobium purpureum* had 20 pairs. These results were in line with Hartati *et al*. (2017) that orchids (Orchidaceae) generally had a metacentric chromosome shape.

These parameters were often observed by taking pictures when chromosome was in the metaphase of mitotic division (Chen *et al*., 2017). Karyotype analysis was carried out by measuring the samples, followed by pairing and alignment to produce an image arranged in pairs (Liu *et al*. 2023).

Based on the results, the karyotype pattern for *D. lamelatum*, *D. macrophyllum*, *D. mirbelianum*, and *D. secundum* was 2n = 38 m, while *D. purpureum* had 2n = 40 m. The pattern observed in all species was composed of the same chromosome shape, namely metacentric, which could be further divided into either symmetry or asymmetry. If all chromosomes in one set had the same size and type, they were considered to be symmetrical and vice versa (Saha, 2022).

Chromosome that had been arranged into karyotypes could be clarified using ideograms. An ideogram was a chart that described chromosome based on the relative sizes and homologous groups. These charts were arranged based on the order of chromosomes from largest to smallest. In ideograms, the long arm position was below, while the short variant was placed above (Muliawati *et al*., 2023).

The determination of chromosomal asymmetry index values is based on a comparison between the chromosome long arm and the short arm (Eroglu, 2015). Based on Table 4, All Dendrobium species have the same and considerable intrachromosomal asymmetry index values (A1). This is because all species have a metacentric chromosomal shape. Neto *et al*. (2017) stated that, intrachromosomal asymmetry index (A1) in Orchidaceae ranged from small asymmetry to moderate asymmetry. A considerable degree of asymmetry can be caused by the loss of chromosome segments due to polyplody, amplification in different heterochromatin regions, or due to hybridization between species with different chromosome sizes. While the interchromosomal asymmetry index (A2) in all species shows a fairly small value. This is directly proportional to the deviation of the size of the chromosomes. The smaller value of the interchromosomal asymmetry index (A2), the chromosomal deviation in one karyotype pattern will also be small (Muliawati *et al*., 2023).

Based on the above research results on various Dendrobium species, we can be concluded that *D. lamelatum*, *D. macrophyllum*, *D. mirbelianum*, and *D. secundum* have a chromosome number 2n = 38, while *D. purpureum* has a chromosome number 2n = 40. *D. lamelatum* has a long arm 2.67 ± 0.54 µm and a short arm 2.51 ± 0.55 µm. *D. macrophyllum* has long arms 1.86 ± 0.37 µm and short arms 1.54 ± 0.41 µm. *D. mirbelianum* has long arms 6.31 ± 0.17 µm and short arms 5.28 ± 0.16 µm. *D. purpureum* has long arms 2.62 ± 0.64 µm and short arms 2.45 ± 0.60 µm. *D. secundum* has long arms 2.89 ± 0.51 µm and short arms 2.70 ± 0.49 µm. *D. lamelatum*, *D. macrophyllum*, *D. mirbelianum*, and *D. secundum*, *D. purpureum* has a metacentric chromosome shape. The karyotype pattern of *D. lamelatum*, *D. macrophyllum*, *D. mirbelianum*, and *D. secundum* is 2n = 38 m, while for *D. purpureum* is 2n = 40 m. The index value (A1) in all species shows a metacentric shape, while the index value (A2) in all species is shows a deviation in chromosome size is small.

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References

Aoyama, M., Alonso, C. and Baltierra, X. C. (2013). Chromosome numbers of some terrestrial orchids in Chile. Chromosome Botany, 8:23-27.

Apriliyania, R. and Wahidah, B. F. (2021). Propagation of *Dendrobium* sp. in vitro: success factors. Phylogeny: Journal Biology Student, 1:33-46.

Atoche, J. A., Iiyama, C. M. and Cardoso, J. C. (2021). Polyploidization in orchids: from cellular changes to breeding applications. Plants, 11:1-21.

Bunnag, S. and Hongthongkham, J. (2015). Polyploid induction of *Dendrobium draconis* Rchb.f. Acta Horticulture, 1087:445-451.

Chen, J., Xia, N., Wang, X., Beeson Jr., R. C. and Chen, J. (2017). Ploidy level, karyotype, and DNA content in the Genus Lonicera. HortScience, 52:1680-1686.

Chen, WH., Kao, YL., Tang, CY. Tsai, C. C. and Lin, T. Y. (2013). Estimating nuclear DNA content within 50 species of the genus *Phalaenopsis Blume* (Orchidaceae). Scientia Horticulture, 161:70-75.

Choi, B., Gang, G. H., Kim, H., Byun, H., Kwak, M., So, S., Myeong, H. H. and Jang, T. S. (2021). Cytological study of *Cypripedium japonicum* Thunb. (Orchidaceae Juss.): An endangered species from Korea. Plants, 10:1-10.

De, L. C., Rao, A. N., Rajeevan, P. K., Srivastava, M. and Chhetri, G. (2015). Morphological characterization in *Dendrobium* species. Journal of Global Biosciences, 4:1198-1215.

Eroglu, H. E. (2015). Which chromosomes are subtelocentric or acrocentric? A new karyotype symmetry/asymmetry index. Caryologia: International Joyrnal of Cytology, 68:239-245.

Grosso, V., Farina, A., Giorgi, D., Nardi, L., Diretto, G. and Lucretti, S. (2018). A high-throughput flow cytometry system for early screening of in vitro made polyploids in *Dendrobium* hybrids. Plant Cell Tissue Organ Culture, 132:57-70.

Hartati, S., Darsana, L. and Cahyono, O. (2014). Study of cytological characterization of orchids in the context of preserving germplasm. Caraka Tani 29:25-30.

Hartati, S., Nandariyah., Yunus, A. and Djoar, D. W. (2017). Short communication: Cytological studies on black orchid hybrid (*Coelogyne pandurata* Lindley). Biodiversity, 18:555-559.

Hartati, S., Parjanto., Sukaya., Muliawati, E. S., Nandariyah., Yuniastuti, E., Manurung, I. R. and Purmiyoto, C. W. W. (2022a). Chromosome of *Phaius tankervilleae* and *Phaius amboinensis* Orchid. International Conference on Sustainable Agriculture and Environment, 1114:1-5.

Hartati, S., Sukaya and Samanhudi (2022b). Karyotype and ploidy of *Vanda dearei* and *Vanda celebica* orchid using flow cytometry analysis. Journal Indonesian Agronomy, 50:337-342.

Hsu, C. C., Chen, S. Y., Lai, P. H., Hsiao, Y. Y., Tsai, W. C., Liu, Z. J., Chung, M. C., Panaud, O. and Chen, H. H. (2020). Identification of high-copy number long terminal repeat retrotransposons and their expansion in *Phalaenopsis* orchids. BMC Genomics, 21:1-13.

Huy, N. P., Tam, D. T., Luan, V. M., Hoang, T. T., Hien, V. T., Ha, N., Duy, P. N. and Tan, N. D. (2019). In vitro polyploid induction of *Paphiopedilum villosum* using colchicine. Scientia Horticulture, 252:283-290.

Kondo, H., Phlaetita, W., Mii, M., Kikuchi, S., Deguchi, A. and Miyoshi, K. (2020). Efficient chromosome doubling of an interspecific hybrid *Dendrobium Stardust* 'fire bird' by treatment of amiprofos-methyl to protocorm like body. Biotechnology, 56:738-749.

Kurniawan, F. Y. and Riyadi, A. D. (2021). Morphological and chromosomal characterization of the orchid *Peristylus goodyeroides* Lindl. From Curug Setawing, Kulonprogo. Journal Math and Science Education, 12:110-122.

Kursel, L. E. and Malik, H. S. (2016). Centromere. Current Biology, 26:481-492.

Lalla, M. and Sudiarta, I. M. (2022). Development of orchid plants in the Motilango Pine Forest Tourism Area, Tibawa District, Gorontalo Regency. J Community Service, 3:87-91.

Lee, Y. I., Chang, F. C. and Chung, M. C. (2011). Chromosome pairing affinities in interspecific hybrids reflect phylogenetic distances among lady's slipper orchids (*Paphiopedilum*). Annals of Botany, 108:113-121.

Lee, Y. I., Tseng, Y. F., Lee, Y. C. and Chung, M. C. (2020). Chromosome constitution and nuclear DNA content of *Phalaenopsis* hybrids. Scientia Horticulture, 262:1-9.

Liu, Y., Duan, S. D., Jia, Y., Hao, L. H., Xiang, D. Y., Chen, D. F. and Niu, S. C. (2023). Polyploid induction and karyotype analysis of *Dendrobium officinale*. Horticlturae, 9:329.

Moraes, A. P., Koehler, S., Cabral, J. S., Gomes, S. S. L., Viccini, L. F., Barros, F., Felix, L. P., Guerra, M. and Martins, E. R. F. (2017). Karyotype diversity and genome size variation in *Neotropical Maxillariinae* orchids. Plant Biology, 19:298-308.

Muliawati, E., Hartati, S., Parjanto. Sukaya., Nandariyah., Yuniastuti, E., Manurung, I. R. and Purmiyoto, C. W. W. (2023). Karyotype of *Phaius tankervilleae* and *Phaius amboinensis* orchid. E3S Web of Conferences, 373:1-6.

Neto, E. M., Nollet, F., Moraes, A. P. and Felix, L. P. (2017). Intrachromosomal karyotype asymmetry in Orchidaceae. Genetics and Molecular Biology, 40:610-619.

Niu, Z., Zhu, F., Fan, Y., Li, C., Zhang, B., Zhu, S., Hou, Z., Wang, M., Yang, J., Xue, Q., Liu, W. and Ding, X. (2021). The chromosome-level references genome assembly for *Dendrobium officinale* and its utility of functional genomics research and molecular breeding study. Acta Pharmaceutica Sinica B, 11:2080-2092.

Nugraheni, K. A., Kurniawan, F. Y., Riyadi, A. D. R., Kinasih, A., Ilmam, H. P. L. and Semiarti, E. (2022). Cytological analysis of *Aerides odorata* Lour. from Sleman, Special Region of Yogyakarta. Advances in Biological Sciences Research, 22:545-552.

Ramesh, T., Kumari, K. G., Muruganantham, P. and Vanithamani, J. (2022a). Cytological studies on some endemic terrestrial orchids. Plant Archives, 22:151-157.

Ramesh, T., Kumari, K. G., Muruganantham, P., Praveena, R., Vanithamani, J. and Koperuncholan, M. (2022b). Studies on chromosome variation in *Vanda* species of Orchidaceae. Plant Archives, 22:130-135.

Saha, M. L. (2022). Botany: Addressing the challenges and opportunities for the 21st century. Dhaka (BD): SRL Printing Press.

Sharma, H. B. and Bhattacharyya, D. (2023). Karyomorphological studies of some lithophytic species of the genus *Dendrobium* Sw. (Orchidaceae) from Manipur, India: a cytotaxonomic approach. Nucleus, 66:145-150.

Sharma, S. K. and Mukai, Y. (2015). Chromosome research in orchids: current status and future prospects with special emphasis from molecular and epigenetic perspectives. Nucleus, 58:173-184.

Silva, G. M., Varella, T. L., Karsbug, I. V., Santana, T. N., Carvalho, I. F., Anez, R. B. S. and Rossi (2017). Cytogenetic characterization of species and hybrids of orchids of *Cattleya* genus. Ctytologia, 82:137-140.

Wang, Y., Chen, Y., Wei, Q., Chen, X., Wan, H. and Sun, C. (2022). Characterization of repetitive sequences in *Dendrobium officinale* and comparative chromosomal structures in *Dendrobium* species using FISH. Gene, 846:1-12.

Yenchon, S. and Te-chato, S. (2014). Polyploid induction of *Dendrobium formosum* by colchisine treatment in vitro. Acta Horticulture, 1025:81-88.

Yuan, Y., Liu, X., Wang, J. and Zhang, J. (2018). Morphological and microscopic identification of three major medicinal *Dendrobium* species in the Ta-pieh Mountains area. Microscopy Research Technique, 1-11.

Zhang, Y., Zhang, G. Q., Zhang, D. Liu, X. D., Xu, X. Y., Sun, W. H., Yu, X., Zhu, X., Wang, Z. W., Zhao, X., Zhong, W. Y., Chen, H., Yin W. L., Huang, T., Niu, S. C. and Liu, Z. J.

(2021). Chromosome-scale assembly of the *Dendrobium chrysotoxum* genome enhances the understanding of orchids evolution. Horticulture Research, 8:183.
Zheng, S. G., Hu, Y. D., Zhao, R. X., Yan, S., Zhang, X. Q., Zhao, T. M. and Chun, Z. (2018). Genome-wide research and applications on *Dendrobium*. Planta, 248:769-784.

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