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Comparative Cytogenetic Characterization of *Grammatophyllum martae*, *Dendrobium violaceoflavens*, and *Dendrobium Tiara Beauty* × *D. Graty*

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Abstract. Orchids are among the most popular ornamental plants widely favored by the public. Plant breeders continuously strive to improve the quantity, quality, and cytological characteristics of orchids to support successful breeding programs. This study aimed to determine the cytological characteristics of *Grammatophyllum martae*, *Dendrobium violaceoflavens*, and the hybrid *D. Tiara Beauty* × *D. Graty*, including chromosome number, chromosome size, chromosome shape, karyotype, and chromosome asymmetry index. The research was conducted at the Plant Breeding Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, from July to August 2025. The number, size, shape, and karyotype pattern of chromosomes were analyzed descriptively. The results showed that *G. martae*, *D. violaceoflavens*, and the hybrid *D. Tiara Beauty* × *D. Graty* have the same chromosome number, 2n = 38, composed of metacentric chromosomes. The chromosomes of *G. martae* had a total arm length of 1.10 ± 0.28 µm, *D. violaceoflavens* 1.48 ± 0.40 µm, and the hybrid *D. Tiara Beauty* × *D. Graty* 1.30 ± 0.36 µm. The intrachromosomal (A1) and interchromosomal (A2) asymmetry indices of the three observed orchids were low, indicating that their karyotypes tend to be symmetrical, as they are composed of metacentric chromosomes with minimal size variation.

Keywords: Chromosome; Karyotype; Metacentric; Orchidaceae; Squash technique.

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Introduction

Orchids are one of the most popular ornamental plants belonging to the family Orchidaceae, widely appreciated for their diverse flower shapes and attractive patterns. However, data indicate that orchid production in Indonesia has declined. Production gradually decreased from 2021 to 2023, from 3,999,203 plants to 3,785,454 plants for potted orchids, while cut orchid production declined from 11,351,615 stems to 2,522,933 stems (1). This condition encourages plant breeders to improve the quality, quantity, and genetic diversity of orchids.

Hybridization among orchid parents has long been carried out and has successfully produced superior hybrids (2). Parent plants are selected based on desirable traits so that their offspring inherit these characteristics. Information on plant traits can be observed through morphological and cytological characteristics. Morphological traits are advantageous because they can be obtained easily and quickly; however, the results are often inconsistent due to their susceptibility to environmental conditions and human error during cultivation. In contrast, cytological characteristics are more consistent and less influenced by environmental factors. Cytological traits can be identified through chromosome analysis.

Information on chromosome characteristics plays an important role in supporting the success of orchid breeding programs. The purpose of chromosome analysis is to obtain genetic information, including chromosome number, size, and shape of an organism (3). The results of chromosome analysis in orchids can be used to analyze phylogenetic relationships among plant species, detect genetic abnormalities, and serve as a reference for hybridization. Therefore, this study is necessary to determine the cytological characteristics of two orchid genera.

Materials and Methods

The study was conducted at the Plant Breeding Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, from July to August 2025. The plant materials used in this study were *G. martae*, *D. violaceoflavens*, and the hybrid *D. Tiara Beauty* × *D. Graty* (Figure 1). The main instrument used was a light microscope. One sample was used for each species. The method applied to obtain chromosome preparations was the squash technique.

The research procedure began with preparing root tip samples measuring 1–2 cm, collected between 05:00 and 06:00 a.m. The root tips were cut using forceps and washed with distilled water to remove any adhering debris. The cleaned roots were then immersed in a hydroxyquinoline solution and stored in a refrigerator for 3 hours at approximately 20°C. Afterward, the root tips were rinsed again with distilled water to remove any remaining solution. The next step was fixation by soaking the root tips in 45% acetic acid for 10 minutes. This was followed by hydrolysis using a mixture of 1 N HCl and 45% acetic acid in a 3:1 ratio. Hydrolysis was carried out in a water bath at approximately 60°C for 10–15 minutes. The roots were then stained in 2% aceto orcein solution for about 1 hour. The stained samples were placed on a glass slide and sliced thinly to approximately 0.5 mm using a razor blade. Finally, the samples were covered with a cover glass and gently pressed using a pencil eraser to produce chromosome preparations.

The observed parameters included chromosome number (2n), chromosome size, chromosome shape, karyotype formula, and chromosome asymmetry indices. Chromosome size was determined by measuring the lengths of the long arm (q), short arm (p), and total chromosome length (q + p). Chromosome shape was identified based on the arm ratio (r = q/p) according to the classification proposed by Mitrenina et al. (2024). Karyotype analysis was performed by arranging chromosomes according to their size and centromere position. Chromosome asymmetry was evaluated using the intrachromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2) following standard cytogenetic procedures.

(a) (b) (c)

Figure 1. Flower images of (a) *G. martae*, (b) *D. violaceoflavens*, and (c) the hybrid *D. Tiara Beauty* × *D. Graty*

Results and Discussion

Chromosome number

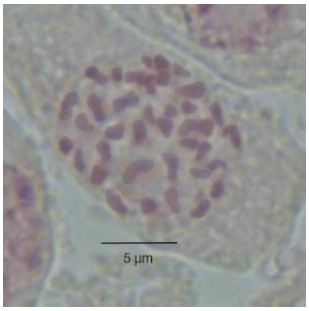
Based on Figure 2, the chromosome numbers of *G. martae*, *D. violaceoflavens*, and the hybrid *D. Tiara Beauty* × *D. Graty* were 2n = 38. This result is consistent with (4), who reported that the majority of orchid species have a chromosome number of 2n = 38. Chromosome numbers within the same genus are generally similar, unless hybridization occurs between individuals with different ploidy levels or mutations arise.

The chromosome counts obtained for *G. martae*, *D. violaceoflavens*, and the hybrid confirm that chromosome numbers within Orchidaceae tend to be highly conserved. These three orchids are therefore compatible for hybridization. According to (5), one of the requirements for successful hybridization is a close evolutionary relationship. Incompatible crosses typically fail due to genetic mismatches that lead to reproductive barriers.

The conserved chromosome number (2n = 38) among the studied orchids suggests genomic stability within Orchidaceae. Similar chromosome numbers may facilitate chromosome pairing during meiosis and increase the likelihood of successful hybrid formation. This cytogenetic compatibility is particularly important in orchid breeding programs because it may contribute to higher fertility and genetic stability in hybrid progenies.

(a)

(b)



(c)

Figure 2. Chromosome numbers of (a) *G. martae* (2n = 38), (b) *D. violaceoflavens* (2n = 38), and (c) the hybrid *D. Tiara Beauty* × *D. Graty* (2n = 38).

Chromosome size

Based on Table 1, each orchid species exhibited different average chromosome arm lengths: *G. martae* (1.10 ± 0.28 µm), *D. violaceoflavens* (1.48 ± 0.40 µm), and the hybrid *D. Tiara Beauty* × *D. Graty* (1.30 ± 0.36 µm). According to (6), chromosome arm lengths of 0.1–2.9 µm are categorized as short, 3–4.9 µm as medium, and >5 µm as long. Thus, the chromosomes of all three orchids fall within the short category and show relatively minor differences.

Although all chromosomes were classified as short, differences in chromosome size may reflect variation in genome organization among the studied orchids. Larger chromosome sizes in *D. violaceoflavens* may indicate a greater amount of repetitive DNA sequences or heterochromatin compared with *G. martae* and the hybrid. Variations in heterochromatin content can influence chromosome condensation, gene expression patterns, and genome evolution. These genomic differences may contribute to variations in morphological traits, growth characteristics, and environmental adaptation among orchid species. Furthermore, the intermediate chromosome size observed in the hybrid suggests the inheritance of chromosomal characteristics from both parental lineages, a phenomenon commonly observed in interspecific hybrids.

Table 1. Chromosome size of *G. martae*, *D. violaceoflavens*, and the hybrid *D. Tiara Beauty* × *D. Graty*

Species	Long arm (q) (µm)	Short arm(p) (µm)	Total arm (q+p) (µm)
G. martae	0.58±0.15	0.52±0.14	1.10±0.28
D. violaceoflavens	0.78±0.22	0.69±0.19	1.48±0.40
D. Tiara Beauty x *D.* Graty	0.68±0.19	0.62±0.18	1.30±0.36

The chromosome sizes ranged from 1.10 to 1.48 µm. According to (7), differences in chromosome size may be influenced by genetic factors and protein composition. Variations in heterochromatin content also contribute to size differences: smaller

chromosomes contain less heterochromatin, while larger ones contain more (8). Chromosome size data are useful in plant breeding, as they enable more accurate identification of gene locations.

Chromosome shape

Based on Table 2, the chromosomes of *G. martae*, *D. violaceoflavens*, and the hybrid *D. Tiara Beauty* × *D. Graty* are metacentric. This classification is based on the arm ratio values ranging from 1 to 1.7. According to (9), chromosome arm ratios (r) of 1–1.7 are classified as metacentric (m), 1.7–3.0 as submetacentric (sm), 3.0–7.0 as acrocentric (t), and >7 as telocentric (T). The arm ratios were 1.13 ± 0.12 for *G. martae*, 1.13 ± 0.08 for *D. violaceoflavens*, and 1.10 ± 0.11 for the hybrid.

Table 2. Chromosome shape of *G. martae*, *D. violaceoflavens*, and the hybrid *D. Tiara Beauty* × *D. Graty*

Species	Arm ratio ($\bar{X}$ ± SD) (µm)	Chromosome type
G. martae	1.13±0.12	38 m
D. violaceoflavens	1.13±0.08	38 m
D. Tiara Beauty x *D.* Graty	1.10±0.11	38 m

According to (10), most members of the Orchidaceae family have metacentric chromosomes. This finding aligns with (11) findings, which state that orchid chromosomes are generally metacentric. This finding aligns with the findings of (12), who stated that the genus Dendrobium has metacentric chromosomes. The lengths of the long and short arms of metacentric chromosomes are nearly identical. According to (13), the long and short arms of a chromosome are identical when the centromere is located in the center of the chromosome.

The presence of exclusively metacentric chromosomes in all three orchids indicates a highly symmetrical chromosome structure. Metacentric chromosomes are generally considered more stable during mitosis and meiosis because the centromere is positioned near the chromosome midpoint, reducing the likelihood of unequal chromosome segregation. Such stability may contribute to normal cellular division and genetic integrity. The uniform occurrence of metacentric chromosomes also supports the low chromosome asymmetry values obtained in this study. It suggests that major structural chromosomal rearrangements, such as inversions or translocations, have not played a significant role in the divergence of these taxa.

Karyotype

Based on Figures 3, 4, and 5, the karyotype patterns of *G. martae*, *D. violaceoflavens*, and the hybrid *D. Tiara Beauty* × *D. Graty* are identical, namely 2n = 2x = 38m, where *m* indicates metacentric chromosomes. All observed orchids are diploid, consisting of two genome sets, each with 19 chromosomes. According to (14), karyotype analysis can be used to detect chromosomal aberrations in both structure and number that occur during cell division. The identical chromosome number (2n = 38) and karyotype formula (2n = 2x = 38m) observed in all taxa indicate that no numerical or structural chromosomal aberrations were detected. The slight variation in chromosome size is likely associated with differences in chromatin condensation or heterochromatin content rather than chromosomal rearrangements.

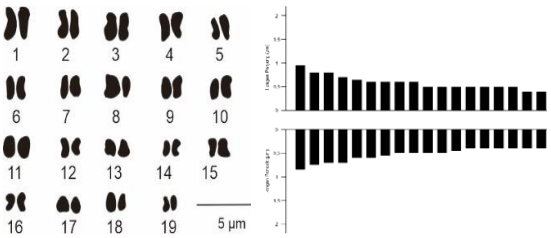


Figure 3. Karyogram and idiogram of *G. martae*

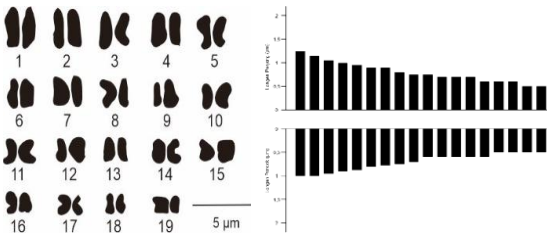


Figure 4. Karyogram and idiogram of *D. violaceoflavens*

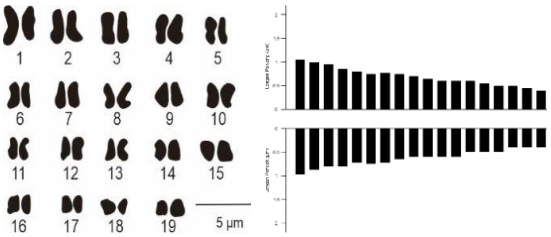


Figure 5. Karyogram and idiogram of the hybrid *D. Tiara Beauty* × *D. Graty*

Figures 3, 4, and 5 show that the idiograms of each orchid exhibit variation in chromosome size and shape. These variations may result from structural changes in chromosomes. According to (15), differences in chromosome size may be caused by varying

degrees of chromatin condensation or differences in heterochromatin accumulation. Heterochromatin plays a role in suppressing gene expression, maintaining chromosome structure, and preventing undesirable genetic recombination (16).

The identical karyotype formula (2n = 2x = 38m) observed in all taxa demonstrates a high degree of cytogenetic similarity despite belonging to different taxa and including a hybrid genotype. Karyotype conservation is generally associated with genomic stability and may indicate that chromosome evolution within these orchids has occurred primarily through small-scale genetic modifications rather than major chromosomal rearrangements. The successful formation of the hybrid *D. Tiara Beauty* × *D. Graty* may therefore be attributed, at least in part, to the high level of chromosomal compatibility between the parental genomes. Such compatibility is advantageous in breeding programs because it can promote stable inheritance of desirable traits in subsequent generations.

Chromosome asymmetry index

The intrachromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2) range from 0 to 1. Based on Table 3, the A1 values were 0.10 for *G. martae*, 0.11 for *D. violaceoflavens*, and 0.09 for the hybrid *D. Tiara Beauty* × *D. Graty*. These low values indicate that the karyotypes of all three orchids are symmetrical. According to (17), lower A1 values correspond to more symmetrical karyotypes, as they are predominantly composed of metacentric chromosomes.

Table 3. Chromosome asymmetry index of *G. martae*, *D. violaceoflavens*, and the hybrid *D. Tiara Beauty* × *D. Graty*

Spesies	A1	A2
G. martae	0.10	0.26
D. violaceoflavens	0.11	0.27
D. Tiara Beauty x *D. Graty*	0.09	0.28

Based on Table 3, the A2 values were 0.26 for *G. martae*, 0.27 for *D. violaceoflavens*, and 0.28 for the hybrid. These values indicate low variation in chromosome size among the three orchids. According to (18), lower A2 values reflect minimal chromosome size variation, whereas values approaching one indicate greater variation. Increased heterogeneity in chromosome size leads to higher interchromosomal asymmetry (19). Chromosome shape and size tend to be uniform when both A1 and A2 values are low (20).

The low A1 and A2 values observed in all three orchids indicate highly symmetrical karyotypes characterized by minimal differences in chromosome arm ratios and chromosome lengths. Symmetrical karyotypes are often regarded as cytologically stable and evolutionarily conserved because they are less affected by structural chromosome alterations. The similarity of asymmetry indices among the studied orchids suggests that they share a comparable chromosomal organization and may have experienced similar evolutionary pressures during diversification. Moreover, the maintenance of a symmetrical karyotype in the hybrid indicates that hybridization did not result in substantial chromosomal restructuring, further supporting the cytogenetic compatibility of the parental species.

Conclusion

Grammatophyllum martae, *Dendrobium violaceoflavens*, and the hybrid *D. Tiara Beauty* × *D. Graty* possess the same chromosome number (2n = 38). The total chromosome lengths were 1.10 ± 0.28 µm in *G. martae*, 1.48 ± 0.40 µm in *D. violaceoflavens*, and 1.30 ± 0.36 µm in the hybrid. All chromosomes were metacentric, resulting in the same karyotype formula, 2n = 2x = 38m. The low intrachromosomal asymmetry index (A1) indicates highly symmetrical chromosome structures, while the low interchromosomal asymmetry index (A2) reflects minimal variation in chromosome size. These findings demonstrate a high degree of cytogenetic similarity among the studied orchids and suggest strong chromosomal compatibility that may support future orchid breeding programs.

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