ISSN 2085-2916 e-ISSN 2337-3652
Tersedia daring http://jai.ipb.ac.id

J. Agron. Indonesia, Desember 2022, 50(3):337-342
DOI: https://dx.doi.org/10.24831/jai.v50i3.43825

Karyotype and Ploidy of *Vanda dearei* and *Vanda celebica* Orchid Using Flow Cytometry Analysis

Kariotipe dan Ploidi pada Anggrek Vanda dearei and Vanda celebica menggunakan Analisis Flow Cytometry

Sri Hartati[1,2*], Sukaya[1], and Samanhudi[1,2]

[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret
Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia
[2]Center for Research and Development of Biotechnology and Biodiversity, Universitas Sebelas Maret
Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia

Received 25 October 2022/Accepted 23 December 2022

ABSTRACT

The diversity of the Vanda orchid is one of the advantages of plants that are used to make new hybrids. The genus of Vanda has a primary chromosome number (x) = 19 and varies in ploidy level. This study aims to identify the Vanda orchid's karyotype pattern and ploidy level through flow cytometry. The materials used are orchids Vanda celebica, Vanda dearei, and its hybrids V. dearei x V. celebica and V. celebica x V. dearei. The results showed the number of chromosomes of V. dearei is 2n = 2x = 40, V. celebica is 2n = 2x =38, the hybrid V. celebica x V. dearei is 2n = 2x = 38, and the hybrid of V. dearei x V. celebica is 2n = 2x = 38. The karyotype V. celebica is 2n = 2x = 17m+2sm, V. dearei is 2n = 2x = 40 =18m+2sm, the hybrid of V. celebica x V. dearei is 2n = 2x = 38 = 17m+1sm, and hybrid of V. dearei x V. celebica is 2n = 2x = 38 =17m+1sm. All observed ploidy levels of orchids are diploid.

Keywords: cytology, diploid, genetics, hybrid

ABSTRAK

Keanekaragaman anggrek Vanda merupakan salah satu keunggulan tanaman yang dapat digunakan untuk membuat hibrida baru. Sifat anggrek ditentukan oleh banyak gen, sehingga perlu dilakukan persilangan agar diperoleh keragaman genetik yang lebih besar. Persilangan antara tetua dapat menghasilkan keturunan dengan jumlah kromosom yang berbeda dari tetuanya. Salah satu metode yang berperan dalam mengetahui tingkat ploidi adalah analisis kariotipe dan flow cytometry. Penelitian ini bertujuan untuk mengidentifikasi pola kariotipe dan tingkat ploidi anggrek Vanda melalui flow cytometry. Bahan yang digunakan adalah anggrek V. celebica, V. dearei, hibrida dari V. dearei x V. celebica, dan V. celebica x V. dearei. Hasil penelitian menunjukkan jumlah kromosom V. dearei 2n = 2x = 40, V. celebica 2n = 2x = 38, hibrida dari V. celebica x V. dearei 2n = 2x = 38, dan V. dearei x V. celebica 2n = 2x = 38. Susunan kariotipe anggrek V. celebica adalah 2n = 2x = 17m+2sm, V. dearei adalah 2n = 2x = 40 =18m+2sm, hibrida dari V. celebica x V. dearei adalah 2n = 2x = 38 = 17m+1sm, dan V. dearei x V. celebica adalah 2n = 2x = 38 = 17m+1sm. Semua tingkat ploidi anggrek yang diamati adalah diploid.

Kata kunci: sitologi, flow cytometry, genetika, kariotipe

INTRODUCTION

Indonesia is a country with quite a lot of orchids. There are about 5,000 species scattered throughout Indonesia's forests (Suryani, 2015). Vanda is one of the orchids with various patterns, so it is always sought after by fans. The diversity of the Vanda orchid is one of the advantages of plants that are used to make new hybrids (Hartati, 2014). Breeding methods through hybridization and selection become a way for breeders to produce new varieties with the expected characteristics, starting from color, flower shape, aroma, plant shape, early maturity, and resistance to pests and diseases chromosomes number. The difference in the appearance of the chromosomes can be seen with a light microscope with a magnification of 1,000x because the size and position of the centromere are different.

Vanda is a genus of orchids in the Vandaceous group that can grow in various climates, from hot tropical climates to cold, snowy climates. The distribution is extensive,

* Corresponding author. e-mail: tatik_oc@yahoo.com

from the coast to the mountains. Vanda is a type of orchid with high economic value because it has advantages in its flowers' beauty (Rupawan *et al*., 2014). Normal plant mating produces new plants that have even (2x) chromosomes. If the chromosome is 3x, the orchid parent is fertilized. Parents who have abnormal chromosomes produce fruit that falls off quickly. Parents with 4x chromosomes will produce more significant fruit, while parent plants with 6x and 8x chromosomes will produce poor orchids. Many genes determine the nature of orchids, and it is necessary to cross them to obtain greater genetic diversity (Mulkan, 2011).

One of the main cytological methods that play a role in theoretical and empirical research is the Karyotype (Liang and Chen, 2015). The karyotype is a sequence of chromosomes arranged from the longest to the shortest. Ramadhani (2012) explains that a karyotype is a process of chromosome analysis carried out by classifying chromosomes based on the length and shape of the chromosomes used to make idiograms. Each plant family and within different populations of the same species have different karyotypes (Shi *et al*., 2009).

Observing the level of ploidy through observing the number of chromosomes has weaknesses. This is because the tissue consists of many cells and chromosome multiplication only occurs in actively dividing cells. Hence, the possibility of a mixoploid is also significant and will be difficult to observe if only on the number of chromosomes. Therefore, to determine the ploidy level of plants, it is necessary to observe ploidy using flow cytometry. Flow cytometry is responsible for rapid genome size screening on multiple populations of the same species as well as on multiple individuals of each population and supports hybridization (Siljak-Yakovlev *et al*., 2008).

MATERIALS AND METHODS

Plant Material

The research was carried out at the Cytology Laboratory of the Biology Research Center (LIPI) Bogor, conducted from March 2019 to June 2019. The materials used are orchids *V. celebica* (Figure 1), *V. dearei* (Figure 1), a hybrid of *V. dearei* x *V.celebica*, and a hybrid of *V. celebica* x *V. dearei*. The chemicals used are Hydroxyquinoline 0.002M, glacial acetic acid 45%, 1N HCl solution, acetic acid 45%, acetoorcein 2%, PI (Propidium Iodide), and RNAse buffer (1 mL).

Karyotype Pattern

Root tips that have been taken from plants were pre-treated with Hydroxyquinoline 0.002M and stored in a refrigerator for 3-4 hours at 5 °C. Fixation using.45% glacial acetic acid for 10 minutes. Hydrolysis was done by immersing the roots in 1N HCl solution and 45% acetic acid with a ratio of 1: 3 for 1-5 minutes at 60 °C. Staining to clarify, the chromosomes used acetoorcein 2% and squashed after it was sealed with transparent nail polish. The preparations were then observed under a light microscope.

Preparations of orchid roots were observed at 15 times ocular lens magnification and 100 times objective lens magnification using a light microscope. Chromosome images from microscope observations were taken using the obtilab application, which was then processed using Corel Draw X7 and Microsoft Excel for chromosome character analysis. The variables observed were the number of chromosomes, chromosome size, and chromosome shape.

Flow Cytometry Analysis

The standard used is the chromosomal number of black orchid (*Coelogyne pandurata*) is 2n = 2x = 36 (Hartati *et al.*, 2017). Ploidy analysis used a Partec CyFlow chamber (Partec GmbH) with a 920 mW diode pumped solid state laser at 488 nm and a diode laser at 638 nm (25 mW). Leaf pieces (0.5 cm^2) were chopped with a razor blade in a petri dish containing 250 ml of extraction buffer. The Kit series used is CyStain PI absolute P (code 05-5022) from Sysmex Company. Kit for 250 tests contains 125 ml extraction buffer, 500 mL staining buffer, 2 x 1.5 mL Propidium Iodide Solution, and 1 x 5 mg RNAse A. Extraction buffer used Stock solution 200 µL RNAse solution 2 µL. After 30-90 seconds, the extraction buffer was filtered through a Partec 30 ml CellTrics filter. The staining used was 120 µL PI (Propidium Iodide) stock solution and 60 µl RNAse stock solution (1 mL) (Sysmex), then incubated for 30 minutes before being analyzed by flow cytometry. The tool used for flow cytometry analysis is CyFlow space Partec, while for analyzing histograms using FloMax software.

RESULTS AND DISCUSSION

Karyotype Pattern

The chromosome number of *V. celebica* is 2n=38, *V. dearei* is 2n = 40, and their hybrid is 2n = 38 (Table 1). Karyotype of *V. celebica* is 2n = 34 m + 4 sm. The karyotype of *V. dearei* is 2n = 39 m + 1 sm. The karyotype of hybrid *V. celebica* x *V. dearei* is 2n = 38 m, and the karyotype of hybrid *V. dearei* x *V. celebica* is 2n = 38 m (Figure 1-5). Aziz (2020) states that chromosomes can vary from species to species or may be different types of wild and cultivars. Zhao (2014) states that homologous chromosome variations in an individual are caused by the minichromosome between secondary and centromere narrowing. According to Chen

Table 1. The number of chromosome *Vanda dearei, Vanda celebica,* hybrid of *V. celebica* with *V. dearei,* and *hybrid of V. dearei* with *V. celebica*

Orchid	Chromosome number
V. celebica	2n = 38
V. dearei	2n = 40
V. celebica x *V. dearei*	2n = 38
V. dearei x *V. celebica*	2n = 38

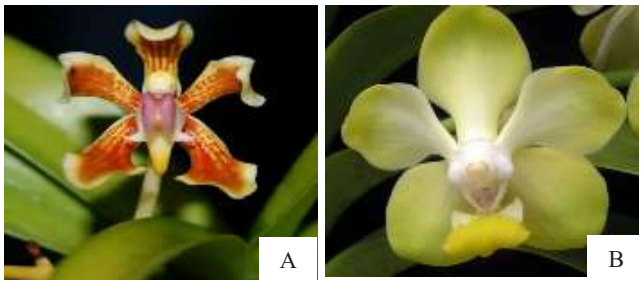


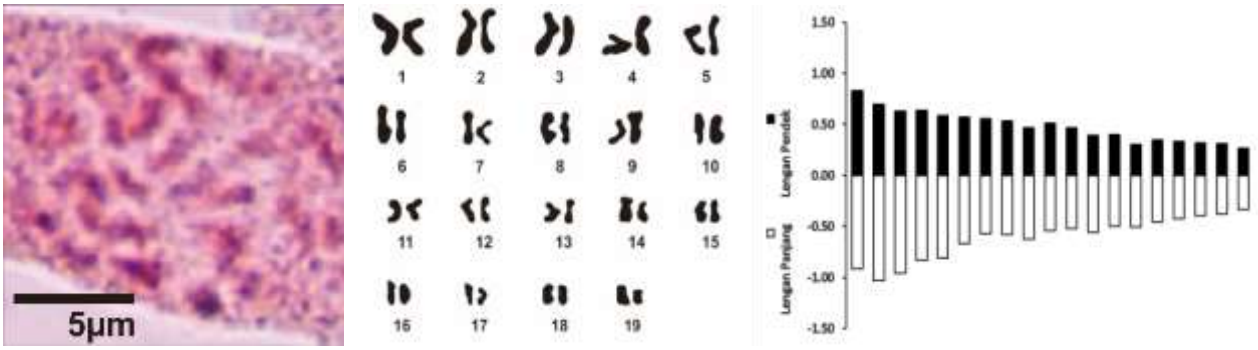
Figure 1. (A) *Vanda celebica* dan (B) *Vanda dearei*

(2017), the Polo karyotype of the *Vanda* orchid varies greatly. According to Chen (2017), the high karyotype variation indicates the large genetic cohesiveness of a species, so it has wide adaptability.

Chromosomes in plants have a unique genetic structure, starting from the centromere's number, size, shape, and position. The unique genetic structure of chromosomes facilitates the characterization of chromosomes in a species. Chromosome characterization is displayed by pairing each homologous pair ultimately and arranging it from the largest to the smallest in the karyogram. The karyotype and its features are schematically presented as idiograms or karyograms (Weiss-Schneeweiss and Schneeweiss, 2012). A karyogram is presented through a single image in the form of a diagram called an ideogram. Arrangement of karyotypes with parameters of chromosome length and number, centromere position, secondary indentation size and position, satellite size and position, degree and distribution of heterochromatin, comparison of chromosome pairs, and comparison of the chromosome arm. Using chromosomes as genetic markers are carried out by analyzing the arrangement of chromosomes (Aziz, 2019). According to Tabassum *et al.* (2014), karyotype analysis provides the basis for the development of biological systems and relative relationships. Karyotypes are used to determine the number of chromosomes and chromosomal aberrations in the chromosome structure during cell division and to determine the relationship between the two and abnormalities in the anatomy, morphology, and physiology of organisms. According to Hartati (2014), the deviation from the number and structure of chromosomes that occurs during division is known from the presence of a karyotype. Chromosome aberrations are related to the anatomical structure, morphology, and physiological abnormalities of the organism.

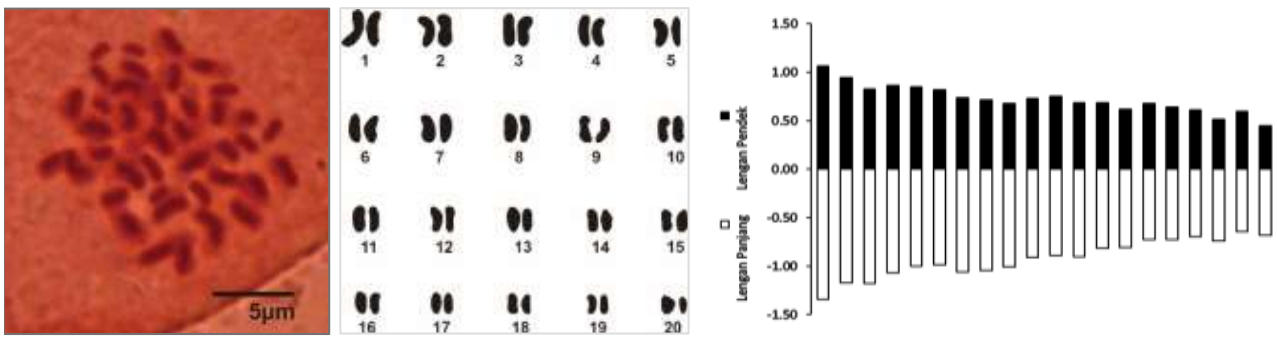
Flow Cytometry Analysis

The histogram in Figure 6 and Table 2 shows that all 4 Vanda species and their hybrid have the ploidy level are diploid (2x). Control samples of diploid plants were calibrated at channel 200. Diploid plants showed peaks at



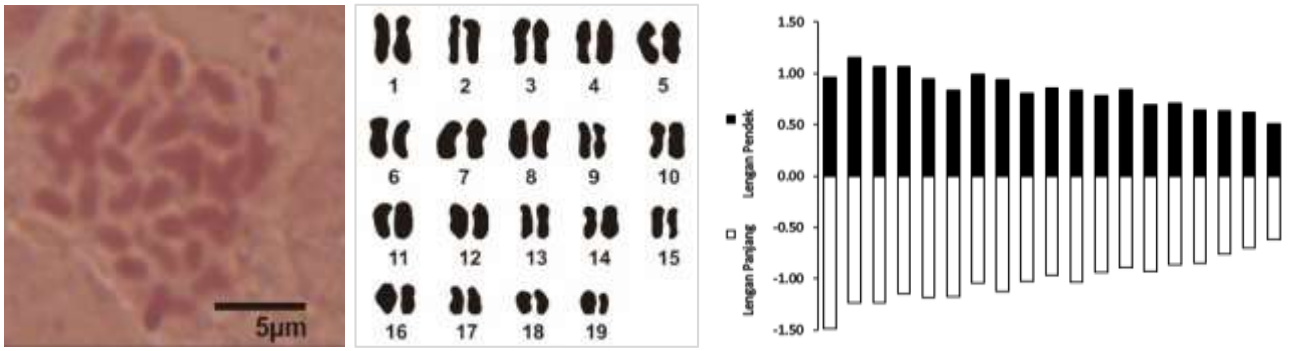
(A) (B) (C)

Figure 2. (A) Chromosome number of *V. celebica*, (B) Karyotype of *V. celebica*, (C) Karyogram of *V. celebic*



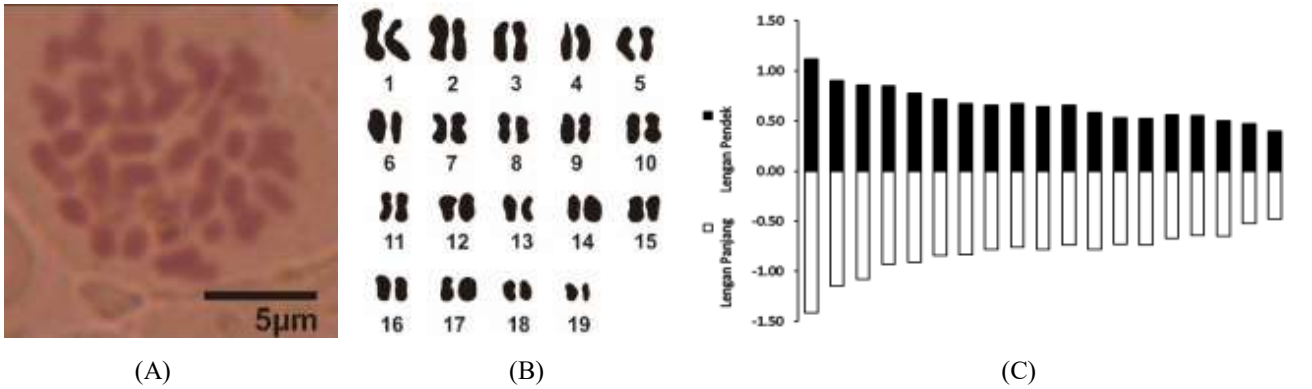
(A) (B) (C)

Figure 3. (A) Chromosome number of *V. dearei*, (B) Karyotype of *V. dearei*, (C) Karyogram of *V. dearei*



(A) (B) (C)

Figure 4. (A) Chromosome number of *V. dearei* x *V. celebica*, (B) Karyotype of *V. dearei* x *V. celebica*, (C) Karyogram of *V. dearei* x *V. celebica*



(A) (B) (C)

Figure 5. (A) Chromosome number of *V. celebica* x *V. dearei,* (B) Karyotype of *V. celebica* x *V. dearei,* (C) Karyogram of *V. celebica* x *V. dearei*

channel 200, triploid plants at channel 300, tetraploid plants at channel 400, and mixoploid plants showed more than one peak on different channels. The observation shows no difference in the ploidy level of hybrids from the parents based on identification through flow cytometry. In contrast to the results of research by Hartati *et al.* (2017), which showed a change in the ploidy level of hybrids from the parents. The parent C. *pandurata* is diploid with the number of chromosomes 2n = 2x = 36, the parent C. *rumphii* is tetraploid with the number of chromosomes 2n = 4x = 72, and the crossing of the two parents produces a hybrid that is triploid with the number of chromosomes 2n = 3x = 54.

Grosso *et al.* (2018) also conducted flow cytometry analysis on Dendrobium hybrid PLBs. According to Grosso *et al.* (2018), flow cytometry was chosen to evaluate DNA content in Dendrobium hybrid explants due to the problematic explants and tiny chromosome size. Flow cytometry analysis was performed to assess the original ploidy level of explants and endopolyploid tendency during tissue culture in PLB, observing the polyploidization tendency after pretreatment of PLB in PLB liquid culture and detecting the ploidy level in regenerated plantlets. Another study by Jones and Kuehnle (1998) shows that the number of chromosomes in 37 Dendrobium species is uniform 2n = 2x = 38. However, the average chromosome size among Dendrobium species varies, ranging from 1.53 pg 2C-1 (D. *cruentum* Rchb.f.) to 4.23 pg 2C-1 (D. *spectabile* (Bl.) Miq.). This proves that, in some cases, flow cytometry can help distinguish varieties or species that have almost the same morphological characters but with different karyotype profiles. In addition, according to Hajrudinović *et al.* (2015), results from flow cytometry are essential for the identification of crossing systems and analysis of ploidy dynamics in Sorbus.

Table 2. Ploidy level of *V. celebica, V. dearei, V. celebica* x *V. dearei,* and *V. dearei* x *V. celebica*

Orchid	Mean	CV (%)	Ploidy
V. celebica	185.71	4.62	2x
V. dearei	211.67	4.61	2x
V. celebica x *V. dearei*	212.28	8.38	2x
V. dearei x *V. celebica*	213.42	4.77	2x

Note: Ploidy compared to standart

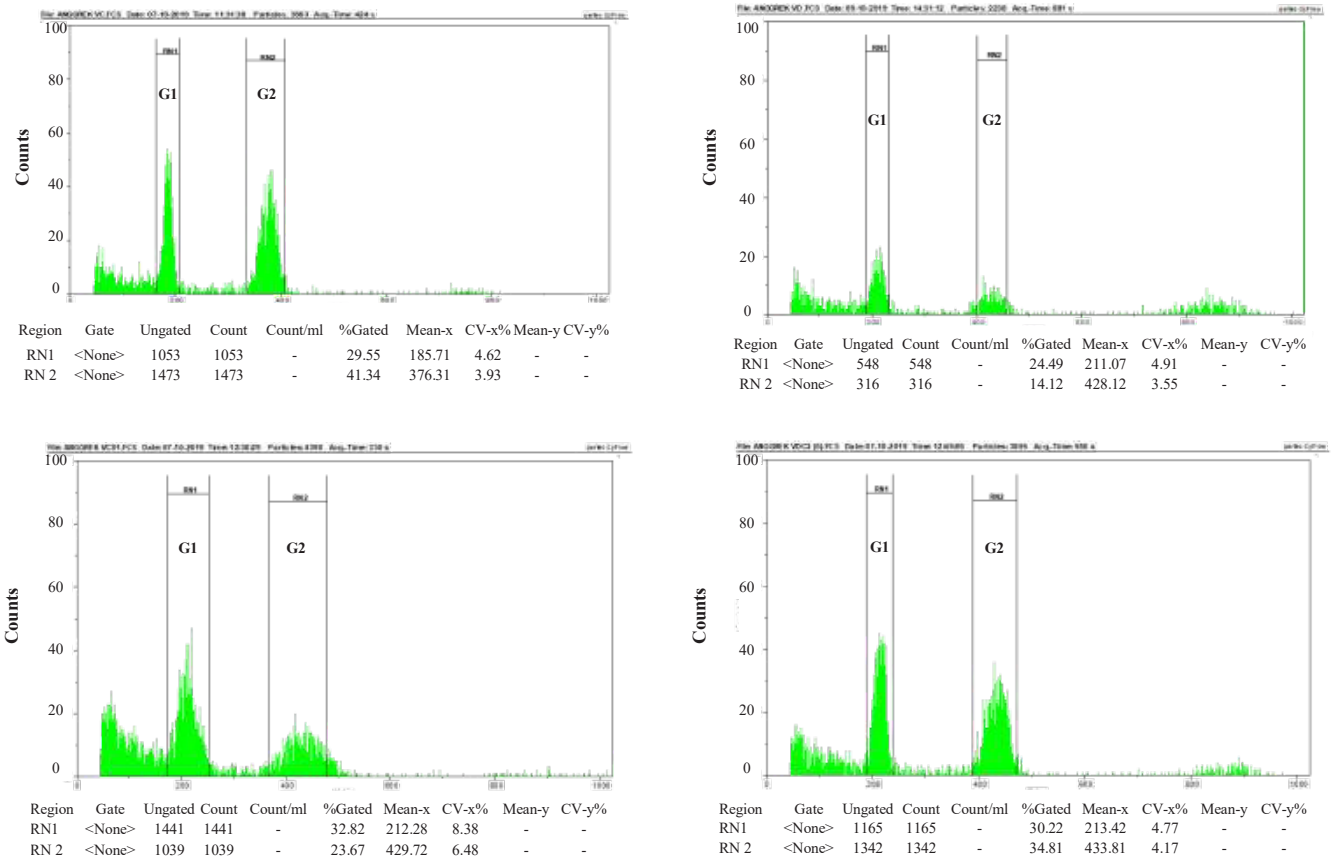


Figure 6. Histograms of relative nuclear DNA content of (A) *V. celebica*, (B) *V. dearei*, (C) *V. celebica* x *V. dearei*, (D) *V. dearei* x *V. celebica*

CONCLUSIONS

The number of chromosomes of *Vanda dearei* 2n = 2x = 40, *Vanda celebica* 2n = 2x = 38, the hybrid of *V. celebica* x *V. dearei* 2n = 2x = 38, and *V. dearei* x *V. celebica* 2n = 2x = 38. The karyotype arrangement of *V. celebica* is 2n = 2x = 17 m + 2 sm, *V. dearei* is 2n = 2x = 40 = 18 m = 2 sm, the hybrid of *Vanda celebica* x *Vanda dearei* is 2n = 2x = 38 = 17 m + 1 sm, and *Vanda dearei* x *Vanda celebica* is 2n = 2x = 38 = 17 m + 1 sm. All observed ploidy levels of orchids were diploid.

ACKNOWLEDGMENTS

This research was carried out with financial support from the Directorate General of Higher Education, Ministry of Education Culture, Research, and Technology with the Fundamental Research Program scheme in 2022 with contract number 254/UN27.22/PT.01.03/2022 to fund this work.

REFERENCES

Aziz, I.R. 2019. Plant chromosomes as genetic markers. J. Technoscience 13:125-131.

Aziz, I.R. 2020. Chromosome variations of the Rutaceae family in Indonesia. J. Techoscience 14:115-127.

Chen, J., N. Xia, X. Wang, R.C. Beeson, C. Jianjun. 2017. Ploidy level, karyotpype, and dns content in the genus lonicera. HortScience 52:1680-1686.

Grosso, V., A. Farina, D. Giorgi, L. Nardi, G. Diretto, S. Lucretti. 2018. A high-throughput flow cytometry system for early screening of in vitro made polyploids in Dendrobium hybrids. PCTOC. 132:57-70. Doi:10.1007/s11240-017-1310-8. http://dx.doi.org/10.1007/s11240-017-1310-8.

Hajrudinović, A., S. Siljak-Yakovlev, S.C. Brown, F. Pustahija, M. Bourge, D. Ballian, F. Bogunić. 2015. When sexual meets apomict: Genome size, ploidy level and reproductive mode variation of *Sorbus aria s.l.* and *S. austriaca* (Rosaceae) in Bosnia and Herzegovina. Ann. Bot. 116:301-312. Doi:10.1093/aob/mcv093.

Hartati, S., Nandariyah, A. Yunus, D.W. Djoar. 2017. Short communication: Cytological studies on black orchid hybrid (*Coelogyne pandurata* Lindley). Biodiversitas. 18:555-559. Doi:10.13057/biodiv/d180216.

Hartati, S., B. Agus, Ongko. 2014. Increasing genetic diversity of dendrobium spp. through hybridization to support the development of orchids in Indonesia. Caraka Tani J. Agric. Sci. 29(2).

Hartati, S., D. Linayati, C. Ongko. 2014. Study of orchid characterization by cytology in order to conserve germplasm. J. Agric. Sci. 29:25-30.

Jones, W.E., A.R. Kuehnle. 1998. Ploidy identification using flow cytometry in tissues of Dendrobium species and cultivars. Lindleyana. 13:11-18.

Kanwal, K.S. 2014. Conservation of *Phauis tankervilleae* a valuable orchid of Arunachal Pradesh, India. Indian Forester 140:1263-1264.

Liang, G., Chen HJPo. 2015. Scaling chromosomes for an evolutionary karyotype: a chromosomal tradeoff between size and number across woody species. PLoS ONE 10:e0144669. Doi:10.1371/journal.pone.0144669.

Mulkan, Y.H. 2011. A complete dialelic cross of two orchid parents, seed germination and in vitro enlargement and acclimatization of phalaenopsis plantlets. Thesis. Lampung University. Indonesian.

Ramadhani, D., Y. Lusiyanti, Z. Alatas, M. Lubis, V.A. Suvifan. 2012. Semi automation of karyotype for detection of chromosome aberrations due to radiation exposure. JFN 6:178-186.

Rupawan, I.M., Z. Basri, M. Bustami. 2014. Growth of Vanda orchids (*Vanda spp*) on various media compositions in vitro. eJ Agrotechbis 2:488-494.

Shi, L.R., H.F. Li, R.Y. Gao, H.Q. Han. 2009. Chromosome number and karyotype analysis of *Althaea rosea* [J]. Jiangsu Agri. Sci. 5:173-174.

Siljak-Yakovlev, S., V. Stevanovic, M. Tomasevic, S.C. Brown, B. Stevanovic. 2008. Genome size variation and polyploidy in the resurrection plant genus Ramonda: Cytogeography of living fossils. Environ. Exp. Bot. 62:101-112. Doi:10.1016/j.envexpbot.2007.07.017.

Suryani, R. 2015. Outlook of orchids. Center for Agricultural Data and Information System Secretariat General of the Ministry of Agriculture 2015 (PUSLOHORTI).

Tabassum, N., U. Rafique, K.S. Balkhair, M.A. Ashraf. 2014. Chemodynamics of methyl parathion and ethyl parathion: adsorption models for sustainable agriculture. BioMed Res intern. Doi:10.1155/2014/831989.

Weiss-Schneeweiss, H., Schneeweiss. 2012. Karyotype diversity and evolutionary trends in angiosperms. J Plant Genome Diversity 2:209-230.

Zhao, C., F. Li, S. Gao. 2014. Research on chromosome karyotype analysis of *Plumbago auriculata*. Open Accses Lib. J. 1:e404.