

Short Communication: Cytological studies on black orchid hybrid (*Coelogyne pandurata* Lindley)

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Abstract. Hartati S, Nandariyah, Yunus A, Djoar DW. 2017. Cytological studies on black orchid hybrid (*Coelogyne pandurata* Lindley). *Biodiversitas* 18: 555-559. Black orchid (*Coelogyne pandurata* Lindley) is one of the rare species of *Coelogyne* Lindley, protected by the government of Indonesia. The cytological character of orchid is very important to study to support the success of breeding. The study aimed to assess the chromosomal number, karyotype pattern and ploidy level the hybrid of Black Orchid (*Coelogyne pandurata*). This study shows that the chromosomal number of the hybrid of *Coelogyne pandurata* ($2n = 36$) $><$ *Coelogyne rumphii* ($2n = 72$) is $2n = 54$. Ploidy analysis by flow cytometry shows that hybrid shows triploid ($2n = 3x = 54$) different from the parent *Coelogyne pandurata* which is diploid ($2n = 2x = 36$) and the parent *Coelogyne rumphii* which is tetraploid ($2n = 4x = 72$). However, both parents and their hybrid performed the same karyotype pattern, which is metacentric. The chromosome size showed a variation in length from the longest *Coelogyne pandurata* $2.98 \pm 0.15 \mu\text{m}$ to the shortest *Coelogyne rumphii* $2.24 \pm 0.15 \mu\text{m}$. The hybrids have a range of $2.50 \pm 0.10 \mu\text{m}$ to $2.85 \pm 0.10 \mu\text{m}$. Karyotype patterns of black orchid (*C. pandurata*), *C. rumphii* and the hybrid is metacentric.

Keywords: Chromosome, cytology, *Coelogyne*, flow cytometry, ploidy

INTRODUCTION

One of the rare species of *Coelogyne* Lindl., protected by the government of Indonesia is black orchid (*Coelogyne pandurata* Lindley). This species of orchid, naturally found in eastern Kalimantan, is very exciting with the characters of large green flowers with a black tongue protruding from its center. The black color is a rare trait needed by experts' cruciferous plant breeding to produce new shades of color more attractive interest. Therefore, this orchid has high economic value because of its potential as a valuable holding crosses. However, the existence of black orchid is currently being endangered and now hard to find even in its native habitat, so the saving cultivation should be done before extinction occurs. *Coelogyne* orchid others include *Coelogyne rumphii* which have small yellow flowers with brown tongue. Orchid plants are plant species that have a very large diversity of phenotypes. Kinship was based phenotypic analysis of a number of appearances on the phenotype of an organism. Phylogenetic relationship between two individuals or populations can be measured by the number of characters in common with the assumption that different characters are caused by differences in genetic makeup.

The introduction of natural orchid character based on cytology would strongly support the success of orchids plant breeding. However, research on natural orchid plant cytology is very rarely done. Orchids are a diploid number of chromosomes, one pair of chromosomes consists of two sets of homologous chromosomes. Therefore, variations in the number of chromosome sets (ploidy) in the bark of

plants included in the group euploidy, which state that the number of chromosomes is observed from a living creature is a multiple of the number of chromosomes essentially. Differences chromosome generally describes the genetic and protein differences in the content of an individual. The main variations that can be observed that the absolute size or length, morphology, the relative size and number of chromosomes. Individuals within a species have the same chromosome number but different species in a single genus have different numbers of chromosomes. The shape, size and number of chromosomes of each species are always fixed, so it can be used for the purpose taxonomy, knowing diversity, kinship and the evolution although in certain circumstances also occur variation.

Based on the above the writer is interested in conducting research on identification of Black Orchid (*C. pandurata*) and *C. rumphii* as the parents and their F1 hybrid based on cytological characters in Laboratory Research Center for Biology LIPI, Bogor Indonesia. The objectives of this research were to: (i) determine the chromosome numbers, shape, size, karyotype pattern and the ploidy level of Black Orchid (*C. pandurata*) and *C. rumphii* as the parents and their F1 hybrid. The results give direct evidence of genome homology between the parental species.

MATERIALS AND METHODS

Plant material

Coelogyne pandurata and *Coelogyne rumphii* were taken from the living a collection of Bogor Botanical

Garden, Indonesian Institute of Sciences (LIPI), Bogor, West Java, Indonesia and hybrid from *C. pandurata* \times *C. rumphii* were produced by hand pollination. The plants of interspecific F1 hybrids were cultivated in the Laboratory of Tissue Culture, Faculty of Agriculture, Sebelas Maret University, Surakarta, Central Java, Indonesia. This research was conducted in the Cytology and Genetics Laboratory, Research Center for Biology, Indonesian Institute of Sciences, Bogor, West Java, Indonesia.

Chromosome preparation

Chromosome analysis of the parent orchid and their hybrid was taken using the root tips. Calculation of the number of chromosomes, carried out at the Research Center for Biology LIPI Bogor Indonesia, was done based on the method of Manton (1950). Pieces of roots are soaked in a solution of 0.002 M 8-Hydroxyquinoline at ca 4°C for 3-5 hours and then fixed in 45 % acetic acid for 10 minutes. They were macerated in a mixture of 1 N HCl and 45 % acetic acid (1:3) at 60°C for 1-5 minutes and then stained with 2 % aceto-orcin by the usual squash method. After that piece meristem pressed on object glass and then observed under a microscope with a magnification x 1000 for the calculation of the number of chromosomes. The plates were considered for the karyotype analysis in each species in the present investigation Chromosome size can be determined by measuring the length of the chromosome arm. The chromosome shape was established on the recommendation for varying values of r (Ciupercescu et al. 1990).

Ploidy analysis was conducted using a space CyFlow® (Partec GmbH) equipped with a diode pumped solid-state laser 920 mW) at a wavelength of 488 nm and a laser diode at a wavelength of 638 nm (25 mW). Leaf pieces (0.5 cm²) chopped using a razor blade in a petri dish containing 250 mL of extraction buffer. After 30-90 seconds of extraction buffer was filtered using a Partec 30 mL Cell Trics filters. Using PI staining buffer (propidium iodide) and RNase (1 ml), incubated for 30 min and then analyzed by a flow cytometry before. As used control *Coelogyne* 2n = 36. The observed variables include the number, size and shape of the chromosome, karyotype pattern and the ploidy level. The data were analyzed descriptively.

RESULTS AND DISCUSSION

In plant taxonomy, chromosome observation is very important. The number of chromosomes is cytological characters most easily observed when compared to other chromosomal characteristics such as size and shape of chromosomes. The composition can be used to determine the karyotype chromosomal aberrations both in total and chromosome structure that occurs at the time of cell division and with abnormalities that occur in the anatomy, morphology, and physiology of a living being.

Chromosome analysis showed a cross between *Coelogyne pandurata* and *Coelogyne rumphii* produce different numbers of chromosomes between the elders and the individual F1 crosses results. This study shows that the

parent *C. pandurata* has a chromosomal number of 2n = 36 and the parent *C. rumphii* has a chromosomal number of 2n = 72. The hybrid of the cross of *C. pandurata* \times *C. rumphii* has a chromosomal number of 2n = 54 (Table 1 and Figure 2). This study is in line with the previous research done by Balanos-Villegas et al. (2008). They found that the hybrid *Doritaenopsis* had a different number of chromosome (2n = 72) with the parent of *Phalaenopsis* sp (2n = 38). Davina et al. 2009 explain the chromosome studies in Orchidaceae vary chromosome number from genus level, at genus *Oncidium* the eight *Oncidium* species analyzed showed 2n = 108 (*Oncidium bifolium* Sims.), 2n = 56 (*O. divaricatum* Lindl., *O. fimbriatum* Lindl., *O. longipes* Lindl. and *O. riograndense* Cogn.), 2n = 42 (*O. edwallii* Cogn. and *O. longicornu* Mutel.) and 2n = 84 (*O. pubes* Lindl.) and also reported that the chromosomal number of 19 orchids being studied varied from the lowest of 2n = 26 *Eltroplectris schlechteriana* to the highest of 2n = 108 *Catasetum fimbriatum*. Furthermore, Ramesh and Renganathan (2013a) reported that from five species of *Coelogyne* spp., in all other species studied in the present report of chromosome numbers are in correlation with those of earlier reports such as *C. corymbosa* 2n = 26 and *C. fimbriata* 2n = 22. The first records of chromosome number have been made in 3 species namely, *C. barbata* 2n = 18, *C. breviscapa* 2n = 32 and *C. cristata* 2n = 26. Most of the species of chromosome should have been originated by aneuploidy. In studies Cox et al. (1998) the number of chromosomes Genus *Paphiopedilum*, i.e. sect. *Barbata* 2n = 28-42, sect. *Pardalopetalum* 2n = 26, sect. *Coryopedilum* 2n = 26, sect. *Cochlopetalum* 2n = 32-37, sect. *Brachypetalum* 2n = 26, sect. *Parvisepalum* 2n = 26, Genus *Phragmipedium* 2n = 18-28, Genus *Cypripedium* 2n = 20.

Genome analysis provides valuable information about species relationship and therefore, plays an important role in plant breeding program. The number and form of chromosomes in each cell plant species are fixed. Each cell has a characteristic number of chromosomes and each chromosome in one species also has a distinctive structure. Consistency chromosome widely used by taxonomists to help solve problems related to plant morphology. Levels can ploidy affect the size of the growth rate, plant, stress tolerance and other important agronomic characteristics (Ferchichi et al. 2006; Walker et al. 2006).

Begum and Alam (2005) showed that of the seven species of orchids being observed, four species had the chromosome number of 2n = 38, karyotype patterns in species *Peristylus constrictus*, *Luisia grovesii*, *Sarcanthus appendiculatus* and *Rhynchostylis retusa*. While, three other species had the number of chromosomes with different patterns of karyotype is *Oberonia iridifolia* 2n = 30, *Pholidota pallida* 2n = 40, and *Phaius tankervilleae* 2n = 52. The species of the orchid family has 2n = 20 chromosomes, namely, *Gastrochilus indicus* and *Bulbophyllum atropurpureum* diploid, having somatic chromosome 30 that was *Eulophia epidendrea*, *Malaxis versicolor* and *Oberonia verticillata* as triploid, somatic chromosome 40 *Coelogyne ovalis*, *Eria reticosa* and *Spathoglottis plicata* as tetraploids, species diploid, triploid and tetraploid species were an example for euploids. *Eria*

pauciflora ($2n = 38$), *Habenaria grandifloriformis* ($2n = 22$), *Habenaria rariflora*, ($2n = 42$), *Habenaria viridiflora* ($2n = 22$), *Luisia birchea* and ($2n = 38$), *Nervilia plicata* ($2n = 24$) all this species as aneuploids (Ramesh and Renganathan 2013b). In studies Skarma et al. 2010 the number of chromosomes in all three species genus *Cymbidium* i.e. *C. hookerianum*, *C. eburneum* and *C. mastersii* $2n = 40$.

Orchids usually have metacentric shaped chromosomes. The Ramesh and Renganathan (2013a) states that orchids generally have shaped metacentric chromosomes. The results of this study confirmed that all observed orchids have metacentric chromosome. This study showed that *C. pandurata* has the longest chromosome, $2.98 \pm 0.15 \mu\text{m}$ with length ratio $1.26 \pm 0.12 \mu\text{m}$, while *C. rumphii* has the shortest chromosome, $2.24 \pm 0.15 \mu\text{m}$ with length ratio $1.40 \pm 0.13 \mu\text{m}$. The hybrid of male *C. pandurata* \times female *C. rumphii* has a length of $2.85 \pm 0.14 \mu\text{m}$, and the hybrid of female *C. pandurata* \times male *C. rumphii* has a length of $2.50 \pm 0.10 \mu\text{m}$ with length ratio $1.08 \pm 0.05 \mu\text{m}$ to $1.23 \pm 0.07 \mu\text{m}$. Genome size can affect the cellular parameters, such as cell size, and nucleotypic effects can have an impact on important characteristics such as growth and yield (Turpeinen et al. 1999; Walker et al. 2006). Truta et al. (2013), states that the karyotype with metacentric and submetacentric chromosome types are considered primitive

and least developed, because they are not supported restructuration and significant genetic rearrangements during evolution. Jorapur and Kulkarni (1980) reported detailed karyological studies in a few members of Orchidaceae. The trend toward karyotype asymmetrization by increasing the number of telocentric chromosomes is a progressive step in the evolution of karyotype and have an impact on the evolution of species. Phylogenetic analyses (Pinheiro et al., 2009) indicated that *Epidendrum fulgens* and *E. puniceoluteum* are closely related species with divergent karyotype and chromosome numbers ($2n = 2x = 24$ and $2n = 4x = 52$, respectively).

Analysis of flow cytometry

In addition to observing the number, shape, and size of chromosome ploidy analysis was also performed by *flow cytometry* analysis to support the results of cytology. Based on the histogram (Figure 3 and Table 1) *C. pandurata* had $2x$ ploidy level (diploid) so that the chromosome number of $2n = 2x = 36$, *C. rumphii* have $4x$ ploidy level (tetraploid) so that the chromosome number of $2n = 4x = 72$ and the hybrid has a $3x$ ploidy level (triploid) so that the number of chromosomes $2n = 3x = 54$, then the results of *flow cytometry* analysis to supplement the results of cytological analysis.

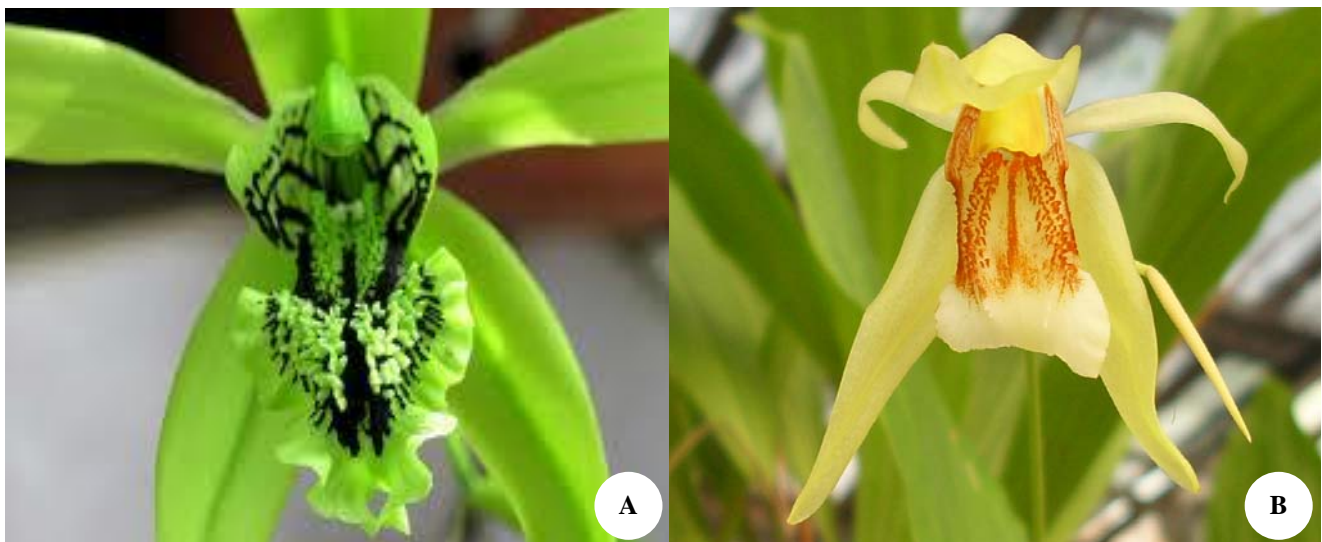


Figure 1. The flower of *Coelogyne* from Bogor Botanical Garden, Indonesia in 2012. A. *Coelogyne pandurata*, B. *Coelogyne rumphii*

Table 1. Chromosome number, karyotype and ploidy *Coelogyne pandurata* and *Coelogyne rumphii*

Orchid	Chromosome (2n)	Total chromosome length (μm)	Length ratio (μm)	Ploidy level	Centromere position
<i>C. pandurata</i>	36	2.98 ± 0.15	1.26 ± 0.12	2x	metacentric
<i>C. rumphii</i>	72	2.24 ± 0.15	1.40 ± 0.13	4x	metacentric
Hybrid ($\text{♀}C. \text{rumphii} \times \text{♂}C. \text{pandurata}$)	54	2.85 ± 0.14	1.08 ± 0.05	3x	metacentric
Hybrid ($\text{♀}C. \text{pandurata} \times \text{♂}C. \text{rumphii}$)	54	2.50 ± 0.10	1.23 ± 0.07	3x	metacentric

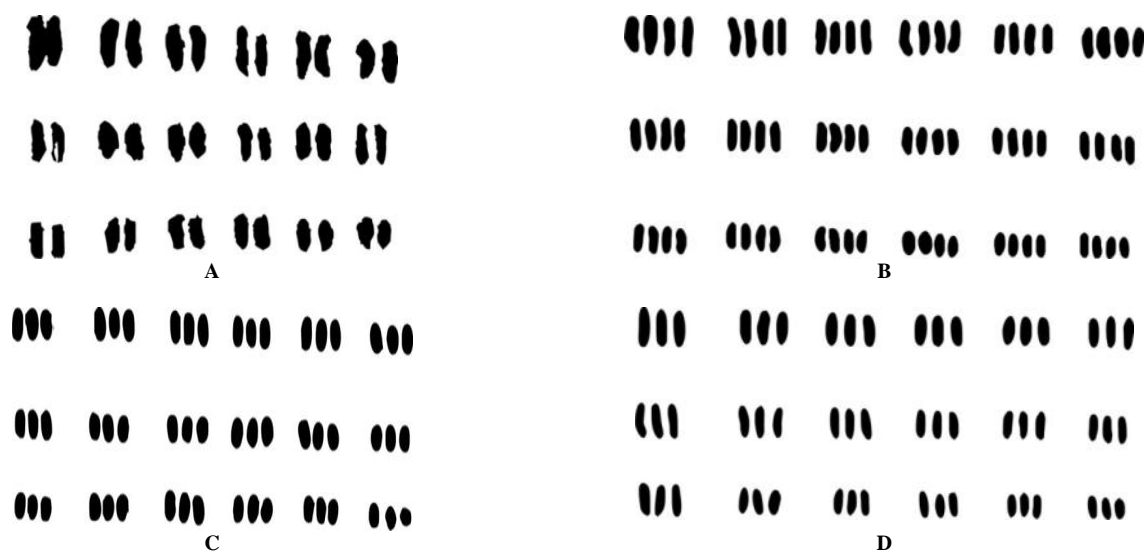


Figure 2. Karyotype of black orchid hybrid (*Coelogyne pandurata* Lindley). A. *C. pandurata* ($n = 36$), B. *C. rumphii* ($n = 72$), C. Hybrid ♀ *C. pandurata* x ♂ *C. rumphii* ($n = 54$), D. Hybrid ♀ *C. rumphii* x ♂ *C. pandurata* ($n = 54$)

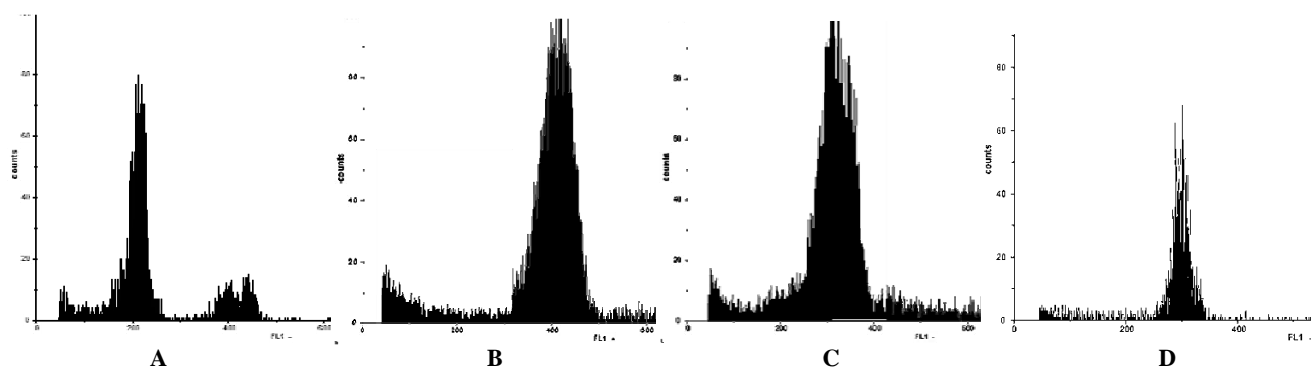


Figure 3. The peak position from *Flow Cytometry* of black orchid hybrid (*Coelogyne pandurata* Lindley). A. *C. pandurata* (2x), B. *C. rumphii* (4x), C. Hybrid ♀ *C. pandurata* x ♂ *C. rumphii* (3x), D. Hybrid ♀ *C. rumphii* x ♂ *C. pandurata* (3x)

This study showed that the change in ploidy level of the hybrid from the parents. The parent *C. pandurata* was diploid and the parent *C. rumphii* was tetraploid and the cross of both parents generated the hybrid which was a triploid. This result indicates that there may appear new character in the hybrid. According to research Travnicek et al. (2012) of the species *Gymnadenia* among this sample was found five different ploidy levels (2x, 3x, 4x, 5x, and 6x) and Travnicek et al. (2011) notice in the previous note that these cytotypes known as tetraploid, hexaploid, octoploid, etc. This result also in line with the research of Aoyama et al. (2013) which states that the results of a cross from the parent in the ploidy level $2n = 2x = 44$ and $2n = 4x = 88$ obtained the hybrid of the ploidy level $2n = 3x = 66$. Moreover, in the study by Lee et al. (2011) on *Paphiopedilum* orchids and hybrid, the parents *P. delenatii*, *P. micranthum*, *P. bellatulum*, *P. rothschildianum* which all

had $2n = 26$, *P. callosum* had $2n = 29$ and *P. glaucophyllum* had $2n = 1$, the hybrid of *P. delenatii* x *P. micranthum* had $2n = 26$, *P. delenatii* x *P. bellatulum* $2n = 26$, *P. delenatii* x *P. rothschildianum* had $2n = 26$, *P. delenatii* x *P. callosum* had $2n = 29$, *P. delenatii* x *P. glaucophyllum* had $2n = 31$. Balanos-Villegas et al. (2008) also explain that the cross of *Doritaenopsis* sp. and *Phalaenopsis* sp. generated the hybrid *Doritaenopsis* I-Hsin Purple Jewel with ploidy level $2n = 3x = 57$.

In conclusion, a chromosomal number of black orchid (*Coelogyne pandurata*) is $2n = 36$, *Coelogyne rumphii* was $2n = 72$ and the hybrid was $2n = 54$. Karyotype patterns of black orchid *C. rumphii*, *C. pandurata* and the hybrid was metacentric. Ploidy level of hybrid from the cross of diploid *C. pandurata* and tetraploid *C. rumphii* was triploid.

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