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Karyotype Analysis of *Phalaenopsis* Hybrids with Colchicine Induction

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Abstract
Phalaenopsis hybrid is one of the important orchids in the horticultural market as an ornamental plant whose demand continues to increase. This causes the need for breeding to obtain new, varied flower characters as desired by polyploidization. Polyploidy induction in Phalaenopsis hybrids was carried out using colchicine. This study aimed to determine the karyotype pattern of Phalaenopsis hybrids. The research was conducted at the Screenhouse of the Faculty of Agriculture and Integrated Laboratory Unit of Sebelas Maret University, Surakarta, in August 2021 - March 2022. Polyploidy induction was carried out by dripping a 1500 ppm concentration of colchicine on the flower buds of a Phalaenopsis hybrid. The method used in this research is squashing. The experimental results showed that the karyotype pattern in the control *Phal*. Golden Tree was 2n=3x-7=50m, while in *Phal*. Fuller Sunset and *Phal*. OX X-ray was 2n = 4x = 76m. The karyotype pattern in orchids with colchicine induction was *Phal*. In Phal, Golden Tree 2n = 6x - 14 = 100 m. Fuller Sunset and *Phal*. OX X-ray 2n = 8x = 152 m.

Keywords: chromosomes; orchids; polyploid.

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Introduction

Phalaenopsis is one of the orchid genera with the highest number of species, comprising approximately 45–50 species distributed across tropical and subtropical regions of Asia, New Guinea, and Australia (1). This genus has significant economic value as a horticultural plant and is widely cultivated in greenhouses (2). Among its members, *Phalaenopsis* hybrids are particularly important in the global ornamental plant market due to their attractive flower morphology, long vase life, and diverse color variations. For instance, in Taiwan, a leading country in *Phalaenopsis* breeding, hybrids of this genus contribute to nearly 50% of the national flower export value (3).

Breeding efforts have focused on developing Phalaenopsis cultivars with superior traits to meet the increasing demand for novel floral characteristics. One such approach is artificial polyploidization, which induces chromosome duplication and can increase flower size, thicker leaves, and improved overall plant vigor. Previous studies have shown that polyploid *Phalaenopsis* plants often display enhanced ornamental traits compared to their diploid counterparts, making them more desirable in the floriculture industry (4). Colchicine is commonly used to induce polyploidy through soaking or cotton-mediated application.

While several studies have reported the successful induction of polyploidy in orchids, many have relied primarily on morphological or flow cytometry analyses to confirm polyploidy. However, these methods may not provide detailed chromosomal insights, particularly regarding structural variations or chromosomal behavior. Karyotype analysis, which involves examining chromosome number, size, and morphology, offers a more comprehensive understanding of chromosomal changes and is critical for validating the success of polyploidization and its genetic consequences (5).

Despite its importance, limited studies have applied detailed karyotype analysis to evaluate colchicine-induced polyploidy in *Phalaenopsis* hybrids. Therefore, this study aims to analyze the karyotype patterns of colchicine-treated *Phalaenopsis* hybrids to confirm polyploidization and provide a deeper understanding of chromosomal alterations associated with induced polyploidy. This approach complements previous methods by offering cytogenetic evidence that strengthens the interpretation of phenotypic and molecular data in orchid breeding programs.

Materials and Methods

Three hybrid orchids were used as research materials: Phalaenopsis Golden Tree, *Phalaenopsis* Fuller Sunset, and *Phalaenopsis* OX X-ray. The orchid is in the bud stage and comes from Green Leaves Orchids, Salatiga. The three orchids were examined at the screenhouse of the Faculty of Agriculture and the UPT Integrated Laboratory, Sebelas Maret University, Surakarta. The method used in this research is squashing, which is one method for making preparations.

The stages of work begin by dripping 1,500 ppm colchicine on cotton, wrapping it into the bud, and covering it with carbon paper. Furthermore, sepal retrieval was carried out between 07.30 and 08.05 WIB, where the buds were in the prometaphase or metaphase stage in mitotic division. The sepals were then immersed in aquadest and put in a refrigerator at a temperature of 5-8°C for 24 hours. Furthermore, the sepals were immersed in 45% acetic acid for ± 1 hour at room temperature. The sepals were then rinsed with aquadest 3 times and then put in 1 N HCl for 15 minutes at room temperature. The sepals were rinsed again with Aquadest 3 times. The sepals were further stained by immersion in 2% aceto-orcein for ± 24 hours at room temperature. The next stage is squashing by taking the sepal pieces along and placing them on the slide, and then closing it with a cover glass. Gently tap the sepals protected by the cover glass with a pencil so that the cells spread out and don't accumulate. The sepals are then pressed using the thumb to straighten the chromosomes. Observations were made using a microscope, and an Optilab microscope camera was used to capture clear images of chromosomes.

The chromosome images obtained from microscopic observation were further processed using CorelDRAW X7 (Corel Corporation, licensed version) for clear visualization and labeling of chromosome structures. The Image Raster software (Raster 3.0, licensed freeware version) was used to calculate chromosome length, centromere position, and arm length ratio to perform quantitative measurements. Microsoft Excel 2019 (licensed institutional version) was employed for data tabulation, arm ratio calculation, and the chromosome length distribution tables.

The chromosome arm ratio, the ratio between the long and short arms, was used to classify chromosomes based on centromere position (i.e., metacentric, submetacentric, subtelocentric, or telocentric). These classifications were then used to identify homologous chromosome pairs and construct idiograms. The resulting idiogram diagrams were edited and finalized in CorelDRAW X7 for publication-quality output. All software used in this study, including CorelDRAW X7, Image Raster 3.0, and Microsoft Excel 2019, was legally obtained and used under institutional or academic licenses.

Results and Discussion

A karyotype refers to the structural arrangement of chromosomes based on their number, size, and morphology. It is crucial in identifying chromosomal abnormalities or induced variations during cell division, particularly in plant breeding, where polyploidization is applied. According to (6), the standard karyotype is constructed by arranging homologous chromosome pairs from the longest to the shortest, with satellite chromosomes at the end.

In this study, the karyogram results (Figure 1) of control plants showed that each chromosome pair had a similar shape and size, with all chromosomes identified as metacentric. The control karyotype formulas were *Phalaenopsis* Golden Tree 2n = 3x - 7 = 50m, *Phal.* Fuller Sunset 2n = 4x = 76m, and *Phal.* OX X-ray 2n = 4x = 76m. These results suggest differences in ploidy level among cultivars, which may influence their respective breeding histories and parentage. Different species have different karyotypes, which can also occur between plant families, subfamilies, and genera (7).

Figure 1. The orchid chromosome karyogram a. *Phal*. Golden Tree control; b. *Phal*. Golden Tree with 1,500 ppm colchicine; c. *Phal*. Fuller Sunset control; d. *Phal*. Fuller Sunset with 1,500 ppm colchicine; e. *Phal*.OX X-ray control; f. *Phal*. OX X-ray with colchicine 1,500 ppm

Colchicine at 1,500 ppm resulted in a notable increase in chromosome number in all cultivars tested. Treated karyotype patterns were *Phal.* Golden Tree 2n = 6x - 14 = 100m, *Phal.* Fuller Sunset 2n = 8x = 152m, and *Phal.* OX X-ray 2n = 8x = 152m. This confirms the success of artificial polyploidization, as the chromosome number doubled compared to the controls. These findings are consistent with previous research by (8), who demonstrated that colchicine treatment in *Phalaenopsis* increased chromosome number and enhanced flower size and petal thickness.

The observed chromosome numbers support the hypothesis that most *Phalaenopsis* species have a basic chromosome number of x = 19, with common complements being 2n = 38. Exceptions such as the *Aphyllae* subgenus, which may have 2n = 34 or 36, have also been reported (8). The variation in ploidy level in this study aligns with reports from (9), who showed that chromosome doubling significantly alters plant vigor, size, and morphology in *Phalaenopsis* hybrids.

Idiograms constructed based on the chromosome length and morphology (Figure 2) represent the chromosomal organization in both treated and control plants. Idiograms illustrate chromosome size and shape using chromatin condensation patterns (10). All idiograms in colchicine-treated plants consistently showed only metacentric chromosomes, suggesting no significant structural alterations such as translocations or inversions, which might otherwise produce submetacentric or telocentric shapes.

However, subtle differences in idiogram structure may indicate microstructural changes. According to (11), colchicine induces polyploidy and can promote chromosomal structural changes, which may lead to new plant genotypic and phenotypic variations. Although

such alterations were not detected in the present study, further analysis using fluorescence in situ hybridization (FISH) or flow cytometry could help confirm chromosomal stability in colchicine-induced polyploids.

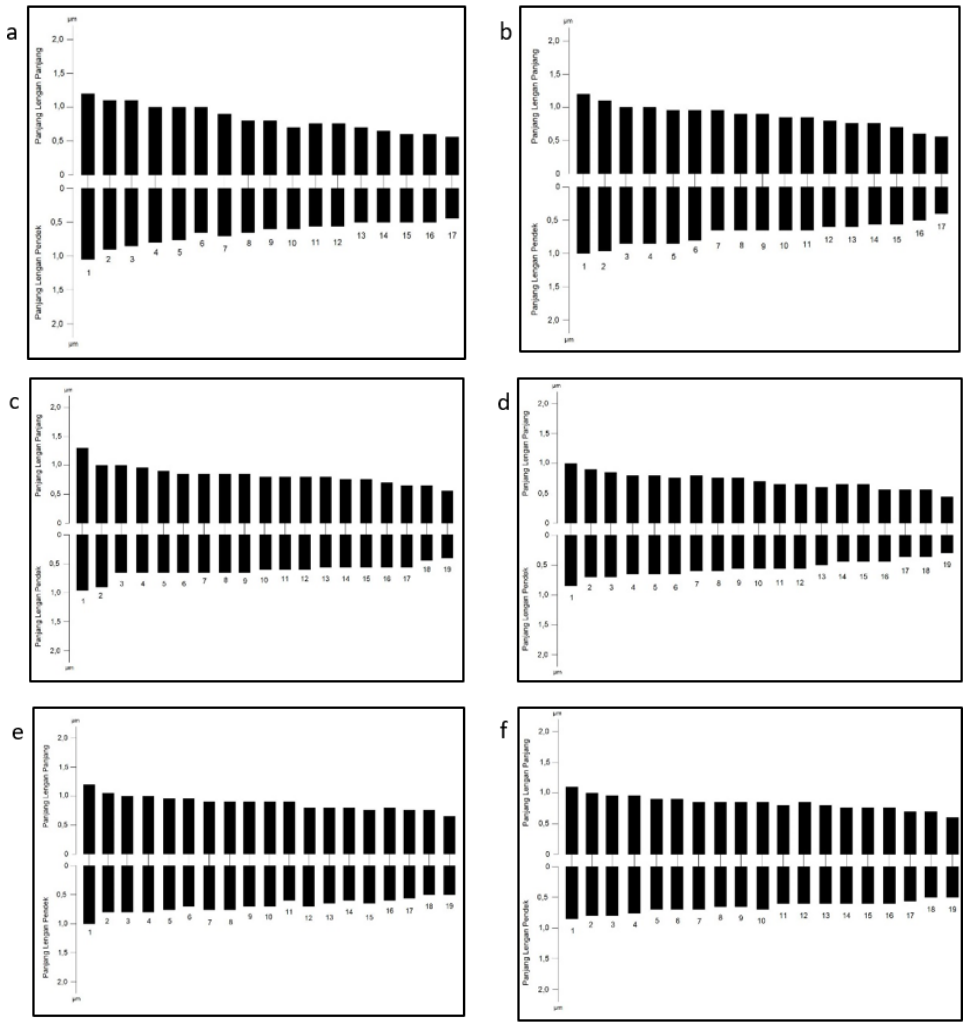

Figure 2. The orchid chromosome ideogram a. *Phal*. Golden Tree control; b. *Phal*. Golden Tree with 1,500 ppm colchicine; c. *Phal*. Fuller Sunset control; d. *Phal*. Fuller Sunset with 1,500 ppm colchicine; e. *Phal*. OX X-ray control; f. *Phal*. OX X-ray with colchicine 1,500 ppm

The karyotype of individuals within the same species typically exhibits consistent chromosome size and morphology; however, variations may occur due to structural chromosomal changes. These changes can result from mechanisms such as genome duplication, segmental duplication, inversion, translocation, centric fission, Robertsonian fusion, or deletion, all commonly associated with evolutionary processes (12). The consistent increase in chromosome number across treated samples supports the reliability of colchicine as an effective polyploidization agent for *Phalaenopsis*. Furthermore, maintaining metacentric morphology implies that colchicine treatment under the tested conditions did not induce significant chromosomal abnormalities. These findings reinforce the potential of induced polyploidy as a reliable strategy in Phalaenopsis breeding programs to enhance ornamental characteristics.

Conclusion

The two hybrid orchids, *Phalaenopsis* 'Fuller Sunset' and *Phalaenopsis* 'OX X-ray' control, exhibited identical karyotype patterns of 2n = 4x = 76m. In contrast, the power of *Phalaenopsis* 'Golden Tree' showed a karyotype of 2n = 3x – 7 = 50m. Treatment with 1,500 ppm colchicine induced a different karyotype pattern: *Phalaenopsis* 'Fuller Sunset' and 'OX X-ray' became 2n = 8x = 152m, while *Phalaenopsis* 'Golden Tree' exhibited 2n = 6x – 14 = 100m.

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Conflict of Interest

All authors declare no conflicts of interest.

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