Induction of Polyploidy Using Colchicine in Flower Buds from Phalaenopsis Hybrids

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Abstract. Phalaenopsis hybrid is one of the orchids that is in great demand in the horticultural market as cut and potted flowers. One of the attempt to obtain new variation flower characters in Phalaenopsis hybrids is polyploidization using colchicine. The purpose of this study was to determine the cytological character of orchids (number, size, shape of chromosomes). 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 by dripping 1500 ppm concentration of colchicine on flower bud of Phalaenopsis hybrid. The method of this research using squash preparation for chromosome analysis. The results of this research showed that number of chromosomes in control *Phalaenopsis* Golden Tree 2n = 3x − 7 = 50, *Phalaenopsis* Fuller Sunset and *Phalaenopsis* OX X-ray 2n = 4x = 76. *Phalaenopsis* Golden Tree which were treated with 1500 ppm colchicine each underwent polyploidization with number of chromosomes 2n = 6x − 14 = 100, *Phalaenopsis* Fuller Sunset and *Phalaenopsis* OX X-ray 2n = 8x = 152. *Phalaenopsis* Golden Tree, *Phalaenopsis* Fuller Sunset and *Phalaenopsis* OX X-ray has a relatively small chromosome size, and has the same chromosome shape, namely metacentric.

Keywords: orchid · chromosome · karyotype

1 Introduction

Orchid is one of horticultural plants that are in great demand by the public as an ornamental plant. This is because orchids have a variety of types, uniqueness, and attractiveness that are different from other plants. The uniqueness and attractiveness of this plant lies in the flowers such as the diversity of the labelum which is the differentiation of the

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petals that function for self-pollination and even attract pollinators [1]. In addition, the uniqueness of flowers in orchids also lies in the diversity of shapes, colors, and aromas. Based on these characteristics, one of the most popular genera is Phalaenopsis. This orchid has a unique labellum which plays an important role in attracting pollinating insects [2]. These orchids, especially Phalaenopsis hybrids, are also in great demand in the horticultural market as cut and potted flowers because flowering every year easily and have a shelf-life of 3–4 months [3]. These things cause it to be necessary to do breeding on this orchid in order to obtain a new variety of flower characters as desired. It can be obtained by polyploidization.

Polyploidization is a change in the number of chromosomes that occurs during mitosis or meiosis and produces polyploids. Polyploidization can increase genetic diversity and improve flower characteristics. Polyploidy plants generally have a larger size than normal plants, so that polyploidized plants are favored by flower lovers. This can be achieved by artificial polyploidization through the induction of chemical mutagens such as colchicine. Colchicine induction is the most effective and widely used to produce polyploid individuals. This has been proven by giving colchicine to hybrid Vanda [4], *Phalaenopsis pulcherrima* [5], and *Phalaenopsis amabilis* [6]. This induction can be done on the flower. Plantlets suspected to be polyploid with colchicine treatment on flower buds of *Phal. Amabilis* [6]. This causes the need to prove the polyploid by chromosomal analysis. The results of the analysis are in the form of genetic information which will later be useful as a basis in plant breeding to obtain superior varieties and improve the properties of ornamental plants. In addition, the information can also improve understanding of the basic biological events that make individual species special, and enable the development of agronomic species [7]. Therefore, research is needed to prove the effect of colchicine on chromosomes and to know the morphology of chromosomes and karyotype patterns in Phalaenopsis hybrids.

2 Material and Methods

The research location was in the screenhouse of the Faculty of Agriculture and the UPT Integrated Laboratory, Sebelas Maret University, Surakarta. The research was carried out from August 2021 to March 2022. Research material: flower buds of *Phalaenopsis* Golden Tree, *Phalaenopsis* Fuller Sunset and *Phalaenopsis* OX X-ray. The method used in this research wa the squash method, which was one of the methods used to make preparations.

Research implementation. Colchicine application: 1,500 ppm colchicine was dropped on cotton, wrapped in orchid flower buds and covered with carbon paper. Preparation of preparations: (a) Materials were collected by taking sepals, starting at 07.30–08.05 WIB. (b) The sepals are immersed in a vial containing aquadest for 24 h at a temperature of 5–8 °C in a refrigerator. (c) Fixation was done by immersing the material in a vial containing 45% acetic acid for ± 1 h. The material was then shaken with aquadest 3 times. (d) Hydrolysis was carried out by immersing the material in a vial containing 1 N HCl for 15 min. The material was washed using aquadest 3 times. (e) Staining was done by immersing the material for ± 24 h in a vial containing 2% aceto-orcein. (f) Squashing was done by taking the pieces of sepals along ± 0.5 mm and

placed on the slide. The preparation was covered with a cover glass and squeezed using the thumb or a pencil that was tapped slowly.

Observation variables include: number, size and shape of chromosomes and karyotype pattern. The observations were analyzed descriptively to identify the morphological characteristics (number, size and shape) of the chromosomes of several Phalaenopsis hybrids. The next step was to analyze the karyotype symmetry which includes the calculation of the intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2). The calculation of A1 follows the way of Zarco [8].

Intrachromosomal asymmetry index:

$$A1 = 1 - [\sum_{n=i}^{i} (bi/Bi)/n] \quad (1)$$

where: bi is the average short arm of each pair of homologous chromosomes, Bi is the average long arm of each pair of homologous chromosomes, and n is the number of pairs of homologous chromosomes.

The formula for the interchromosomal asymmetric index is $A2 = SD/\overline{X}$. Standard deviation of chromosome length in a karyotype. $\overline{X}$ is the average length of chromosomes in a karyotype. The standard deviation of chromosome length in a karyotype. $\overline{X}$ is the average length of the chromosomes in a karyotype.

3 Results and Discussion

3.1 Chromosome Number

The chromosomes number was a variable that easier to be studied than other characteristics such as karyotype, shape, and size of chromosomes. The results of the study on plants without colchicine treatment (control) showed that the chromosome number of *Phal*. Golden Tree was $2n = 3x - 7 = 50$, *Phal*. Fuller Sunset and *Phal*. OX X-ray had a chromosome number of $2n = 4x = 76$ (Fig. 1). This was in accordance with the opinion of Kuo et al. [9] that the basic chromosome number of Phalaenopsis reaches $x = 19$. This study proves that the chromosomes in the three types of orchids are polyploid. This was in accordance with the opinion of Evans and Bosa [10] that species of the same genus can have different chromosome numbers due to polyploidy, that's because basically all species have the same basic chromosome from generation to generation. Lee et al. [11] also stated that the number of chromosomes in most of the Phalaenopsis hybrid cultivars was polyploid and some cultivars were hyperploid and hypoploid. The number of polyploids also occurs in other types of orchids such as the result of a cross between *Coelogyne pandurata* and *Coelogyne rumphii* with a triploid chromosome number ($2n = 3x = 54$) [12].

Phal. Golden Tree, *Phal*. Fuller Sunset and *Phal*. OX X-ray treated with 1,500 ppm colchicine, the chromosome number doubled (polyploidization). *Phal*. Golden Tree chromosome number increased to $2n = 6x\text{-}14 = 100$, *Phal*. Fuller Sunset and *Phal*. OX X-ray to $2n = 8x = 152$ (Fig. 1). This number indicates that the chromosomes in the three types of orchids treated with colchicine were twice the number of normal chromosomes. This is like the opinion of Azmi et al. [6] that colchicine treatment of

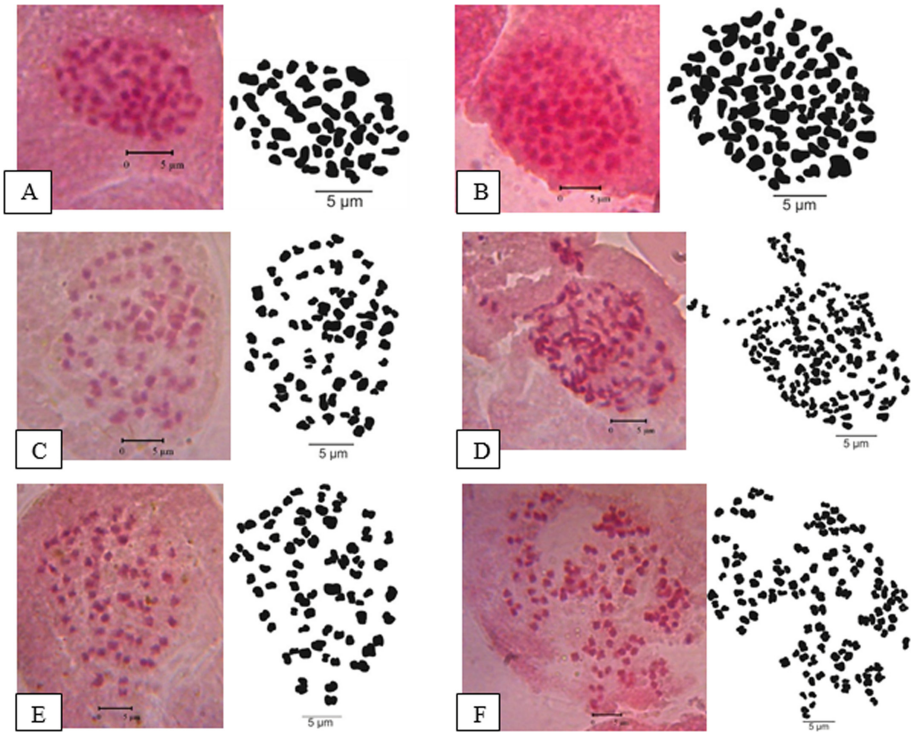

Fig. 1. The orchid chromosomes A. *Phal*. Golden Tree control, 2n = 50; B. *Phal*. Golden Tree with 1,500 ppm colchicine, 2n = 100; C. *Phal*. Fuller Sunset control, 2n = 76; D. *Phal*. Fuller Sunset with 1,500 ppm colchicine, 2n = 152; E. *Phal*. OX X-ray control, 2n = 76; F. *Phal*. OX X-ray with colchicine 1,500 ppm, 2n = 152

500 mg L^{-1} for 5 days can cause *Phal. Amabilis* with normal chromosomes 2n = 38 to 2n = 76.

An increase in the number of chromosomes with colchicine treatment can result in changes in the treated part, which is larger or thicker than normal. Giving colchicine to *Phal. Amabilis* [13] can also cause thicker leaves. Therefore, increasing the number of chromosomes by giving colchicine is expected to increase the size of the flowers to be larger and thicker than normal.

3.2 Chromosome Size and Shape

Chromosome size can be determined by measuring the length of chromosome's arm. Measurement of long arm length (q) and short arm length (p) to determine the total length of chromosomes (q + p). The results showed that the average chromosome size differences in the controls were *Phal*. Golden Tree 1.49 ± 0.12, *Phal*. Fuller Sunset 1.60 ± 0.14, and *Phal*. OX X-ray 1.65 ± 0.13.. This is in accordance with the statement of Lin et al. [14] that the chromosome size in Phalaenopsis is between 1.5–3.5 m. Sharma and Mukai [15], also added that there is a diversity of sizes in the Orchidaceae family

which ranges from 0.5 to 8.0 μm. In addition, plants of the same species usually have the same chromosomes number, but the chromosomes size and shape can different and varied. Variations are generally accompanied by numerical changes with insignificant differences in chromosomal size and shape [16]. These variations can have neutral, beneficial or damaging properties for plants [7].

The results showed average chromosome size difference in the colchicine treatment of 1,500 ppm were *Phal*. Golden Tree 1.65 ± 0.13, *Phal*. Fuller Sunset 1.41 ± 0.12, and *Phal*. OX X-ray 1.56 ± 0.11. The average length of chromosomes from the colchicine treatment of 1,500 ppm in Table 1 shows a smaller size than the control. This was because the chromosomes in plants treated with colchicine have a large number so they tend to be smaller in size than controls. This was similar to *Paphiopedilum villosum* with colchicine treatment which experienced chromosome doubling to become tetraploid so that the chromosome size was smaller than the control (diploid) [17].

The chromosomes shape can be determined by centromere position and classified by calculating the ratio of chromosome arms (r = q/p). Based on Table 1, the average chromosomal arm ratio in control *Phal*. Golden Tree was 1.28 ± 0.13. *Phal*. Fuller Sunset has a chromosome arm ratio of 1.33 ± 0.14. The chromosomal arm ratio in the control *Phal*. OX X-ray was 1.31 ± 0.15. The results of these calculations show that all the chromosomes in the three orchids are metacentric. Orchids generally have metacentric chromosomes [18], as in some orchids of the genus Cattleya [19] and Coelogyne [12].

The average chromosomal arm ratio in the colchicine treatment was 1,500 ppm, namely *Phal*. Golden Tree 1.26 ± 0.15. *Phal*. Fuller Sunset has a chromosome arm ratio of 1.31 ± 0.14 (Table 1). The chromosomal arm ratio on *Phal*. OX X-ray was 1.27 ± 0.14 (Table 1). The results of these calculations showed *Phal*. Golden Tree, *Phal*. Fuller Sunset and *Phal*. OX X-ray with 1,500 ppm colchicine treatment had a metacentric shape in each pair of chromosomes.

The chromosomes shape can be determined by centromere position. The chromosomes centromere of *Phal*. Golden Tree, *Phal*. Fuller Sunset and *Phal*. OX X-rays in both control and colchicine treatment were located in the middle or in the median position.

3.3 Chromosomal Asymmetry Index

Intrachromosomal asymmetry index (A1) was used for determine variation of chromosome shape in a karyotype with a value ranging between zero and one. The value of A1 can help in determining the evolutionary relationship between plant groups [20]. The results showed that the A1 values in the control were *Phal*. Golden Tree of 0.57 ± 0.0018, *Phal*. Fuller Sunset of 0.67 ± 0.0011, and *Phal*. OX X-ray of 0.68 ± 0.0012. The A1 values in orchids treated with colchicine were *Phal*. Golden Tree 0.79 ± 0.0008, *Phal*. Fuller Sunset 0.84 ± 0.0006, and *Phal*. OX X-ray 0.84 ± 0.0004. The A1 value calculated is close to 0. The smaller A1 value or close to 0 indicates that the number of metacentric chromosomes is increasing.

The value of the interchromosomal asymmetry index (A2) was used for determine the dispersion of chromosome size in a karyotype. The smaller the value indicates the deviation or dispersion of chromosome size in one karyotype is not too large. The A2 value in the control *Phal*. Golden Tree was 0.23 ± 0.0379. The value of A2 on *Phal*. Fuller Sunset control is 0.23 ± 0.0431. *Phal*. OX X-ray in the control has an A2 value of

0.17 ± 0.0387. The results showed that the A2 values in orchids treated with colchicine were *Phal*. Golden Tree 0.22 ± 0.0295, *Phal*. Fuller Sunset 00.22 ± 0.0196 and *Phal*. OX X-ray 0.16 ± 0.0205. The results of these calculations explain that the A2 value is small or close to zero in both control and treatment. According to Neto et al. [21], the A2 value tends to approach zero in some species of Epidendroideae and Orchidoideae subfamilies. These results indicate that the size dispersion in one karyotype is not too large or small. The smallest dispersion of chromosome size was found in *Phal*. OX X-ray with colchicine treatment.

4 Conclusion

Colchicine treatment of 1,500 ppm on *Phal*. Golden Tree, *Phal*. Fuller Sunset and *Phal*.OX X-ray affected the chromosome number to twice the control chromosome. The chromosomes number in control was *Phal*. Golden Tree $2n = 3x - 7 = 50$, while in *Phal*. Fuller Sunset and *Phal*. OX X-ray $2n = 4x = 76$. The number of chromosomes treated with colchicine was *Phal*. Golden Tree $2n = 6x\text{-}14 = 100$, while in *Phal*. Fuller Sunset and *Phal*. OX X-ray $2n = 8x = 152$. Chromosome size in the 1,500 ppm colchicine treatment was smaller than the control. All chromosomes, both control and treated with colchicine, were metacentric.

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