



Research Article

## Morphological characteristics of *Phalaenopsis* hybrids treated with 1500 ppm colchicine

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### ABSTRACT

The increasing number of *Phalaenopsis* sp. enthusiasts needs to be balanced with the availability of good-quality cultivars, one of which can be achieved by improving flower morphological characteristics through colchicine induction. Colchicine-induced plants often exhibit superior traits such as larger, thicker, and more durable flowers. This study aimed to investigate the effect of a 1500 ppm colchicine concentration on the floral morphological characteristics of the *Phalaenopsis* sp., with a particular focus on flower size, petal thickness, and sepal morphology. This research provides genetic diversity information on *Phalaenopsis* sp., which can serve as a basis for future breeding programs and as a reference material for orchid growers and researchers. The materials used were flower buds of three *Phalaenopsis* varieties: *Phalaenopsis Fuller's Sunset*, *Phalaenopsis Golden Tree*, and *Phalaenopsis OX X-Ray*. Each treatment was replicated three times. Morphological data were analyzed qualitatively through scoring and converted into binary data. The results showed that 1500 ppm colchicine affects *Phalaenopsis Fullers Sunset* in the form of rhombus-shaped petals, and on *Phalaenopsis Golden Tree* in the form of pointed lateral sepal tips, concave transverse and longitudinal cross-sections of petals, and on *Phalaenopsis OX X-Ray* in the form of split dorsal sepal edges, and open petal arrangement. Overall, colchicine treatment also induced apparent enlargement of flowers in all three *Phalaenopsis* hybrids.

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**Keywords:** chromosome; flower morphology; mutagenesis; ornamental plant; polyploidy

### INTRODUCTION

The orchid is a decorative plant that is highly valued by the communities. The increasing appreciation for this beauty in society has led to a rise in demand for orchid plants across various markets. In 2019, orchid production in several regions of Indonesia, including Sumatra, Central Java, West Sulawesi, and Maluku, totaled 594,356 plants. In 2020, production increased to 625,403 plants (BPS, 2020). This increasing demand needs to be balanced with production supply that meets the community's needs. Indonesia has considerable potential for developing orchid plants due to its vast orchid germplasm (Hartati et al., 2021).

Orchids are one of the families of Orchidaceae, a family of the largest flowering plants. Orchid plants comprise approximately 26,000 species, divided into 750 genera, and are widely distributed across Indonesia (Hartati et al., 2021). *Phalaenopsis* is an orchid with a variety of flower colors. *Phalaenopsis* is one type of orchid that is an essential commodity among orchid species from Indonesia, with the potential to be developed as a commercial species with improved flower characteristics through polyploidization (Sarathum et al., 2010).

Orchid plants are one type of ornamental plant with high economic value. They have become Indonesia's export commodity, so it is necessary to improve the nature of plants to maintain their competitiveness and quality. One of the plant breeding programs that can be employed to obtain superior plants is through biotechnology techniques, including the use of mutations. This technique is used to obtain polyploid plants. Polyploidy can produce changes in genetic comparison and data interpretation. Polyploid plants exhibit larger organs, greater biomass, higher yields, superior tolerance to biotic and abiotic stresses, as well as higher levels of primary and secondary metabolites (Miri, 2020).

Colchicine is a widely used chemical agent for polyploid induction. It works by inhibiting spindle fiber formation during mitosis, leading to chromosome doubling (Singh et al., 2025). However, it should be noted that morphological changes resulting from colchicine treatment on flowers are temporary and occur only in the treated tissues. These changes are non-heritable and do not affect the whole plant. Heritable polyploidy can be induced if colchicine affects reproductive cells, such as pollen, which can produce progeny with altered ploidy levels after pollination (Zhu et al., 2022).

The effectiveness of colchicine application and polyploid induction depends on several factors, including explant types, plant species, colchicine concentrations, and treatment duration (Hannweg et al., 2013). Concentration and duration of treatment time are the two most important factors. The results of several studies have shown that colchicine has a varying influence on different ornamental plants, particularly orchid species and varieties. The optimum concentration and exposure time of colchicine are different for each species and variety, even within the same plant family (Sarathum et al., 2010).

In *Phalaenopsis* orchids, Hartati et al. (2023) induced polyploidy by applying 1500 ppm colchicine to flower buds. Chromosome analysis using squash preparation revealed that control plants had chromosome numbers of  $2n = 3x - 7 = 50$  (*Phalaenopsis* Golden Tree) and  $2n = 4x = 76$  (*Phalaenopsis* Fuller's Sunset and *Phalaenopsis* OX X-Ray). After colchicine treatment, polyploidization occurred with chromosome numbers of  $2n = 6x - 14 = 100$  (*Phalaenopsis* Golden Tree) and  $2n = 8x = 152$  (*Phalaenopsis* Fuller's Sunset and *Phalaenopsis* OX X-Ray). Based on this, the present study aimed to investigate the effect of a 1500 ppm colchicine concentration on the floral morphological characteristics of the *Phalaenopsis* sp., with a particular focus on flower size, petal thickness, and sepal morphology.

## MATERIALS AND METHODS

The research was conducted in March 2025 at the Screen House, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia. The tools used in this study included rulers, pens, notebooks, a camera, and a computer. The plant materials consisted of three varieties of *Phalaenopsis* sp.: *Phalaenopsis* Fuller's Sunset, *Phalaenopsis* Golden Tree, and *Phalaenopsis* OX X-Ray.

This study was a descriptive observational study focusing on the qualitative morphological traits of flower buds treated with colchicine. Although non-experimental, the study was conducted with replication and standardized procedures to improve reliability. Each variety had three replicate plants, and observations were performed on three flower buds per plant. The selected flower buds were the middle bud in the inflorescence, which represents a typical developmental stage in the plant.

The research method involved direct observation of the morphology of *Phalaenopsis* Fuller's Sunset, *Phalaenopsis* Golden Tree, and *Phalaenopsis* OX X-Ray orchid plants, which had been treated with 1,500 ppm colchicine. The application of colchicine was performed by moistening cotton with 1 mL of a colchicine solution. The bud was then wrapped with cotton and covered with carbon paper on the outside for 2 days. Each accession consisted of three observed plant samples.

Morphological observations were carried out on stems, leaves, and orchid flowers once blooming was complete. Data collection techniques involved qualitative recording

and documentation using the *Orchid Ornamental Plant Characteristics Guide* (BRMP, 2007).

The study focused on ten morphological characteristics observed at full bloom, namely a rounded flower shape, a diameter that can exceed 10 cm, and butterfly-like floral morphology with sepals and petals exhibiting similar coloration. The observations included petal form, dorsal sepal edges, the shape of the lateral sepal end, petal end cross-section, petal arrangement, leaf shape, leaf tip shape, leaf edge shape, leaf surface texture, and leaf color. The observation data were analyzed descriptively.

## RESULTS AND DISCUSSION

### *Petal form*

Observations in Table 1 show that *Phalaenopsis* Fuller's Sunset, colchicine treatment resulted in perfectly rhombic petals, while no colchicine treatment flowers displayed imperfect rhombic petals. This indicates that colchicine can influence petal morphology by inducing genetic changes. Darmono (2015) added that the chemical compounds used for mutation induction easily decompose into free radicals, allowing plant properties to change. In line with this, chemical mutagenesis is recognized as an inexpensive and effective method to generate high levels of novel nucleotide diversity in plant genomes (Jankowicz-Cieslak & Till, 2016). Meanwhile, in *Phalaenopsis* Golden Tree and *Phalaenopsis* OX X-Ray, the petal form remained semicircular in both colchicine-treated and non-colchicine-treated flowers (Figures 2 and 3), showing that colchicine treatment did not alter petal form in these varieties.

Table 1. Qualitative flower character data of the orchid *Phalaenopsis* sp.

Varieties	Petal form	Qualitative character				
		Dorsal sepal edges	Shape the lateral sepal end	Petal end cross-section	Petal arrangement	
<i>Phalaenopsis</i> Fuller's Sunset	C	Perfect rhombus	Not split	Taper	Flat	Open
	NC	Imperfect rhombus	Not split	Taper	Flat	Open
<i>Phalaenopsis</i> Golden Tree	C	Semicircle	Not split	Taper	Uneven	Contact
	NC	Semicircle	Not split	Blunt	Flat	Contact
<i>Phalaenopsis</i> OX X-Ray	C	Semicircle	Split	Blunt	Flat	Open
	NC	Semicircle	Not split	Blunt	Flat	Contact

Note: NC= No Colchicine, C= Colchicine Treatment Dorsal Sepal Edge

### *Dorsal sepal edges*

In *Phalaenopsis* Fuller's Sunset and *Phalaenopsis* Golden Tree, the dorsal sepal edges were non-split in colchicine-treated and non-colchicine-treated flowers, indicating no effect of colchicine on this character. In *Phalaenopsis* OX X-Ray, colchicine treatment caused a slight splitting at the dorsal sepal tip, which was less visible in non-colchicine-treated flowers. This supports Grouh et al. (2011), who stated that colchicine can cause abnormal morphological changes during mutagenesis. Appropriate colchicine concentration and immersion time are essential for effective polyploidy induction (Hailu et al., 2021). The difference in characteristics between the colchicine-treated and non-colchicine-treated orchids was observed in the petal part of the orchid flowers (Figures 1, 2 and 3).

### *Lateral sepal end shape*

The explanation in Table 1 shows that in *Phalaenopsis* Fuller's Sunset, the lateral sepal end remained pointed in colchicine-treated and non-colchicine-treated flowers. In *Phalaenopsis* Golden Tree, colchicine treatment changed the lateral sepal tip from blunt (no colchicine treatment) to pointed (colchicine treatment) (Figure 3). In *Phalaenopsis* OX

X-Ray, the lateral sepal tip was blunt in both treatments (Figure 2). Sukma et al. (2016) stated that this was due to an increase in the diameter of plant cells resulting from an increase in cell ploidy, which could lead to cell differentiation. Colchicine can also cause abnormal morphological effects during the mutagenesis process, which ultimately leads to the formation of larger flower sizes.

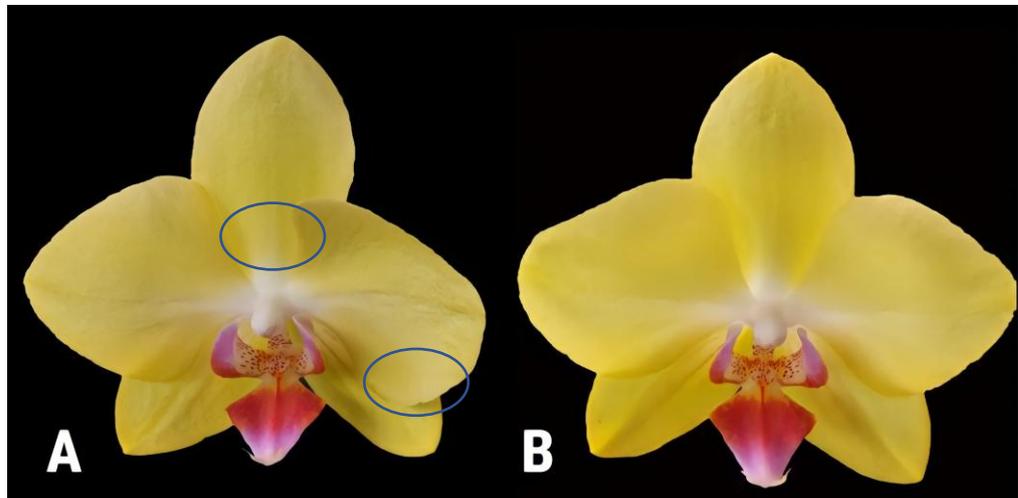


Figure 1. (A) *Phalaenopsis* Fuller's Sunset without colchicine, (B) *Phalaenopsis* Fuller's Sunset with colchicine.

#### *Petal end cross-section*

Based on observations in Table 1, the cross-sectional shape of the petals was flat in non-colchicine-treated flowers of all three varieties. With colchicine treatment, *Phalaenopsis* Golden Tree exhibited an uneven petal tip cross-section (Figure 3), while Fuller's Sunset and OX X-Ray remained flat (Figures 1 and 2). Colchicine solution can stimulate plant growth by increasing enzyme production, where the concentration of colchicine affects the cell division process, ultimately leading to changes in the cell. Cai et al. (2015) also noted that colchicine can cause side effects, including abnormal morphology, such as the thickening of plant parts during the mutagenesis process. Thickening of polyploid plants was also found in *Caladium* "Tapestry" (Tavan et al, 2015). In *Phalaenopsis* orchids, Hartati et al. (2023) induced polyploidy by applying 1500 ppm colchicine to flower buds. Chromosome analysis using squash preparation revealed that control plants had chromosome numbers of  $2n = 3x-7 = 50$  (*Phalaenopsis* Golden Tree) and  $2n = 4x = 76$  (*Phalaenopsis* Fuller's Sunset and *Phalaenopsis* OX X-Ray). After colchicine treatment, polyploidization occurred with chromosome numbers of  $2n = 6x-14 = 100$  (*Phalaenopsis* Golden Tree) and  $2n = 8x = 152$  (*Phalaenopsis* Fuller's Sunset and *Phalaenopsis* OX X-Ray).

#### *Petal arrangement*

Observations in Table 1 show that colchicine treatment affected the petal arrangement differently across the three *Phalaenopsis* hybrids. In *Phalaenopsis* Fuller's Sunset, the petal arrangement remained open in both colchicine-treated and non-colchicine-treated flowers (Figure 1), indicating that this trait is relatively stable under the applied colchicine concentration. In *Phalaenopsis* Golden Tree, the petals were touching in both treatments (Figure 3), suggesting that colchicine did not significantly alter this morphological trait at the applied concentration. In *Phalaenopsis* OX X-Ray, colchicine treatment changed the petal arrangement from touching (no colchicine treatment) to open (colchicine treatment) (Figure 2), indicating a morphological effect of polyploidization induced by colchicine.

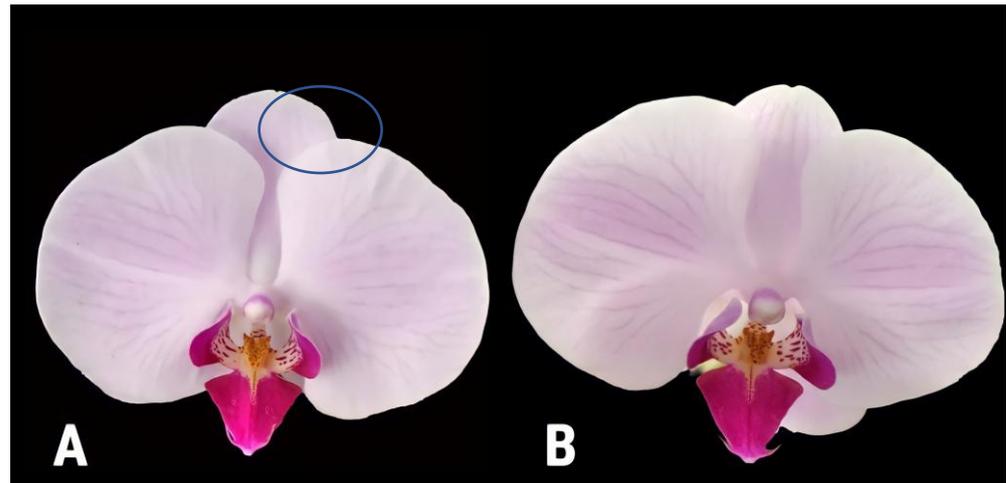


Figure 2. (A) *Phalaenopsis* OX X-Ray without colchicine, (B) *Phalaenopsis* OX X-Ray with colchicine.

The enlargement of cells and organs in colchicine-treated plants is consistent with the mechanism of polyploidization. When cells are exposed to colchicine, chromosome replication occurs without subsequent cell division, increasing cell volume (Fox et al., 2020). This increase in cell size contributes to changes in flower morphology, including the arrangement of petals.

Morphologically, *Phalaenopsis* Fuller's Sunset has rhombus-shaped petals, *Phalaenopsis* Golden Tree has semicircular petals, and *Phalaenopsis* OX X-Ray has semicircular petals. All three hybrids have dorsal sepals that are not split. Lateral sepal tips are sharp in *Phalaenopsis* Fuller's Sunset, blunt in *Phalaenopsis* Golden Tree, and blunt in *Phalaenopsis* OX X-Ray. Petal tips are flat in all hybrids.

Polyploidy refers to a condition in which an organism possesses more than one complete set of chromosomes. This condition can be induced through the application of chemical agents such as colchicine. As a mitotic inhibitor, colchicine disrupts microtubule function, which prevents the formation of the spindle apparatus. When spindle formation is inhibited during cell division, polyploid individuals may be produced. Polyploidy has become a key strategy in the development of *Phalaenopsis* orchid hybrids (De et al., 2014).

The observed differences in petal arrangement, particularly in *Phalaenopsis* OX X-Ray, can be associated with colchicine-induced polyploidy. Hartati et al. (2023) reported that the results of this research showed the number of chromosomes in control *Phalaenopsis* Golden Tree ( $2n=3x-7=50$ ), *Phalaenopsis* Fuller Sunset, and *Phalaenopsis* OX X-ray ( $2n=4x=76$ ). They found that colchicine treatment at the applied concentration effectively induced chromosome doubling, resulting in the production of tetraploid cells in *Phalaenopsis* hybrids. The increase in chromosome number is correlated with morphological changes, including larger cell size, thicker petals, and altered petal arrangement. Similarly, Hartati et al. (2024) highlighted that polyploidization can lead to phenotypic variations in flower morphology, even within the same hybrid.

These results suggest that colchicine-induced polyploidization can lead to superior floral characteristics, including increased organ size and modified petal arrangement, as shown in Table 1 and Figures 1–3. However, the extent of morphological change varies depending on the hybrid. This highlights the importance of considering both colchicine concentration and species-specific response when inducing polyploidy in *Phalaenopsis* orchids.

#### Leaf shape

Table 2 shows that colchicine does not affect leaf shape. This is because colchicine only works on the applied part, the flower bud. To ensure that colchicine treatment does not affect other parts, such as the leaves. This is because colchicine is an immobile element; colchicine consists of the component  $C_{22}H_{25}NO_6$ , which cannot be translocated or

moved from old plant tissues to young plant tissues. Trueman et al. (2013) added that Ca is usually absorbed by plants passively. Ca translocation in plant tissues is very difficult because Ca is immobile; as a result, Ca translocation from young tissues to old tissues is very unlikely.

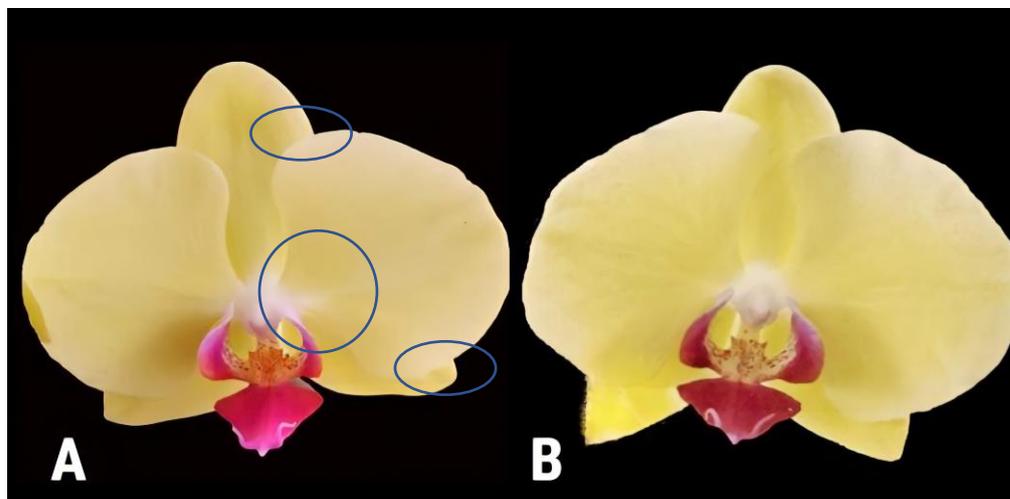


Figure 3. (A) *Phalaenopsis* Golden Tree without colchicine, (B) *Phalaenopsis* Golden Tree with colchicine.

According to Hannweg et al. (2013), the effectiveness of colchicine application in affecting the degree of polyploidization depends on several factors, including the type of plant part applied, plant species, colchicine concentration, and the duration of colchicine treatment. Among these factors, colchicine concentration and the duration of treatment time are the two most important factors supporting the success of polyploidization. It should be noted that the average flower width is not very significant in non-colchicine-treated flowers, as the effectiveness of colchicine use is not optimal. Sarathum et al. (2010) also noted that another factor that could contribute to this is that the colchicine did not absorb at all, or only a small amount of colchicine was absorbed in the plant parts during the first week. Therefore, the variable length of the flower was not much different from the control.

#### Leaf tip shape

Table 2 shows that colchicine does not affect the shape of the leaf tips on *Phalaenopsis* sp. Colchicine only affects the part of the plant where it is applied, in this case, the flower buds, and does not translocate to other parts, such as leaves. Therefore, any chromosomal changes induced in the flower buds are unlikely to persist in the vegetative tissues.

Table 2. Qualitative leaf character data of the orchid *Phalaenopsis* sp.

Varieties		Leaf shape	Leaf tip shape	Leaf edge shape	Leaf surface texture	Leaf color
<i>Phalaenopsis</i> Fuller's Sunset	C	Ovate	Lacerated end split	Whole	Shaved	Green
	NC	Ovate	Lacerated end split	Whole	Shaved	Green
<i>Phalaenopsis</i> Golden Tree	C	Ovate	Lacerated end split	Whole	Shaved	Green
	NC	Ovate	Lacerated end split	Whole	Shaved	Green
<i>Phalaenopsis</i> OX X-Ray	C	Ovate	Lacerated end split	Whole	Shaved	Green
	NC	Ovate	Lacerated end split	Whole	Shaved	Green

Note: NC = No Colchicine, C = Colchicine Treatment Dorsal Sepal Edge.

Sarathum et al. (2010) revealed that colchicine exhibits different absorption rates in various plants, particularly orchids. The survival rates of all plants exposed to colchicine were significantly reduced, and this decline occurred consistently across all treatment durations and dosage levels, indicating that neither the length of exposure nor the

concentration of colchicine prevented the negative impact on plant survival (Wang et al., 2023). This suggests that the effectiveness of colchicine concentration and the timing of administration to induce polyploidy must be precise and appropriate; even within the same plant family, the concentration level and timing of administration may differ. The part of the plant to which colchicine is applied also determines the occurrence of polyploidization. Huy et al. (2019) noted that young tissues containing meristematic cells are preferred for achieving high efficiency in polyploidy formation in colchicine-treated plants.

#### *Leaf edge shape, leaf surface texture, leaf color*

Based on Table 2, it is evident that the shape of the leaf edges in the three varieties remains intact. In contrast, the texture of the leaf surface of the *Phalaenopsis* Fuller's Sunset, *Phalaenopsis* OX X-Ray, and *Phalaenopsis* Golden Tree is bald. Then the leaf color of the three varieties is green, evenly distributed throughout the leaf surface.

Rahayu et al. (2015) also noted that a common problem associated with polyploid induction in vegetative meristem tissues is that polyploid changes occur in only one or a few cells. Hence, the resulting plants usually have a tissue constitution with cells that are still diverse in number, or a mixture of cells of diploid origin with polyploid cells (mixploid). Sukma et al. (2016) also noted that these conditions enable changes that lead to diploid (diplontic selection), specifically competition between diploid and polyploid cells, where diploid cells ultimately dominate. Polyploid cells do not grow optimally, so polyploid plants are not produced.

Several factors that influence the success of colchicine applications, such as sunlight and microorganisms, also impact its effectiveness. Atichart (2013) also noted that a high percentage of contamination by microorganisms can lead to the production of unwanted plants. Azmi et al (2016) added that the high toxicity effect could allegedly cause inhibition of the plant development process. As in high concentrations, at concentrations of 1,000 and 2,000 mg L<sup>-1</sup>.

## CONCLUSIONS

Qualitative morphological characteristics showed that 1500 ppm colchicine induced changes in the three *Phalaenopsis* hybrids compared to non-colchicine-treated flowers. In *Phalaenopsis* Fuller's Sunset, colchicine treatment resulted in perfectly rhombic petals, whereas non-colchicine-treated flowers had imperfect rhombic petals. In *Phalaenopsis* Golden Tree, colchicine treatment caused sharp lateral sepal tips and an uneven cross-section of petal tips. At the same time, non-colchicine-treated flowers showed blunt lateral sepal tips and flat petal tips. In *Phalaenopsis* OX X-Ray, colchicine treatment led to split dorsal sepal edges and an open petal arrangement. In contrast, non-colchicine-treated flowers had non-split dorsal sepals and a touching petal arrangement. These results indicate that colchicine-induced polyploidization can alter specific floral morphological traits in *Phalaenopsis* orchids.

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