

Colchicine-Induced Phenotypic Alterations in *Dendrobium* 'Transient White Rika' and 'Florenza': Valuable Material for Genetics-Based Learning Modules

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Abstract: This study investigates the phenotypic alterations in *Dendrobium* 'Transient White Rika' (RKA) and 'Florenza' (FLO) orchids resulting from colchicine-induced polyploidy during in vitro cultivation. Colchicine, a known mitotic inhibitor, was applied at varying concentrations (0 ppm, 4000 ppm, 5000 ppm, 6000 ppm) to induce chromosome doubling and assess its impact on the vegetative and generative phases of the orchids. A factorial Randomized Block Design (RBD) was employed to analyze the effects of these treatments. The study revealed significant variations in leaf length and color, particularly at higher colchicine concentrations. Morphological changes were most pronounced at 6000 ppm, where the leaf length decreased, and flower morphology exhibited unique features compared to the control. The study also observed a reduction in stomatal density as colchicine concentration increased, correlating with an enlargement of leaf epidermal cells. These findings indicate that colchicine treatment can effectively induce phenotypic changes in *Dendrobium* orchids, contributing to the development of hybrids with superior ornamental qualities. However, the study also notes that the response to colchicine is variable, with certain parameters like the number of new shoots and leaf width not showing significant changes. The results underscore the potential of colchicine-induced polyploidy in orchid breeding, though further research is necessary to optimize treatment protocols and ensure the stability and viability of the resulting hybrids. These findings may serve as valuable material for genetics-based learning modules in biology.

Keywords: Genetic Mutation; Morphology; Polyploidy

Introduction

The demand for orchids is quite high, leading to an increase in production capacity each year. Orchid production ranks among the top 10 largest ornamental plant productions in Indonesia (Central Statistics Agency, 2023). *Dendrobium* is an ornamental plant that has remained popular over time. This type of orchid features a variety of colors and flower shapes, is visually appealing, easy to cultivate, and flowers readily. The growing public demand for *Dendrobium* orchids presents an opportunity for breeders to enhance the characteristics and quality of these orchids.

Phenotypic studies in plant breeding are crucial for optimizing agricultural products by assessing leaf traits quickly and accurately, aiding in the selection of desirable traits for commercial purposes (H. Zhang *et al.*, 2023). The general phenotype of *Dendrobium* orchids includes a slender stem with a diameter of 1.1 cm. The height of mature orchid plants ranges from 20 to 35 cm, with parallel leaf veins. The leaves of *Dendrobium* orchids are 9 to 11 cm long and 3 to 4 cm wide. *Dendrobium* 'Transient White Rika' has fragrant flowers shaped like butterflies, with ivory-white petals and a glossy dark purple labellum, and the flower size ranges from 5 to 7 cm. *Dendrobium* 'Florenza' has bright reddish-

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orange flowers. The blooming period of these *Dendrobium* orchids is approximately one month. The shape, color, and fragrance of these flowers contribute to the increasing demand for orchids.

The improvement of characteristics and quality of *Dendrobium* orchids can be achieved through plant breeding techniques, specifically mutation induction by chromosome doubling (polyploidy) (Amien *et al.*, 2023; Hartati *et al.*, 2014). Currently, the utilization of polyploidy plant breeding techniques using colchicine has been widely reported (He *et al.*, 2016; Revathi & Thomas, 2022; Tharawoot *et al.*, 2012; Tuwo & Indrianto, 2016). Colchicine ($C_{22}H_{25}O_6N$) can inhibit the formation of spindle fibers during cell division, resulting in the formation of polyploid individuals (Bunnag & Hongthongkham, 2015; Rahayu, 2015; Taratima *et al.*, 2023). One of the advantages of polyploid orchids is that they have larger flower sizes and longer-lasting blooms (Vichiato *et al.*, 2014).

Study on the induction of polyploidy in orchids using colchicine has been extensively conducted (Zakizadeh *et al.*, 2020; X. Zhang & Gao, 2020, 2021). Colchicine applied *in vitro* has successfully increased the chromosome number in Phalaenopsis orchids *in vitro* (Rahayu *et al.*, 2015; Wu *et al.*, 2022). Similarly, in *Dendrobium* orchids, both *in vitro* and *in vivo* applications of colchicine have been able to alter chromosomes and characteristics during the vegetative phase (Pham *et al.*, 2019; Vilcherrez-Atoche *et al.*, 2022).

Colchicine ($C_{22}H_{25}O_6N$) is an alkaloid derived from the tuber of *Colchicum autumnale* L. (Family Liliaceae) and is widely used as an antimitotic agent in inducing polyploid plants. This compound can inhibit the formation of spindle fibers during cell division, leading to the formation of polyploid individuals (Hannweg *et al.*, 2016)

Study on the induction of polyploidy in *Dendrobium* orchids using colchicine presents significant implications for the horticultural industry and the flower trade. This study aims to observe phenotypic changes in RKA and FLO *Dendrobium* resulting from tissue culture in the vegetative and generative phases following colchicine treatment.

This study also has great potential as a material for developing learning modules in biology lessons, especially in the genetics sub-material. Learning modules can cover important topics in genetics, such as mutation, polyploidy, and chromosome changes by utilizing polyploidy induction techniques in this study. Students can be introduced to basic genetic concepts through direct observation of changes in plant phenotypes, such as increased flower size and extended flower blooming periods. Modules can be designed to include laboratory experiments that allow students to understand the application of mutation techniques in

plant breeding, as well as how plant breeding can improve the quality and quantity of agricultural products. This study can be used as interesting and relevant teaching materials to improve students' understanding of genetics in the context of life.

Method

This study was conducted in the greenhouse and Botany Laboratory of PGRI Argopuro University, Jember. The study employed a factorial Randomized Block Design (RBD) with two factors with two factors and 5 replications. The first factor was the variety treatment consisting of *Dendrobium* Transient White Rika (ALG) and *Dendrobium* Florenza (FLO), and the second factor was the concentration of colchicine (0 ppm or control, 4000 ppm, 5000 ppm, and 6000 ppm) with a total of 40 plants.

The materials used in this study included two varieties of *Dendrobium* orchid seedlings, Two species of *dendrobium* which are the result of crossbreeding and are not rare or protected species. *Dendrobium* Transient White Rika (ALG) and *Dendrobium* Florenza (FLO), which were obtained from tissue culture and were approximately 3 months old post-acclimatization, colchicine, fungicide, and bactericide. The equipment used in this study included rulers, writing tools, markers, knives, razors, cameras, and pipettes.

The study procedure consisted of several activities, including planting *Dendrobium* orchid seedlings in pots; the seedlings were planted in pots with dried pine bark. The stock solution for colchicine treatment was prepared by mixing colchicine with ethanol. The required amount of stock solution for each treatment was calculated based on the number of samples per treatment and the amount of stock solution applied to each plant.

The application was carried out in the morning around 07:00-08:00 for three consecutive days. The *Dendrobium* seedlings were treated with colchicine according to the given treatments, with a dosage of 0.4 mL. The application was performed by dropping colchicine onto mature young leaves, with four drops per leaf (0.4 mL per drop, 40 mL per leaf). Afterward, the *Dendrobium* seedlings were covered with transparent plastic, which was removed after 8 hours. The purpose of covering was to prevent evaporation on the seedlings, allowing the colchicine solution to be optimally absorbed by the seedlings.

The observed vegetative parameters included the time of new leaf emergence, the number of new shoots, the number of new leaves, leaf length, leaf width, leaf color, and stomatal density. Stomatal density by making a stomata mold using nail polish, then attaching it to a microscope slide and counting the number of stomata in

the field of view. The results are then converted into 1 mm². The generative parameters included flower morphology. Statistical data analysis was conducted using ANOVA with SPSS version 22.

Result and Discussion

The results of this study are very relevant for educational science, especially biology, because they provide concrete examples of the application of genetic concepts, such as mutation, polyploidy, and selection in plant breeding. Through experimental practicums, teachers can teach students about genetic changes in plants, such as polyploidy induction in *Dendrobium* orchids, which facilitates the understanding of material that is often abstract. In addition, this study also opens up insights into the practical application of genetics in the field of horticulture, which can be used to develop more interesting and applicable learning modules.

The following are the results of morphological observations on the parameters of the emergence time of new leaves, the appearance of new shoots, the emergence of new leaves, leaf length and width, and leaf color after colchicine treatment on *Dendrobium* orchids, as presented in Table 1.

Table 1. ANOVA Test Results Observation Parameters

Parameter	Result of ANOVA		
	Variety	Concentration	Interaction of variety and concentration
New shoots	1000 ns	0,875 ns	0,291 ns
New leaves	0,445 ns	0,747 ns	0,266 ns
Leaf Length	0,675 ns	0,041 s	0,912 ns
Leaf Width	0,163 ns	0,340 ns	0,734 ns
Leaf Color	0,004 s	0,064 ns	0,001 s
Height	0,079 ns	0,674 ns	0,914 ns

The table shows that the treatment of varieties and colchicine concentrations had both significant and non-significant effects on several observation parameters. In this study, the colchicine concentration treatment had a significant impact on leaf length and leaf color, influenced by variety treatment and the interaction between variety and concentration. This is indicated by the significance value of ≤ 0.05 . The results of Duncan's test on leaf length (Table 2) and leaf color can be seen in the following tables (Table 3 and Table 4).

Table 2. Duncan Test Results Leaf Length Parameters

Treatment (Concentration)	Average
K1 Control	63.2000 a
K2 4000 ppm	28.1250 b
K3 5000 ppm	8.3333 c
K4 6000 ppm	8.3333 c

Table 3. Duncan Test Results Leaf Color Parameters Varieties Treatment.

Treatment (Variety)	Average
RKA (V1)	49 a
FLO (V2)	44 b

Table 4. Duncan Test Results for Leaf Color Parameters, Varieties and Concentration Interaction Treatments.

Treatments (Varieties and Concentration Interaction)	Average
V1K1	13 a
V1K2	12 a
V1K3	12 a
V1K4	12 a
V2K1	12 a
V2K2	8 b
V2K3	12 a
V2K4	12 a

Based on the results of the ANOVA test in Table 1, it is shown that the variety treatment significantly affected leaf color, the colchicine concentration treatment significantly affected leaf length, and the interaction between variety and concentration treatments had a significant effect on leaf color. Other parameters, such as the number of new shoots, the number of new leaves, leaf width, and plant height, did not show significant differences. This indicates that colchicine induction only had a significant effect on certain parameters.

The parameters that did not show a significant effect may be due to the fact that orchids are perennial plants with slow growth, so their response to chemical treatments does not always manifest phenotypically. In line with study on *Dendrobium nindii* seedlings, which exhibited slower vegetative growth compared to hybrid orchids (Soelistijono *et al.*, 2023). Similarly, study on *Coelogyne Nervosa* A. Rich orchids showed slow growth during conservation methods (Chithra Devi *et al.*, 2021).

The phenotypic traits that emerge due to the influence of colchicine are affected by the number of mutant cells formed. If the number of normal cells is more dominant than the mutant cells, then the effect of colchicine on these cells may not be morphologically apparent. The morphological impact of colchicine is influenced by the number of affected cells, where not all cells divide simultaneously, and therefore, not all interact with colchicine. Only actively dividing cells are affected, resulting in a mixture of normal and mutant cells (cells affected by colchicine) within the same tissue or individual.

Normal diploid cells and mutant tetraploid cells can coexist within the same tissue, a condition known as a mixoploid plant. Mixoploid plants exhibit varying ploidy levels within the same tissue, such as diploid and

tetraploid cells (Hassan *et al.*, 2022; Sánchez-Betancourt & Núñez Zarantes, 2022). Mixoploidy has been observed in bottle gourd offspring, aiding in the development of stable seedless varieties (Hassan *et al.*, 2022). In mixoploid plants, chromosome doubling does not occur in all cells of the treated tissue. Syukur *et al.* (2013) stated that both spontaneous and artificial mutations are usually harmful, and cells carrying the new mutation traits tend to be lost in competition with normal cells.

Based on data from study on the vegetative phase morphology of *Dendrobium* RKA and FLO, the leaf length parameter indicates that as the colchicine concentration increases, the leaves of *Dendrobium* become shorter. A characteristic of polyploid plants is a slower growth rate compared to diploid plants. This slowdown occurs only during the early vegetative growth phase. The delayed emergence of new leaves in orchids treated with colchicine is likely the initial stage of polyploidy, as the increased number of chromosomes in polyploid plants leads to slower cell division, which in turn slows the growth of primordial leaves. Polyploid plants often exhibit a slower growth rate, resulting in delayed flowering and reduced fertility compared to their diploid counterparts, possibly due to irregular meiosis (Sattler *et al.*, 2016).

The leaf color parameter in the variety treatment and the interaction between varieties and colchicine concentration shows different effects (Figure 1). Leaf color variation among different *Dendrobium* orchid species is influenced by genetic, morphological, and environmental factors. Each species exhibits distinct leaf characteristics, which can be classified based on morphology and anatomy. *Dendrobium* orchids display significant genetic diversity, with 1,184 species identified globally. This diversity contributes to variations in leaf morphology, including shape and color (Wahba *et al.*, 2014). Leaf color is an important feature, and environmental factors such as light and growth conditions also play a significant role in the expression of leaf characteristics across *Dendrobium* species (Sudeep *et al.*, 2018). Study shows that anatomical differences, such as stomatal size and epidermal cell shape, vary between *Dendrobium* species, which may correlate with leaf color (Handayani & Pramono, 2022). The presence of flavonoids has been linked to color variation in flowers, suggesting a similar mechanism could influence leaf color (Qiu *et al.*, 2023).

The use of colchicine also affects leaf anatomy, particularly stomata (Figure 2). The higher the concentration of colchicine applied, the lower the stomatal density on the orchid leaves (Safi'i *et al.*, 2024). This is due to the direct relationship between colchicine concentration and the degree of chromosome doubling. Higher chromosome doubling causes enlargement of the leaf epidermal cells (Gantait *et al.*, 2011). Consequently,

the distance between stomata increases, resulting in fewer stomata within a given field of view. These findings are consistent with the study which showed that the number of stomata decreased compared to the control plants; the higher the concentration of colchicine used, the lower the stomatal density (Soetopo *et al.*, 2016).

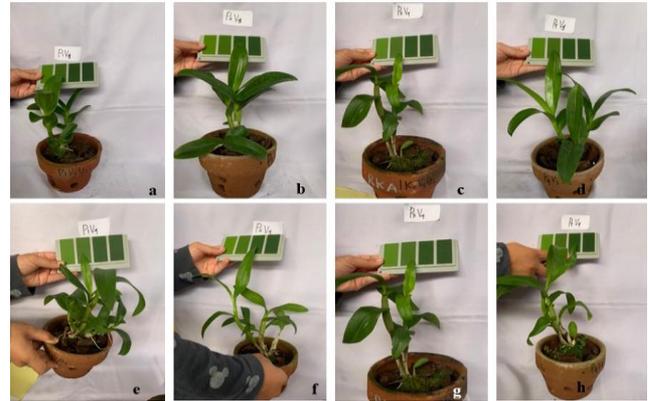


Figure 1. Leaf color: a) RKA variety with 0 ppm; b) RKA variety with 4000 ppm; c) RKA variety with 5000 ppm; d) RKA variety with 6000 ppm; e) FLO variety with 0 ppm; f) FLO variety with 4000 ppm; g) FLO variety with 5000 ppm; h) FLO variety with 6000 ppm.

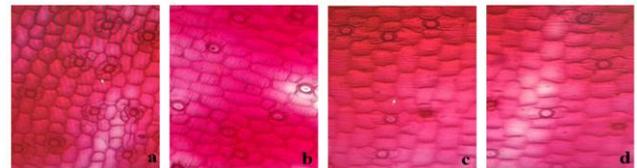


Figure 2. Stomata density: a) 0 ppm concentration; b) 4000 ppm concentration; c) 5000 ppm concentration; d) 6000 ppm concentration

Study has shown that colchicine treatment can lead to larger stomata but lower stomatal density in polyploid *Dendrobium* species. For example, *Dendrobium cariniferum* exhibited a significant decrease in stomatal density along with an increase in stomatal size after colchicine (Revathi & Thomas, 2022; X. Zhang & Gao, 2021). An optimal colchicine concentration (0.05% for 24-96 hours) was found to induce tetraploidy, which correlated with morphological changes, including stomatal characteristics (Revathi & Thomas, 2022). However, other studies reported no significant differences in stomatal density across various colchicine concentrations in specific *Dendrobium* varieties (Sari *et al.*, 2023).

In this study, observable flower morphology was noted in the Transient White Rika variety, while the Florenza variety had not yet flowered. Morphological changes in RKA flowers were apparent at the 6000 ppm treatment level (Figure 3). In Figure 3a, a unique form of RKA flower morphology can be seen, including a

distinctive labellum shape, a combination of colors on the sepals, and a flower size different from the control treatment. The presence of flowers with unique shapes and color combinations may be influenced by colchicine. The impact of colchicine on the flower morphology of *Dendrobium* orchids is significant, primarily through polyploidy induction, which enhances various morphological traits. Study has shown that colchicine treatment can cause substantial changes in flower characteristics, contributing to the development of superior hybrids. In this study, the flower size in the colchicine treatment was smaller than without colchicine treatment (Fig 4 and 5).

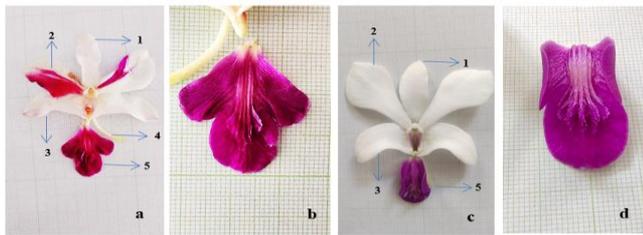


Figure 3. Flower morphology of the Transient White Rika variety. a. Flower with 6000 ppm colchicine; b. Labelum of lower with 6000 ppm colchicine; c. Flower with 0 ppm colchicine; d. Labelum of lower with 0 ppm colchicine

For instance, in *Dendrobium crumenatum*, colchicine treatment at a concentration of 0.05% for 96 hours successfully induced tetraploidy at a rate of 50%, leading to improved flower characteristics and overall plant morphology (Revathi & Thomas, 2022). Additionally, tetraploid plantlets of *Dendrobium cariniferum* showed broader and thicker leaves and increased stem diameter, which is highly valuable for aesthetic and commercial purposes (X. Zhang & Gao, 2021). *Dendrobium wardianum* treated with colchicine also displayed wider, darker green leaves and longer stems, indicating significant morphological improvements due to polyploidy (Wang *et al.*, 2023).

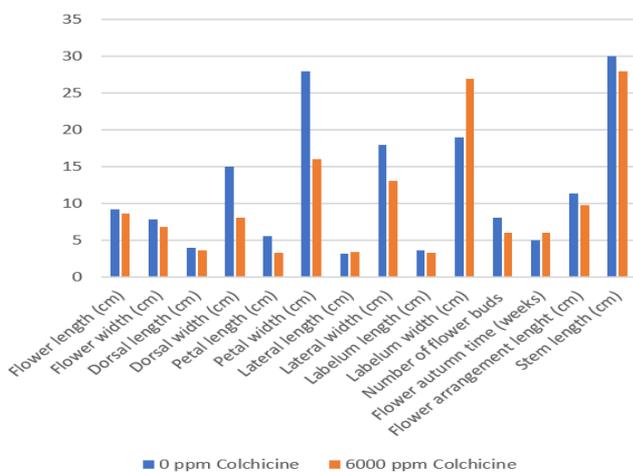


Figure 4. Generative growth parameters of *Dendrobium* Transient White Rika

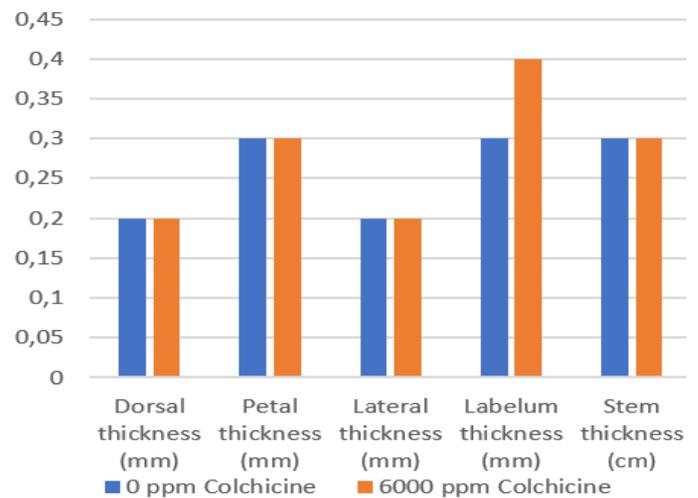


Figure 5. Morphology of the thickness of the dendrobium flower parts

In addition to its impact on leaf and stem morphology, the application of colchicine is also associated with increased flower size and quality. For example, larger flower sizes and extended longevity have been observed in orchid hybrids treated with colchicine. These morphological improvements, such as increased flower width and length, are crucial for breeding programs aimed at enhancing ornamental quality (Hartati *et al.*, 2023). However, while colchicine treatment shows promising results in enhancing flower morphology, it is important to consider potential risks, such as decreased survival rates and the stability of induced polyploids, which could affect long-term breeding outcomes.

Further study is needed to explore the long-term effects of colchicine treatment on the stability of polyploids and the survival of *Dendrobium* plants. Additionally, more in-depth studies on optimal colchicine concentrations are required to maximize morphological benefits while minimizing potential risks. The application of this technique in orchid breeding programs can be further developed to produce hybrids with superior characteristics that meet market demands.

Conclusion

The study revealed significant variations in leaf length and color, particularly at higher colchicine concentrations. Morphological changes were most pronounced at 6000 ppm, where the leaf length decreased, and flower morphology exhibited unique features compared to the control. The study also observed a reduction in stomatal density as colchicine concentration increased, correlating with an enlargement of leaf epidermal cells. These findings

indicate that colchicine treatment can effectively induce phenotypic changes in *Dendrobium* orchids, contributing to the development of hybrids with superior ornamental qualities. This study also provides a valuable reference for developing laboratory-based teaching modules on polyploidy and its impact on plant morphology for biological sciences, especially genetics.

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Author Contributions

DSS and SD write the manuscript; DSS designs the research and oversees the entire process; HRP collects the data. SD and NK are analyzing data

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Conflicts of Interest

The authors declare no conflict of interest.

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