Bioedukasi
Jurnal Biologi dan Pembelajarannya
Vol. 21 No 3, October 2023, page 251-255
e-ISSN: 2580-0094; p-ISSN:1693-3931
DOI: 10.19184/bioedu.v21i3.39770
jurnal.unej.ac.id/index.php/BIOED

Leaf Morphological Response of Several Dendrobium Orchids due to Colchicine Application

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Article Info
Article history:
Received June 08, 2023
Revised October 02, 2023
Accepted October 03, 2023

Keywords:
Colchicine,
Dendrobium,
Leaf Morphology.

ABSTRACT
Dendrobium orchid is an ornamental plant that has a relatively stable market and does not depend on the season. The increasing public demand for Dendrobium orchids is an opportunity to improve the character and quality of Dendrobium orchids, one of which is by inducing mutations through the application of colchicine. This study aims to determine the effect of orchid varieties and colchicine application on the morphology of Dendrobium orchid leaves. This research was conducted at the Sriwijaya Orchid Gallery Cluster B9, Jember. This study used a 2 factorial Randomized Block Design (RBD). The first factor was the treatment of varieties consisting of Dendrobium Venus Purple Algis (ALG) and Dendrobium Transient Pink Frederika (FRD), the second factor was the treatment of colchicine concentrations (0 ppm or control, 4000 ppm, 5000 ppm and 6000 ppm). The results of this study indicated that the application of colchicine had a significant effect on several leaf morphological characters, namely leaf length, width, and color. The best leaf length was obtained at a concentration of 5000 ppm, the best leaf width was obtained at a control concentration of 0 ppm, and the most intense leaf color was obtained at a colchicine concentration of 6000 ppm.

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1. INTRODUCTION

Indonesia is known as a country with abundant orchid species. The distribution of orchids in Indonesia from Papua to Sumatra reaches around 5000 species (Suryani, 2015). The diversity of the orchid germplasm is a good potential and produces high-quality orchids, both in terms of leaf shape and flower color. Orchid plants are not only cultivated for cut flowers but are also widely used as ornamental plants. Data from the Badan Pusat Statistik (2021), shows that orchid production in Indonesia in 2020 has decreased from 11,683,333 stalks to 11,351,615 stalks in 2021. West Java is the area that contributes the most domestic orchid production with a total of 4,836,753 stalks, then Banten with 4,804,840 stalks, and East Java with 629,197 stalks.

Efforts to improve the morphology of Dendrobium orchid leaves do not yet determine the character and quality of Dendrobium orchid morphology, considering the increasing consumer demand for Dendrobium orchid plants. The increasing public demand for Dendrobium orchids is an opportunity to improve the quality and character of Dendrobium orchids. The leaf morphology of the Dendrobium orchid has a unique shape, including elongated round leaves, leaf thickness varies from thin, fleshy, stiff, and has a flat surface. The leaves are not stemmed, the leaves sit completely on the stem. The edge of the leaf is not serrated (flat) with a split leaf tip, the leaf veins are parallel to the leaf edge, and the tip is at the tip of the leaf. The arrangement of the leaves on the Dendrobium orchid is alternate or facing and the colour of the leaves on the Dendrobium orchid.

One way that can be done to improve the character and quality of Dendrobium orchids is through mutation induction with the application of colchicine. Colchicine is a chemical that can cause polyploid individuals. The substance colchicine can cause a doubling of the number of chromosomes, because colchicine, the active spindle thread mechanism, is blocked by colchicine, so that cell division does not occur or the chromosomes are not attracted to each pole during anaphase, while the chromosomes have already replicated so the number doubles (Ermayanti *et al.*, 2018).

Warmadewi (2017), mutation is a change in the gene or chromosome material of a cell that is passed on to its offspring. Mis replication of genetic material during cell division by radiation, chemicals (mutagens), or viruses, or can occur during the process of meiosis (Pham et al., 2019). One type of mutation induction that uses

chemicals and can be done to develop the potential of Dendrobium orchids is through the application of colchicine. The application of colchicine to plants aims to obtain the characteristics of polyploid plants, polyploid plants generally have different characters from diploid plants (Liu et al., 2023)). However, morphological changes do not really bring about bigger changes (Nadhira, 2019)

Nadhira (2019), research results show that the application of colchicine by drip on *Dendrobium bifalce* L. orchids crossed with Dendrobium lithocola at a concentration of 5000 ppm can produce tentraploid plants with a chromosome number of 76. The results of this research also show that the morphological characters in Dendrobium orchids bifalce L. crossed with Dendrobium lithocola had significantly different effects on the characters of leaf number, leaf length, leaf width and leaf colour. Based on several facts obtained from the results of previous research, this research aims to determine the effect of variety type and colchicine concentration on the morphological characters of orchid leaves. Several morphological characters of Dendrobium orchid leaves that were observed included: age at which new leaves emerge, number of new shoots, number of new leaves, leaf length, leaf width, and leaf colour. The results of this research can be used theoretically and practically, not only in the academic community but also in society at large, especially orchid lovers.

2. RESEARCH METHOD

This research was conducted in September 2022 – February 2023 at the Sriwijaya Orchid Gallery Cluster B9, Jember. This study used a 2 factorial Randomized Block Design (RBD). The first factor was the treatment of varieties consisting of Dendrobium Venus Purple Algis (ALG) and Dendrobium Transient Pink Frederika (FRD) and the second factor was the concentration of colchicine (0 ppm or control, 4000 ppm, 5000 ppm and 6000 ppm). The materials used in this study included 2 varieties of Dendrobium orchid seeds, namely Dendrobium Venus Purple Algis and Dendrobium Transient Pink Frederika aged ± 3 months after acclimatization, colchicine, fungicide and bactericide. While the equipment used in this study included a ruler, writing instruments, markers, knives, razors, cameras, and pipettes.

This research procedure consists of several activities, including; Plant Dendrobium orchid seedlings in pots; Seedlings are planted in pots with pieces of pine stems that have been dried; The stock solution for colchicine treatment comes from a mixture of colchicine and ethanol. The stock solution requirement for each treatment is calculated based on the number of samples for each treatment and the number of stock solutions applied to each plant; Application was done in the morning around 07.00-08.00 for 3 consecutive days; Orchid seeds were dripped with colchicine according to the treatment given, namely 0.4 mL. Application was done by dripping colchicine on young leaves that have matured as much as four drops with 0.4 mL (40 mL/drop); Then the Dendrobium seedlings are covered using transparent plastic, and opened after 8 hours. The purpose of the enclosure was to prevent evaporation from dripping on the seeds, so that the colchicine solution can be absorbed optimally by the seedlings.

Observation of Dendrobium orchid leaf morphology was carried out 1 week to 3 months after treatment. Parameters observed include; age of emergence of new leaves, number of new shoots, number of new leaves, leaf length, leaf width, and leaf color. Statistical analysis of the data used the Anova test with the SPSS version 22 application.

3. RESULT AND DISCUSSION

The results of the ANOVA test in this study are presented in Table 1.

Table 1. Anova Test Results for Several Observation Parameters

No.	Parameters	The average of observation parameters		
		Orchids Variety Factor	Colchicine Concentration Factor	Interaction between orchids variety and colchicine concentration
1	New leaf age	0,363^{ns}	0,493 ^{ns}	0,694 ^{ns}
2	Number of new shoots	0,388 ^{ns}	0,967 ^{ns}	0,658 ^{ns}
3	Number of new leaves	0,871 ^{ns}	0,23 ^{ns}	0,915 ^{ns}
4	Leaf length	0,995 ^{ns}	0,102 ^{ns}	0,359 ^{ns}
5	Leaf width	0,747 ^{ns}	0,019 ^{s}	0,315 ^{ns}
6	Leaf colour	0,408 ^{ns}	0,004 ^{s}	0,851 ^{ns}

Note: *s = significant, *ns = non-significant

Furthermore, the Duncan test results for parameters that have a significant influence are listed in Table 2.

Table 2. Duncan Test Results for Some Parameters

No.	Treatments	Leaf Width	Leaf Length	Leaf Color
1	Control (K1)	2,0625[a]	9,6875[a]	2,25[a]
2	4000 ppm (K2)	1,2875[b]	8,75[b]	2,8333[b]
3	5000 ppm (K3)	1,8375[c]	11,3125[c]	3[c]
4	6000 ppm (K4)	0,6875[d]	4,55[d]	3,1429[d]

Note: Numbers followed by the same letters show results that are not significantly different

Based on the results of this research, morphological measurements are an indicator of the presence of polyploid plants. According to Nadhira (2019) leaf morphology observations were carried out to determine the effect of giving colchicine on plants that were displayed phenotypically. In morphology, the ploidy level of a plant is analyzed based on analyzing the ploidy level on its shape, size and colour compared to the diploid plant.

In this study, the treatment of variety types and the combination of variety types and colchicine application did not have a real or significant effect on the observed parameters, while colchicine application had a significant effect on the parameters of leaf length, leaf width and leaf colour. Colchicine derived from *Colchium antumnale* seed extract is able to induce plants to become polyploidy plants at the right concentration and time. As a result, mitosis is hampered, because certain concentrations of colchicine are able to weaken the microtubules that make up the spindle thread (Pradana dan Hartatik, 2019). The change in cells from diploid organisms to polyploid cells in the mitosis phase occurs when the chromosomes that have doubled during interphase fail to separate (Yulia et al., 2022; Zakizadeh et al., 2020)

The method of application of colchicine will affect the concentration and duration of colchicine application. The application of colchicine can be done by dripping in vivo, usually using a higher concentration compared to soaking or giving it to growth media (tissue culture) in vitro, and the application can be done more than once. Apart from influencing the size of the concentration used, the method of application also influences the magnitude of the interaction formed between the subject and colchicine. Giving colchicine at a certain intensity and for a certain time can increase the chances of forming polyploid plants (Lim et al., 2017). The mechanism of action of colchicine is by binding to β-tubulin dimers and inhibiting microtubule assembly, but colchicine does not inhibit the action of microtubules that are already bound. So the effect that occurs is the doubling of the chromosomes in the cell due to the failure of the microtubules to pull the chromosomes towards the poles (Eigsti, 1957)

Giving the mutagen colchicine to each plant has a different response. In the results of this study, the application of colchicine had no significant effect on the parameters of the age at which new leaves emerged, the number of new shoots and the number of new leaves. Giving colchicine gave an inappropriate response and effect, so that the development and formation of leaf primodials was delayed or experienced a setback (Nengsih et al, 2022). Giving colchicine is inhibitory and can suppress leaf growth, thus giving an effect that can reduce the number of leaves (Mahyuni et al, 2015). According to Warmadewi (2017), artificial and spontaneous mutations were usually dangerous and the properties of new mutations carried by cells will tend to be lost in competition with normal cells. Therefore, the result of not appearing the effect of colchicine morphologically can be caused by the number of normal cells that predominate compared to mutant cells (Masruroh, 2018).

In this study, the application of colchicine had a significant effect on leaf length. The results showed that the application of colchicine at a concentration of 5000 ppm produced the longest leaves among the other concentration treatments, while the application of colchicine at a concentration of 6000 ppm produced the shortest leaves. These results indicate that there was a tendency for the leaf length to decrease due to the application of colchicine concentrations with increasing concentrations. According to Nadhira (2019), this was a phenomenon of abnormal cell division due to the treatment of colchicine as a chemical.

On the leaf width parameter, colchicine application had a significant effect. The application of 6000 ppm colchicine produced the smallest leaf width, while the treatment without colchicine (control) produced the largest leaf width among the other treatments. The results of this study indicate a tendency that the higher the concentration of colchicine given the smaller the leaf width. This was presumably because the application of colchicine can affect the dilation of orchid leaves (Revathi & Thomas, 2023). This was in line with the results of research by Nengsih et al (2022) which showed that the largest increase in plant height was in fact found in the control treatment, which was 1.43 cm. According to Maghfirah et al (2018), the higher the concentration of colchicine given, it can affect the mitotic. This causes the cells to become larger and plant growth to be stunted. Plant growth is hampered due to the use of colchicine which limits the assembly of microtubules making up the cell framework, and disrupts the layout of proteins in the cell membrane which was regulated by microtubules and microfilaments, so that the molecules in the cytoplasm were not distributed properly (Nengsih et al, 2022)).

In this study, the application of colchicine also had a significant effect on leaf color. The concentration of 6000 ppm gave a darker leaf color, while the control treatment (without colchicine) produced a lighter (less

intense) leaf color. This was proven, that the tendency of the higher the concentration of colchicine given the color of the Dendrobium orchid leaves were getting darker (dark green).

In this study, colchicine application did not significantly affect the age of emergence of new leaves on leaf morphology in this study. Masruroh (2018), stated that the application of colchicine to several varieties did not result in the plants becoming polyploid and caused negative effects such as the longevity of new leaves on Dendrobium orchid plants. The reason is that the response to colchicine given is not always different from that of normal plants, so that the leaves are less sensitive to the chemical. Leaf growth on orchid plants is slow, so the response to the application of chemicals is not always shown phenotypically (Masruroh, 2018). According to (Widiasteoty et al., 2016) the growth of orchid plants is slow and requires a relatively long time.

The application of colchicine on the parameters of the number of new shoots and the number of new new leaves had no significant effect. As a result, colchicine can be morphologically influenced by the number of cells. This was because colchicine only works on cells that are actively dividing, whereas those consisting of many cells in plant parts or tissues do not divide at the same time (Masruroh, 2018). That mean that not all cells in that part of the plant interact with colchicine, but only with cells that are actively dividing. Cells that interact with colchicine were not always in large numbers, because the effect of colchicine on these cells did not appear morphologically, so normal cells were more dominant than cells affected by colchicine (mutant). This was an evidenced in mixoploid plants, where a plant has two different ploidy levels (Masruroh, (2018).

According to Nengsih et al., (2022), the growth in the number of shoots that appear produced the lowest number of shoots. This was because the absorption of colchicine concentrations has not been able to grow optimally, so that the formation of shoots is hampered. The entry of the given colchicine mutation substance did not affect all plant cells, but only cells that were actively dividing and not at the same time, so that due to colchicine treatment, it caused changes in various plants (Aili et al, 2016). In the study of Damayanti dan Mariska (2003). The application of colchicine concentrations causes physiological damage to plants and inhibits the formation of spindle fibers which stops the mitotic process at the metaphase stage. Based on this research, the growth of Dendrobium orchids on leaf morphology with the results of colchicine application requested by the public is an opportunity to improve the quality and character of Dendrobium orchids in terms of length and colour of Dendrobium orchid leaves.

4. CONCLUSION

Colchicine treatment on Dendrobium Venus Purple Algis (ALG) and Dendrobium Transient Pink Frederika (FRD) with various levels of concentration had a significant effect on leaf length, width and color, but the effect of a single type of Dendrobium orchid and its interaction with colchicine treatment had significant effect on leaf morphology. The application of colchicine with a concentration of 5000 ppm produced the longest leaves, the application of the treatment without colchicine (control) produced the widest leaves among the other treatments, while the application of colchicine with a concentration of 6000 ppm produced the most intense leaf color (dark green) compared to some of the colchicine treatments with lower concentrations.

5. ACKNOWLEDGEMENT

We say thanks to the Institute for Research and Community Service (LPPM) Argopuro PGRI University, Jember as the main donor for carrying out this research.

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