



Morphological Character of The Natural Orchid *Phalaenopsis* Spp. Results of Colchicine Polyploidy Induction

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Abstract

The induction of polyploidy in orchids can lead to morphological changes, including larger leaf and flower sizes, longer bloom durations, and more intense flower colors. Morphological characterization is necessary to determine the morphological changes that occur after colchicine treatment. This study aims to determine the morphological characteristics of natural orchids, *Phalaenopsis* spp, following polyploidy induction using colchicine. The research was conducted from November 2021 to February 2022 at the Screen House, Faculty of Agriculture, UNS. The plant materials used included *P. violacea* from Sulawesi, *P. violacea* from Java, and *P. Amabilis* from Ambon. The treatment used was colchicine 1,250 ppm, which was applied to flower buds using the cotton method with 2 replications. The collected data were analyzed using descriptive statistics and a paired t-test in IBM SPSS Statistics 26 to compare the average performance of mutant plants with that of control plants. Induction of polyploidy using 1,250 ppm colchicine resulted in increased leaf length and width, thicker leaves, longer and thicker flower stalks, larger flowers, and enlarged dorsal and lateral sepals and petals in *P. violacea* from Java, *P. violacea* from Sulawesi, and *P. amabilis* from Ambon.

Keywords: Orchid, *P. violacea* Sulawesi, *P. violacea* Java, *P. amabilis* Ambon

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Introduction

Orchids are one of the horticultural plants classified as ornamental plants. Orchids have high economic value due to their high market demand and tend to appreciate (1). Orchids are traded worldwide, both legally and illegally, as cut flowers and whole plants (2). In Indonesia, the demand for orchids continues to rise, alongside the growing number of people who are fond of orchids and have a passion for caring for ornamental plants, especially orchids (3). Orchid production in Indonesia in 2020 is 11.68 million (4). This figure does not meet all market demand and can still be increased by improving cultivation management to meet existing market needs

The diversity of orchids identified worldwide reaches 25,000 species, with 5,000 of them found in Indonesia. This natural orchid species is utilized as a genetic resource for hybridizing orchids (5). One type of orchid that is

popular and widely used as an ornamental plant is the *Phalaenopsis* genus of orchids. This orchid is a monopodial orchid without pseudobulbs, which has short stems and is covered with leaves (6). *P. violacea* is a species belonging to the genus *Phalaenopsis*. This species is divided into two groups, namely the Sumatran *form* and the Malay *form*, based on their distribution. *P. violacea* has flowers with a dominant *rose-pink color* on the entire surface of the sepals and petals; the lateral sepals do not have bow legs, and the apex of the three sepals forms an equilateral triangle. In addition, *P. violacea* has leaves with a width of less than 8 cm (7). Besides *P. violacea*, another well-known species of the *Phalaenopsis* genus is *P. amabilis*, commonly known as the moon orchid. *P. amabilis* has flowers of large size, luxurious appearance, white flowers, and long-lasting flowers that can even reach 2 months. *P. amabilis* has roots with pointed ends, easy to stick, soft, and

easy to break. The stems of *P. amabilis* are monopodial and short, covered with a waxy coating. *P. amabilis* has thick leaves, which are about 3-8 per plant, are elliptical in shape, 15-30 cm long and 8-15 cm wide, and are dark green in color. *P. amabilis* flowers are white and arranged, have 3 lanceolate-shaped petals, 3 circular flower crowns, and 1 ginostemium (8).

Plant breeding is the process of creating or manipulating genetic diversity in plant species which is then selected according to the desired recombination of these variations. One method of plant breeding is through the use of mutations. Mutations can occur naturally, but with the development of science, they can also be induced intentionally to change the inherited characteristics of organisms and pass them on to their offspring. Mutations may result in an appropriately measurable phenotypic change or may not be expressed (9). Polyploidy is the natural process of increasing the number of chromosomes, resulting in organisms with more than two complete sets of chromosomes. Induction of polyploidy (*artificial induction of polyploidy*) began to be carried out as one of the plant breeding programs with mutation induction methods for ornamental plants, especially orchids. The induction of polyploidy in orchids aims to produce flowers with larger sizes, longer bloom durations, sharper colors, and pathogen-resistant plants. Polyploidy induction is generally carried out by applying antimetabolic agents such as colchicine, *oryzalin*, trifluralin, *propyzamide*, and *amiprophos-methyl* (APM) to tissues, organs, or whole plants (10). Colchicine is one of the most widely used mutagens in inducing polyploidy. Colchicine is a mitotic inhibitor that inhibits chromosome segregation during cell division. The application method for colchicine can be done by dipping, soaking, dripping, or using a cotton swab. Plant organs that can be used for polyploidy induction are also diverse, including seeds, apical meristems, flower buds, and roots (11).

Treatment using colchicine can change the character of natural orchids. This change needs to be known to assess the effect of colchicine application on several species of natural orchids. There are several types of characterization, including morphological, physiological, agronomic, and molecular characterization. Morphological characterization is the process of determining the phenotypic character of a plant based on observations of specific plant parts or the whole plant to determine the genetic relationship between several species or varieties (12). This study aims to determine the morphological

characteristics of natural *Phalaenopsis* spp. Orchids. Polyploidy induction using colchicine.

Materials and Methods

The research was conducted from November 2021 to February 2022 at the Screen House, Faculty of Agriculture, UNS. The plant materials used in this study included three varieties of *Phalaenopsis* orchids, namely *P. violacea* from Sulawesi, *P. violacea* from Java, and *P. amabilis* from Ambon. The treatment used was colchicine 1,250 ppm, which was applied to flower buds using the cotton method. Each treatment was repeated 2 times. Parameters observed included leaf length, leaf width, leaf thickness, flower stalk length, flower stalk diameter, number of flower stalks, flower arrangement length, number of florets, flower length, flower width, flower bloom time, dorsal sepal length, dorsal sepal width, dorsal sepal thickness, lateral sepal length, lateral sepal width, lateral sepal thickness, petal length, petal width, and petal thickness. The collected data were analyzed using descriptive statistics and a paired t-test in IBM SPSS Statistics 26 to compare the average performance of mutant plants with that of control plants.

Results and Discussion

Based on the results of the paired t-test, it was found that the colchicine treatment did not show significant differences in any of the observation parameters, including leaves, stems, and flowers.

Leaf Morphological Character

Based on the results of the paired t-test that has been carried out, it shows that the leaf length of *P. amabilis* treated with colchicine was significantly different from that of control *P. amabilis*. In contrast, the leaf lengths of both *P. violacea* from Sulawesi and *P. violacea* from Java were not significantly different. Additionally, no varieties showed significantly different results after treatment with colchicine (Table 1). This occurred because the effectiveness of colchicine application for polyploidy induction was influenced by several factors, including plant variety, colchicine concentration, and treatment duration (13).

Different varieties can give different results. In addition to varietal differences, the concentration of colchicine and the duration of treatment given also had a distinct impact on each array. This is related to the strength of colchicine exposure to different plant tissues. However, colchicine treatment increased the average leaf length and width in the three varieties, while leaf

thickness only increased in *P. amabilis* from Ambon (Table 1). This is in accordance with the statement of (14) that orchids induced by polyploidy with colchicine, which are tetraploid, have leaves that are longer, wider, and thicker, and leaves that are darker green than diploid orchids.

Flower Stalk Morphological Character

Based on the results of the paired t-test, it was found that the colchicine treatment did not show significant differences in all observed parameters across all varieties. Colchicine treatment also only increased the stalk length of *P. violacea* varieties from Sulawesi and *P. violacea*

from Java, stalk diameters in the three varieties, and flower arrangement length in *P. violacea* varieties from Sulawesi and *P. violacea* from Java (Table 1). According to (15), colchicine can increase the size of plant organs, such as leaves, flowers, and fruit. However, it can also cause shortened stalks or a dwarf type, but with a larger stalk diameter. However, the different varieties may affect the reactions that occur in response to colchicine treatment, as they are related to the distinct tissue characteristics of each variety, which in turn influence the exposure strength of colchicine, including the concentration and duration of treatment.

Table 1. Morphological characteristics of polyploidy-induced orchids using colchicine

Parameter	<i>P. violacea</i> Sulawesi		<i>P. violacea</i> Java		<i>P. amabilis</i> Ambon	
	Control	colchicine	Control	colchicine	Control	colchicine
Leaf						
Leaf length (cm)	14.5	18.5	14.7	17	16.15	17.6 ^a
Leaf width(cm)	5	6.4	4.3	4.8	4.3	5.85
Leaf thickness(cm)	0.0605	0.0455	0.0575	0.1515	0.045	0.048
Flower stalk						
Stem length (cm)	19.75	20.8	6.3	8.3	39.25	38
Stem diameter (cm)	0.2095	0.297	0.16	0.219	0.52	0.584
Number of stalks	1	1 ^b	1	1 ^b	1	1 ^b
Flower arrangement length (cm)	3.15	3.45	2.25	3.3	4	3.65
Flower						
Flower length(cm)	4.5	4.7	3.8	4.4	5.05	5.75
Flower Width(cm)	4.5	4.6	3.65	4	6.6	7.2
Number of buds	1	1.5	1	1 ^b	1	1 ^b
Flower bloom time (days)	25	29	25	28 ^b	30	33.5
Dorsal Sepals						
Dorsal sepal length (cm)	1.9	2	1.75	1.85	2.25	2.95
Dorsal sepal width (cm)	1	1 ^b	0.9	1.06	1.15	2
Dorsal sepal thickness (cm)	0.053	0.0935	0.0505	0.062	0.035	0.044 ^b
Lateral Sepals						
Lateral sepal length (cm)	2.2	2.25	1.9	2.3 ^b	2.35	2.7
Lateral sepal width (cm)	1.5	1.2	1.1	1.4 ^b	1.9	2.065
Lateral sepal thickness (cm)	0.05	0.057	0.047	0.0585	0.033	0.04
Petals						
Petal length (cm)	1.75	1.9	1.3	1.55	3.1	3.7
Petals width (cm)	1	1.4	1.1	1.25	3.2	3.65
Petals thickness (cm)	0.0545	0.0575	0.0455	0.056	0.032	0.0375

a) Significantly different from the control based on the results of the T test, $p = 0.05$

b) T-test cannot be performed because the standard error = 0

Flower Morphological Character

Based on the results of the paired t-test, it was found that the colchicine treatment did not show significant differences in all observed parameters across all varieties. However, colchicine treatment was able to increase the average flower length, flower width, and bloom time for all varieties. In contrast, the increase in the number of florets only occurred in *P. violacea* from Sulawesi (Table 1). This is in accordance with the opinion of (16) that polyploidy induction can produce flowers with a larger size, rounder shape, with more intense color, and have a longer bloom time. However, polyploidy induction will not change the yield potential of a plant.



Figure 1. Comparison of control orchids and those treated with colchicine.

Dorsal and Lateral Sepal Morphological Characteristics

Colchicine treatment can increase the length of the dorsal sepal and the thickness of the dorsal sepal in all varieties, as well as the width of the dorsal sepal in *P. violacea* from Java and *P. amabilis* from Ambon. Whereas in lateral sepals, colchicine treatment increased the average lateral sepal length and lateral sepal thickness in all varieties, and lateral sepal width only increased in *P. violacea* from Java and *P. amabilis* from Ambon (Table 1). This is in accordance with the opinion of (17), which stated that autopolyploidy can cause an increase in the size and thickness of the sepals and petals, increase the duration of flowering, and increase fertility.

Petal Morphological Character

Based on the results of the paired t-test, it was found that the colchicine treatment did not show significant differences in all observed parameters across all varieties. However, colchicine treatment can increase the average petal length, petal width,

and petal thickness in all varieties (Table 1). This is in accordance with the opinion of (18), which stated that colchicine treatment could change the morphology and fertility of the treated plants. Specific changes associated with the ploidy level include larger leaves and petals in tetraploid plants.

Conclusion

Induction of polyploidy using 1,250 ppm colchicine increased morphological traits in *P. violacea* from Java, *P. violacea* from Sulawesi, and *P. amabilis* from Ambon. The changes were observed in leaf length and width (measured in cm), leaf thickness (cm), flower stalk length and diameter (cm), flower size (length and width in cm), and the dimensions of dorsal and lateral sepals and petals (cm). In *P. amabilis*, leaf length showed a statistically significant increase compared to the control, whereas in the two *P. violacea* varieties, the changes were not statistically significant. Overall, the magnitude of morphological changes varied among species, indicating that the effects of colchicine-induced polyploidy are species-dependent. These results suggest that colchicine treatment can enhance ornamental traits in orchids; however, the response may vary across species and cultivars, underscoring the need for species-specific optimization in breeding programs.

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