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Increasing the Growth of *Coelogyne rochussenii* Orchid Plantlets by administering various concentrations of KH_2PO_4 and Pyridoxine

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ABSTRACT

Coelogyne is a native Indonesian orchid with relatively large flower sizes and attractive colors. The purpose of this study was to determine the effect of giving concentrations of Potassium Dihydrogen Phosphate (KH2PO4) and Pyridoxine in Murashige And Skoog media on the growth of the *Coelogyne Rochussenii* De Vriese orchid subculture. The research was conducted at the UPT Tissue Culture Laboratory of Seeds of Food Crops, Horticulture, and Plantation, Riau Province, Jalan Kaharudin Nasution, Kelurahan Simpang Tiga, Kecamatan Bukit Raya, Kota Pekanbaru. The research was conducted for four months, from September to December 2022. The design used in this study was a factorial complete randomized design (CRD) consisting of 2 treatment levels, KH2PO4 and Pyridoxine, with three replications, KH_2PO_4:160 mgl, 170 mg/l), 180 mg/l), 190 mg/l, and Pyridoxine: 0 ppm 0.0 5 ppm, 0.10 ppm, 0.15 ppm. The results of the study by administering various concentrations of Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine did not significantly affect all parameters observed in Murashige and Skoog media on the growth of the *Coelogyne Rochussenii* De Vriese orchid subculture.

Keywords: *Coelogyne Rochussenii,* KH_2PO_4*, Pyridoxine, Plantlate, Orchid.*

1. INTRODUCTION

Orchids are one of the many varieties of flora that are pretty popular among admirers of ornamental plants. Orchids belong to the family Orchidaceae, which contains numerous species. According to Wirawan et al. (2021), there are more than 30,000 species and approximately 800 generations within the family Orchidaceae. The Orchidaceae family can be found nearly everywhere in the world, particularly in the tropics, from lowlands to highlands to the frigid edge. The Coelogyne Rochuscensis De Vriese orchid is a member of the Orchidaceae family.

The epiphytic orchid Coelogyne Rochussenii De Vriese is 92 meters above sea level (Heriansyah et al., 2021). The Coelogyne Rochussenii orchid is threatened with local extinction because the vegetation surrounding its host plant has been altered so that it is no longer natural, indicating its habitat is disturbed.

Coelogyne Rochussenii is a rare epiphytic orchid found in the Bukit Rimbang and Bukit Baling Wildlife Reserves (Puspitaningtyas, 2014). Efforts to preserve the Coelogyne Rochuscenii orchid are, therefore, vitally essential. Because forest land conversion threatens the existence of epiphytic orchids. In addition, the tiny size of orchid seeds and the absence of endosperm as a food reserve inhibit the natural germination process. Consequently, modern plant cultivation techniques, such as tissue culture, are utilized to increase orchid growth and preserve their resilience.

Tissue culture or in-vitro culture is a method for isolating plant parts such as cells, tissues, or organs that are grown aseptically on a medium in a controlled environment so that they can proliferate and regenerate into whole plants (Nurcahyani & Kanedi, 2021). Use of fundamental media, macro or micronutrients, and the correct vitamins are crucial for optimal plant growth.

MS media (Murashige & Skoog) is one of the fundamental media varieties used for all plant types in tissue culture techniques. According to Silalahi and Urban (2015), MS media is extensively used for cultivating a variety of plants because it is more complex and contains nearly all of the nutrients required by plants.

Optimal plant growth requires a balance of macro and micro nutrients. Potassium dihydrogen phosphate ($\mathbf{KH_2PO_4}$) is a nutrient that affects explant proliferation in tissue culture techniques. The compound potassium dihydrogen phosphate ($\mathbf{KH_2PO_4}$) contains two macronutrients, phosphorus and potassium. Phosphorus is required to develop active plant elements, including roots, fruits, and tubers. Phosphorus also contributes to the formation of plant sugars or carbohydrates. The formation of plant roots is affected by the presence of potassium ions, sucrose content, and ferrum ions, which influence the application of phosphorus to the medium (Heriansyah, 2020). Potassium (K) is administered to plants to make them resistant to disease; it also stimulates the roots so that they can support the plant's structure (Sugiarto *et al*., 2019)

According to research conducted by Puri et al. (2022), the optimal concentration of KH_2PO_4 for Dendrobium Sonia orchid explants was 180 mg/l MS medium with a leaf diameter of 4.41 mm, a leaf length of 1.16 cm, and a moist weight of 0.10 mg per plantlet.

Rudiyanto et al. (2018) found that administering 170 mg/l of potassium dihydrogen phosphate (KH_2PO_4) significantly affected the stem height, leaf number, and root number of taka plants. While the results of the study by Afrisco et al., (2022) indicating a KH_2PO_4 concentration of 150 mg/l on MS media is the correct concentration to be given to Dendrobium sp orchid explants with a shoot count of 1.50, the optimal KH_2PO_4 concentration is 200 mg/l on MS media.

If all of a plant's demands are met, including its requirement for vitamins, its growth will be maximized. Vitamins are required by plants as catalysts for numerous metabolic processes. Vitamins are used for cell proliferation and differentiation of in vitro-grown cells and tissues (Dwiyani, 2016). Pyridoxine (Vitamin B6) is one vitamin that can be used in in-vitro cultivation.

Pyridoxine is one of the vitamin B6s found in nature. The presence of nicotinic acid and biotin in Pyridoxine can stimulate the germination process. Vitamin B6 is also essential for biochemical reactions, particularly the metabolism of amino acids.

Based on the research results of Smith et al. (2013) it was explained that the administration of pyridoxine vitamins affected the growth and development of Dendrobium laxiflorum orchid seeds in-vitro. Pyridoxine treatment at a low concentration of 0.1 ppm gave the highest seed growth and development percentage of 57.43%.

According to Hapsoro & Yusnita (2018), Vitamins are organic compounds that are enzyme cofactors, some of which are essential for plant metabolic processes. One of the vitamins used in culture media formulations is Pyridoxine.

With the addition of Pyridoxine (vitamin B6) at concentrations of 0 ppm, 0.1 ppm, 0.3 ppm, and 0.5 ppm, Amalia et al. (2013) determined the percentage of seed growth of D. laxiflorum to be 2.48 percent, 57.69 percent, 53.43 percent, and 54.82 percent, respectively. Based on the obtained percentage results, it can be concluded that Pyridoxine stimulated the growth and development of D. laxiflorum seeds. 57.69% is the most significant proportion of growth and development at a concentration of 0.1 ppm

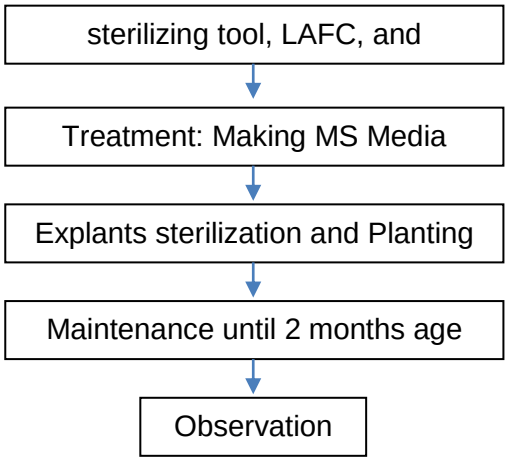
2. MATERIAL AND METHODS

A factorial Completely Randomized Design (CRD) with two factors, KH_2PO_4 and Pyridoxine, was utilized in this study. Each treatment comprised four treatment levels, resulting in a total of sixteen treatment combinations with three replications. Consequently, this investigation included 48 experimental units (bottles). Each experimental element was a bottle containing four explants. The KH_2PO_4 treatment (Factor A) consisted of four concentrations: 160 mg/l, 170 mg/l, 180 mg/l, and 190 mg/l, whereas the Pyridoxine treatment (Factor B) consisted of four concentrations: 0 ppm, 0.05 ppm, 0.10 ppm, and 0.15 ppm.

Laminar Air flow cabinets, measuring cups, beakers, petridishes, pipettes, autoclaves, analytical balances, Erlenmayer, magnetic stirrers, glass stirrers, tweezers, scarpels, spirit lamps, hand sprayers, pH meters, knives, culture bottles, gas burners, volumetric flasks, test tubes, pans, scissors, aluminum foil, and stationery were used in this investigation.

The planting materials (explants) used were two-month-old Coelogyne Rochussenii De Vriese orchids grown at the UPT Tissue Culture Laboratory of Seeds of Food Crops, Horticulture, and Plantation, Riau Province, as well as the chemicals used to produce MS media and the materials required for explant sterilization. The number of shoots (fruits), shoot height (cm), number of leaves (strands), number of roots (fruits), and root length (cm) were observed as parameters.

The research phases are depicted in the accompanying diagram:



3. RESULT AND DISCUSSION

3.1 Number of Shoots (Fruit)

Observations on the number of shoots of Coelogyne Rochussenii revealed that the administration of Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine alone and the interaction of the two had no significant effect on the increase in the number of orchid shoots; the number of orchid shoot results are shown in Table 1.

The data in Table 1 indicates that administering KH_2PO_4 at different concentrations will result in a different number of shoots. Compared to the use of a standard dose of KH_2PO_4 (170 mg/l MS media), the highest number of shoots was observed at a concentration of 180 mg/l, with a difference of 0.09 strands, while the number of shoots decreased at a concentration of 190 mg/l.

The addition of Potassium Dihydrogen Phosphate (KH_2PO_4) to MS media alone had no significant effect on the number of shoots produced by the Coelogyne Rochussenii De Vriese orchid at concentrations of 150 mg/l, 170 mg/l, and 190 mg/l, as determined by Hamzah et al., (2022). In this study, the most significant number of shoots was obtained at a concentration of 180 mg/l MS medium, with a total of 1.56 shoots. The highest number of shoots was obtained at a concentration of 190 mg/l MS medium, with a total of 4.17 shoots.

Table 1. Average Number of Shoots (Fruits) of Coelogyne Rochussenii Orchid with Potassium Dyhydrogen Phosphate (KH_2PO_4) and Pyridoxine in MS Media

KH_2PO_4 Treatment	Pyridoxine Treatment				Average
	0.0 ppm	0,05 ppm	0,10 ppm	0,15 ppm	
160 mg/l	1,44	1,33	1,56	1,44	1,44
170 mg/l	1,44160 mg	1,56	1,56	1,33	1,47
180 mg/l	1,56	1,44	1,67	1,56	1,56
190 mg/l	1,56	1,56	1,44	1,44	1,50
Average	1,50	1,47	1,56	1,44	
KK=	11,81%				

Numbers followed by the same letter in the same column show no significant difference according to the Tukey Advanced Test (BNJ) at the 5% significance level.

Compared to the study of Afrisco et al. (2022), administration of 150 mg/l KH_2PO_4 in MS media produced the highest number of shoots on Dendrobium sp orchid explants, with 1.50 shoots. The higher KH_2PO_4 (180 mg/l) required an additional 30 mg/l to produce 1.56 shoots.

Administration of Pyridoxine alone had no significant effect on the number of branches. With 1.56 shoots, the G2 treatment (Pyridoxine 0.10 ppm) produced more shoots than the other treatments. There is a decrease in the number of seedlings when the dose of vitamins is increased, and the number is even lower compared to the treatment without vitamins (Control).

According to Silalahi (2015), vitamins are organic substances that are cofactors or coenzymes in metabolic processes. Various vitamins, such as Pyridoxine (B6), are added to the medium for optimal growth. Common vitamin concentrations range from 0.1 to 5 mg/l.

The interaction between Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine had no significant effect on the number of orchid shoots, according to the data in Table 1. Even the number of seedlings produced exceeded the effectiveness of a single treatment.

(Supatmi et al., 2007) The KH_2PO_4 compound serves as a source of macronutrients required by plants in large quantities. There is no singular effect and interaction between the administration of Potassium Dihydrogen Phosphate and Pyridoxine because the interval or distance between doses between treatments is too short; consequently, no significant results are achieved.

3.2. Plant Height (cm)

Observations on the height of the shoots of Coelogyne Rochussenii orchids with KH_2PO_4 and Pyridoxine either singly or in interaction did not significantly affect the growth of plant shoot height.

Table 2. Average Shoot Height (cm) of Coelogyne Rochussenii Orchids treated with Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine in MS Media

KH_2PO_4 Treatment	Pyridoxine Treatment				Average
	0.0 ppm	0,05 ppm	0,10 ppm	0,15 ppm	
160 mg/l	1,13	1,23	1,14	1,18	1,17
170 mg/l	1,21	1,17	1,27	1,21	1,21
180 mg/l	1,22	1,23	1,27	1,20	1,23
190 mg/l	1,19	1,22	1,21	1,22	1,21
Average	1,19	1,21	1,22	1,20	
KK=	6,42%				

Numbers followed by the same letter in the same column show no significant difference according to the Tukey Advanced Test (BNJ) at the 5% significance level.

Providing a lower concentration of Dihydrogen Phosphate (KH_2PO_4) (160 mg/l) than the standard dose (170 mg/l) will result in a 0.04 cm decrease in shoot height, whereas increasing the concentration to 180 mg/l will increase shoot height. This indicates that a concentration of 180 mg/l is more optimal for stimulating the growth of orchid shoots' height.

Silalahi (2015) states that element P is required to stimulate growth in meristematic tissue as an enzyme activator. A surplus of element P can inhibit the growth of explants due to absorption competition with other elements.

Comparing the results of this study with those of Rudiyanto et al. (2018), the administration of 170 mg/l KH_2PO_4 affected the height of the Taka leontopetaloides plant's shoots. In contrast, the highest orchid plant shoots were found at a dose of 180 mg/l KH_2PO_4. This demonstrates that 10 mg/l MS media containing the orchid Coelogyne Rochuscenii requires a higher KH_2PO_4 concentration than the standard dose.

According to Table 2, the interaction between Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine did not significantly affect the height of the orchid shoots. With a shoot height of 1.27 cm, the C3G2 treatment combination (KH_2PO_4 180 mg/l and Pyridoxine 0.10 ppm) produced the highest average value compared to the other treatment combinations. This outcome exceeded that of the single treatment. indicating that the combination of KH_2PO_4 and Pyridoxine would be more effective for stimulating the height growth of Coelogyne Rochussenii orchid, despite the fact that there was no significant difference between the regimens.

3.3. Number of Leaves (strands)

The administration of Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine alone or in combination had no significant effect on the number of leaves of the Coelogyne rochusseni orchid plant (Table 3).

The administration of Potassium Dihydrogen Phosphate (KH_2PO_4) at varying concentrations produced a variety of leaves, with the administration of 180 mg/l KH_2PO_4 into MS media producing a greater number of leaves than the other three treatments, with a difference of 0.04 leaves compared to the standard dose of KH_2PO_4 on MS media (170 mg / l). This indicates that this quantity of KH_2PO_4 produced the greatest number of leaves, even when compared to the higher dose (190 mg/l). According to Nurwahyuni (2015), the standard dose of KH_2PO_4 in Murashige and Skoog (MS) media is 170 mg/l. However, this study required a higher dose to generate more Coelogyne Rochussenii orchid leaves.

Table 3. Average Number of Leaves (strands) of Coelogyne Rochussenii Orchid with Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine in MS Media

KH_2PO_4 Treatment	Pyridoxine Treatment				Average
	0.0 ppm	0,05 ppm	0,10 ppm	0,15 ppm	
160 mg/l	4,00	4,11	4,11	4,11	4,08
170 mg/l	4,11	4,22	4,11	4,00	4,11
180 mg/l	4,00	4,11	4,22	4,22	4,14
190 mg/l	4,00	4,00	4,22	4,00	4,06
Average	4,03	4,11	4,17	4,08	
KK=	7,11%				

Numbers followed by the same letter in the same column show no significant difference according to the Tukey Advanced Test (BNJ) at the 5% significance level.

Comparing the findings of this study to those of Puri et al., (2022), it was determined that 180 mg/l of KH_2PO_4 was the optimal concentration for the growth of 4.41 strands of Dendrobium sonia leaves. In this study, the same concentration produced the greatest number of leaves, but there were only 4.14 of them. This can be attributed to the use of various orchid species, whose growth responses vary.

The administration of Pyridoxine alone at varying concentrations did not substantially affect the number of orchid leaves. However, the G2 (Pyridoxine 0.10 ppm) treatment produced a greater number of leaves, 4.17 in total. As with the G3 treatment, the number of leaves decreased when the pyridoxin dose was increased.

According to Ritonga (2011), plants use the vitamin pyridoxine as an antioxidant. Thus, it can be stated that this vitamin aids in maximizing plant growth.

Adding KH_2PO_4 and Pyridoxine had no discernible effect on the number of leaves. However, the C3G2 treatment (180 mg/l KH_2PO_4 and 0.10 ppm Pyridoxine) produced a greater number of leaves, specifically 4.22 leaves. The combination treatment is superior to the single treatment because the number of leaves produced is increased.

3.4 NUMBER OF ROOTS (fruit)

The number of roots of the Coelogyne rochusseni orchid plant was not significantly affected by the administration of Potassium Dihydrogen Phosphate (KH_2PO_4), Pyridoxine alone, or the combination of the two. For more comprehensive data, see Table 4.

Table 4. The average number of roots (fruits) of Coelogyne Rochussenii orchids by administering Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine in MS Media

KH_2PO_4 Treatment	Pyridoxine Treatment				Average
	0.0 ppm	0,05 ppm	0,10 ppm	0,15 ppm	
160 mg/l	2,11	2,33	2,33	2,22	2,25
170 mg/l	2,22	2,33	2,44	2,22	2,31
180 mg/l	2,22	2,11	2,33	2,22	2,22
190 mg/l	2,22	2,22	2,44	2,22	2,28
Average	2,19	2,25	2,39	2,22	
KK=	10,47%				

Numbers followed by the same letter in the same column show no significant difference according to the Tukey Advanced Test (BNJ) at the 5% significance level.

At a KH_2PO_4 concentration of 170 mg/l, a single treatment of adding Potassium Dihydrogen Phosphate (KH_2PO_4) to the MS medium used to cultivate the Coelogyne rochusseni MS orchid produced 2.31 times more roots. It differs from the previous parameters (shoot height, number of shoots, and number of leaves) in that the standard dosage of KH_2PO_4 for MS media is required for maximum root growth.

These results differ from those of Afrisco et al. (2022), who used a higher concentration of Potassium Dihydrogen Phosphate (KH_2PO_4) to produce 3.14 explants of Dendrobium Sp orchids in MS media (190 mg/l). In contrast, the dose administered in this study was 20 mg/l (170 mg/l) with 2.31 roots.

Treatment with varying concentrations of Pyridoxine alone had no significant effect on the number of roots. However, the G2 (Pyridoxine 0.10 ppm) treatment generated the greatest number of roots, with 2.39. If the dose of Pyridoxine is increased, the number of roots will decrease (G3). This indicates that excessive quantities of Pyridoxine can inhibit the growth of the roots of the Coelogyne Rochussenii orchid.

According to Widiastoety et al. (2015), vitamins play a role in the development process as catalysts in metabolic processes.

Vitamins are used for cell proliferation and differentiation of in vitro-grown cells and tissues (Dwiyani, 2016). Vitamins can reduce stress on explants, enhance growth, and reduce abnormal aggregate growth in cell suspension cultures, which are all advantages (Sulichantini, 2015).

The interaction between the administration of Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine at varying doses did not significantly affect the number of orchid plant roots. However, the combination in the C2G2 treatment that produced the greatest number of roots was 170 mg/l KH_2PO_4 and 0.10 ppm Pyridoxine, which produced 2.44 pods.

The P nutrient in a compound plays an essential role in plant growth and production due to its function (Wicaksono et al., 2019). Pyridoxine, meanwhile, functions as a vitamin to reduce stress, stimulate root growth, and optimize plant metabolism, and it can be used as a tonic to stabilize plant growth (Kholifah, 2022). Therefore, combining the two will result in greater growth than using them separately.

3.4 Root Length(cm)

The results of data analysis on the root length of the Coelogyne Rochusseni orchid plants showed that the administration of Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine alone, and the interaction between the two did not significantly affect the root

length of the orchid plants. Data analysis results can be seen in Table 5.

The best root length for the orchid Coelogyne Rochusseni growth was found in the addition of 160 mg/l KH_2PO_4, which was 1.48 cm. This shows that to stimulate the growth of root length, the dose of Potassium Dihydrogen Phosphate (KH_2PO_4) given is lower than the standard dose (170 mg/l) even though the difference is small, 0.02 cm.

This study's results were compared with those of a study by Hamzah et al. (2022). The highest yield of root length was given a KH_2PO_4 concentration of 190 mg/l MS medium with a root length of 1.24 cm, different from the results obtained in this study where the amount of KH_2PO_4 given was lower (160 mg/l) but was able to produce longer root length of 1.48 cm.

Data on tables. The effects of administering Pyridoxine alone were not significantly different. However, the treatment that generated the longest roots was G2 (Pyridoxine 0.10 ppm), which measured 1.48 cm.

According to Nurwahyuni (2015), the typical Pyridoxine concentration in MS media (Murashige and Skoog) is 0.05 ppm. While the dose in this study was 0.10 ppm (0.05 ppm) higher, it was not statistically significant.

Using information in table 5, the combination of Potassium Dihydrogen Phosphate (KH2PO4) and Pyridoxine did not substantially affect root length. However, the treatment combination, that generated the greatest root length was C1G2, at 1.50 cm. Where C1 (KH_2PO_4 160 mg/l) is the treatment, and G2 (Pyridoxine 0.10 ppm) is the control.

Silalahi (2015) states phosphorus (P) is an indispensable plant nutrient. According to Widiastoety et al. (2015), adding vitamins to the culture medium can stimulate the growth of orchid plant tissues and organs, including the roots.

Table 5. Average Root Length (cm) of Orchid Coelogyne Rochussenii with Potassium Dihydrogen Phosphate (KH_2PO_4) and Pyridoxine in MS Media

KH_2PO_4 Treatment	Pyridoxine Treatment				Average
	0.0 ppm	0,05 ppm	0,10 ppm	0,15 ppm	
160 mg/l	1,44	1,48	1,50	1,49	1,48
170 mg/l	1,46	1,49	1,44	1,47	1,46
180 mg/l	1,44	1,46	1,47	1,43	1,45
190 mg/l	1,47	1,44	1,49	1,49	1,47
Average	1,45	1,47	1,48	1,47	
KK=	4,30%				

Numbers followed by the same letter in the same column show no significant difference according to the Tukey Advanced Test (BNJ) at the 5% significance level.

Overall, the Potassium Dihydrogen Phosphate and Pyridoxine treatments, both alone and in interactions, had no significant effect due to the use of dosages with insufficient intervals between treatments, rendering the results insignificant. In general, this study reveals that increasing the number of shoots, shoot height, and number of foliage results in plant growth. Potassium Dihydrogen Phosphate must be added at a larger concentration, whereas a lower concentration is required for the growth of root number and root length. For the pyridoxine treatment alone, you can use a media concentration of 0.1 mg/l MS, which produces consistent data for all treatments. Therefore, this concentration is recommended for promoting the growth of Coelogyne Rochusseni orchid explants.

CONCLUSION

Giving various concentrations of Potassium Dihydrogen Phosphate (KH_2PO_4¬) and Pyridoxine alone or in interaction did not significantly affect the explants of Coelogyne Rochussenii orchids.

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