

Optimizing Tissue Culture Growth of Black Orchid (*CoelogynePandurata L.*) Using Fertilizer and Natural Organic Supplements

ABSTRACT

This research aims to determine the growth of black orchids (*Coelogynepandurata* Lindley) on fertilizer plus several types of organic supplements and to determine the type of media that has the best influence on black orchid tissue culture. The research was conducted in March 2023 at the Tissue Culture Laboratory, Faculty of Agriculture, Mulawarman University. The research was carried out using the Completely Randomized Design (CRD) method with a single factor arranged using 8 treatments, each treatment repeated five times. The eight treatments consisted of $p_1 = \text{Growmore } 1 \text{ g L}^{-1}$, $p_2 = \text{Growmore } 1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1}$, $p_3 = \text{Growmore } 1 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1}$, $p_4 = \text{Growmore } 1 \text{ g L}^{-1} + \text{sweet corn seed extract } 100 \text{ g L}^{-1}$, $p_5 = \text{Growmore } 1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1}$, $p_6 = \text{Growmore } 1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1} + \text{sweet corn seed sprout extract } 100 \text{ g L}^{-1}$, $p_7 = \text{Growmore } 1 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1} + \text{corn seed extract sweet } 100 \text{ g L}^{-1}$, and $p_8 = \text{Growmore } 1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1} + \text{sweet corn seed extract } 100 \text{ g L}^{-1}$. The data was analyzed using variance and continued with the DMRT test (Duncan Multiple Range Test) with a level of 5%. The results of the research show that the addition of Ambon banana extract, mung bean sprouts, or sweet corn seeds to foliar fertilizer can improve the culture of black orchids using tissue culture. The addition of sweet corn seed extract to the media was able to produce the highest number of leaves obtained in the p_4 treatment (Growmore $1 \text{ g L}^{-1} + \text{sweet corn seed extract } 100 \text{ g L}^{-1}$) namely 6.20 leaves and the p_6 treatment (Growmore $1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1} + \text{sweet corn seed extract } 100 \text{ g L}^{-1}$) produces 6.40 leaves. The addition of mung bean sprout extract produced the highest number of roots, namely in the p_3 treatment (Growmore $1 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1}$) and p_5 (Growmore $1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1} + \text{extract mung bean sprouts } 100 \text{ g L}^{-1}$) as many as 13.00 pieces and the longest root length in treatment p_5 (Growmore $1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1}$) namely 7, 08 cm. The best media was found in treatment p_5 (Growmore $1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1}$) producing the highest plant height (5.02 cm), the highest number of roots (13.00 pieces), the longest root length (7.02 cm) and the highest wet weight (0.45 g).

Keywords: black orchid, tissue culture, corn, mung bean sprouts, banana

1. INTRODUCTION

Indonesia has many types of plants, including epiphytic plants. One of them is a type of orchid which is estimated to have more than 5000 orchid species. Indonesia, which is rich in orchid plants, is very profitable because it is supported by the suitability of the climate and the many potential types of orchids. Indonesia is a country with the second largest level of orchid germplasm wealth after Brazil. Many of these orchid species are endemic to Indonesia (Astarini et al., 2015).

Orchids are members of the Orchidaceae family, which is the largest group of flowering plants. Orchidaceae itself consists of 30,000 species and approximately 800 different genera. Orchid plants can be found almost everywhere in the world, especially in tropical areas from the lowlands to the highlands, even in snowy border areas (Salisbury F. B, et al, 1992).

Indonesian orchid species are unique and only found on certain islands, for example, the black orchid which is only found in Kalimantan. One type is *Coelogynepandurata* Lindl. With the common name black orchid (Pranata, 2005; Adi et al., 2014).

Currently, the population of black orchids is very declining and is even threatened with extinction because black orchids are in great demand by the public so their existence in nature is threatened due to excessive harvesting. Another factor causing the decline in the presence of black orchids is external factors in the form of damaged growing habitat due to logging and land conversion (Dwiyani et al., 2022). Internal factors such as the flowering period are very short (wither quickly) and the flowers are relatively difficult to cross (Fadelah, 2006).

This problem can be overcome by orchid propagation techniques through tissue culture (Zakiah & Turnip, 2023). Efforts to save and propagate black orchids using tissue culture techniques were chosen because this technique has several special advantages, namely rapid multiplication of explants, genetic uniformity, pathogen-free aseptic conditions, plant selection, microplant stock that can be reproduced at any time, controlled environment, preserving germplasm, producing plants throughout the year, and multiplying plants that are difficult to propagate conventionally vegetatively (Zulkarnain, 2011).

Using tissue culture techniques, we can carry out various efforts to preserve and develop orchids. Tissue culture has proven to be very useful for certain groups of plants that are difficult to propagate using conventional techniques (Fay, M.E., 1994). The main use of tissue culture is to obtain new plants in large quantities, in a relatively short time, which have the same physiological and morphological characteristics as the parent plant. It is hoped that this tissue culture technique can produce superior new plants (Von-Arnold S, et al, 2002)

Media in tissue culture is an important factor determining the success of tissue culture propagation. The planting medium must contain all the substances needed for the growth and development of an explant. Increasing the production of black orchids using tissue culture techniques qualitatively and quantitatively, can be done by modifying the media by adding complex organic materials to optimize growth.

Foliar fertilizer is widely traded in the community and organic materials are easily obtained so they are widely used as substances that can stimulate the growth of explants. The use of foliar fertilizer and organic materials used in basic media can encourage the growth of black orchid explants because they contain macro and micronutrients, sources of minerals, vitamins, amino acids, and growth regulators (Hasanah, et al, 2014) Based on the description Therefore, further observations were carried out to determine the growth and development of one-year-old black orchid (*Coelogyne pandurata* Lindley) plant explants using tissue culture on organic material media.

2. MATERIALS AND METHOD

2.1. Time and Place

The research was carried out in March 2023. Located at the Tissue Culture Laboratory, Faculty of Agriculture, Mulawarman University.

2.2. Materials and Tools

The materials used in the observations were 1-year-old black orchid plant explants on agar planting media with the addition of leaf fertilizer and organic material extract from ambon banana, mung bean sprout, and sweet corn.

The tools used in the research were a Laminar Air Flow Cabinet (L AFC), autoclave, analytical balance, microscope, culture bottle, erlenmeyer, beaker glass, petri dish, pan, stove, oven, bunsen, tweezers, scalpel, scissors, hand sprayer, rack culture. Meanwhile, the tool used during the observation was a microscope.

2.3. Research Design

The research was conducted using the Completely Randomized Design (CRD) method with a single factor using eight treatments, each treatment repeated five times. The eight treatments consist of $p_1 = \text{Growmore } 1 \text{ g L}^{-1}$; $p_2 = \text{Growmore } 1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1}$; $p_3 = \text{Growmore } 1 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1}$; $p_4 = \text{Growmore } 1 \text{ g L}^{-1} + \text{sweet corn kernel extract } 100 \text{ g L}^{-1}$; $p_5 = \text{Growmore } 1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1}$; $p_6 = \text{Growmore } 1 \text{ g L}^{-1} + \text{Ambon banana extract } 100 \text{ g L}^{-1} + \text{sweet corn seed extract } 100 \text{ g L}^{-1}$; $p_7 = \text{Growmore } 1 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1} + \text{sweet corn kernel extract } 100 \text{ g L}^{-1}$; and $p_8 = \text{Growmore } 1 \text{ g L}^{-1} + \text{ambon banana extract } 100 \text{ g L}^{-1} + \text{mung bean sprout extract } 100 \text{ g L}^{-1} + \text{sweet corn seed extract } 100 \text{ g L}^{-1}$.

2.4. Research Procedures

1. Explant preparation

The planting material used for this research was black orchid explants (*Coelogyne pandurata* L.) resulting from a year-old tissue culture.

2. Plant Maintenance

The culture bottles are placed on the culture rack in the incubation room with lighting using fluorescent lamps. Room conditions are set at a temperature of 25-28°C using air conditioning. Cleanliness of the culture room is carried out using disinfectants. Culture bottles were sterilized using a hand sprayer containing 70% alcohol.

2.5. Parameters

1. Increase in plant height

Plant height data was collected on orchid plants that were one year old after planting in tissue culture media. Plant height is measured from the base of the stem to the longest leaf.

2. Number of leaves

The leaves that are counted are those that have opened completely. Observations of the number of leaves were obtained by counting the entire number of leaves at the time of observation.

3. Number of shoots

Data on the number of shoots was obtained by counting all the number of shoots growing at the time of observation. The shoot criteria are calculated when the newly emerged shoot has a small protrusion on the surface of the explant that points upwards.

4. Number of roots

Calculation of the number of roots is done by counting the number of all roots formed. The roots that are counted are roots that have grown at least 1 cm.

5. Root length

Root length data was obtained by measuring the longest root from the base of the root to the tip of the root.

6. Plant wet weight

Plant wet weight data was taken by weighing fresh plants using a digital scale.

2.6. Analysis Data

The data obtained will be analyzed using variance, to compare the two treatment averages followed by the DMRT test at the 5% level.

3. RESULTS AND DISCUSSION

3.1. Plant height

The results of further analysis using DMRT 5% of plant height aged 48 weeks after inoculation, some treatments showed plant height values were significantly different from plant height in treatment p_5 , namely p_2 , p_3 , p_4 , p_5 , p_6 , p_7 , and p_8 while treatments p_2 , p_3 , p_4 , p_6 , p_7 , and p_8 are not significantly different. The highest plant height data was from treatment p_5 with an average value of 5.02 cm, while the lowest data was from treatment p_1 with an average value of 1.86 cm.

Table 1. The average height of black orchid plants in each treatment medium (cm)

Treatments	Replication					Average (cm)
	1	2	3	4	5	
p_1	2,70	1,90	1,80	1,70	1,20	1,86 ^a
p_2	2,70	2,50	2,80	2,50	1,50	2,40 ^{ab}
p_3	5,70	3,40	2,70	2,40	2,00	3,24 ^{ab}
p_4	3,40	3,00	3,50	2,00	2,30	2,84 ^{ab}
p_5	7,20	6,00	2,80	5,60	3,50	5,02 ^c
p_6	4,40	5,40	3,00	3,00	2,30	3,62 ^b
p_7	4,20	3,00	3,40	3,30	1,60	3,10 ^{ab}
p_8	4,60	4,10	2,60	2,40	1,60	3,06 ^{ab}

Note: Numbers followed by the same letter indicate that they are not significantly different in the 5% DMRT test

Based on the results of the analysis of variance and further tests, DMRT 5% showed a real effect on plant height, meaning that the composition of the media contained therein was able to stimulate the plant height obtained. The use of Growmore leaf fertilizer 1 g L^{-1} and the addition of organic materials such as ambon banana extract and mung bean sprout extract (p_5) with a concentration of 100 g L^{-1} for each organic material is the best treatment combination and has a big influence on height growth. Black orchid culture plants. Compared with previous research which showed that p_8 treatment (Growmore 1 g L^{-1} + ambon banana extract 100 g L^{-1} + mung bean sprout extract 100 g L^{-1} + sweet corn seed extract 100 g L^{-1}) showed high explant value. The best with an average of 4.00 mm .

Based on previous research, the results of the 5% DMRT test when compared with observations of cultures that were one year old, there was a difference in the results of the treatment given to black orchid cultures as in p_1 in the previous study, the average explant height was 2.75 mm (0.27 cm).) whereas in culture observations at one year of age the height growth was an average of 1.86 cm , in p_2 of the previous study it was 1.63 mm (0.16 cm) and at one year of age the average height was 2.40 cm , in p_3 of the study previously 3.63 mm (0.36 cm) and at the age of one year the average height was 3.24 cm , in p_4 previous research it was 2.25 mm (0.22 cm) and at the age of one year the average height was 2.84 cm , in p_5 of the previous study the increase in culture height was an average of 3.63 mm (0.36 cm) while in cultures that were one year old the height growth was an average of 5.02 cm , in p_6 of the previous observation the increase in culture height an average of 2.25 mm (0.22 cm) while in observations of one year old cultures the increase in height was an average of 3.62 cm , in p_7 previous studies the increase in culture height was an average of 3.00 mm (0.3 cm) while the increase the average height of one year old cultures is 3.10 cm , in p_8 previous studies the increase in culture height was an average of 4.00 mm (0.4 cm) while in observations of one year old cultures the average height increase was 3.06 cm . A comparison of these data shows that there is a significant increase in the growth of one-year-old cultures on foliar fertilizer and organic material media.

3.2. Number of Leaves

The results of further analysis using DMRT 5% of the number of leaves were treatments that showed the value of the number of leaves was significantly different from the number of leaves in treatment p_1 (control) as a comparison. These treatments are p_2 , p_4 , p_6 , and p_7 , but are not significantly different from treatments p_3 , p_5 , and p_8 . The highest number of leaf data is the p_6 treatment with an average value of 6.40 pieces, while the lowest data is p_8 with an average value of 2.40 pieces.

Table 2. The average number of black orchid leaves in each treatment medium

Treatments	Replication					Average (Leaves)
	1	2	3	4	5	
p_1	3,00	4,00	3,00	3,00	2,00	3,00 ^{ab}
p_2	5,00	5,00	4,00	6,00	4,00	4,80 ^{cde}
p_3	4,00	2,00	3,00	7,00	2,00	3,60 ^{abc}
p_4	7,00	7,00	5,00	6,00	6,00	6,20 ^{ef}
p_5	4,00	4,00	4,00	4,00	4,00	4,00 ^{bcd}
p_6	6,00	6,00	8,00	6,00	6,00	6,40 ^f
p_7	7,00	5,00	5,00	6,00	4,00	5,40 ^{def}
p_8	3,00	3,00	2,00	2,00	2,00	2,40 ^a

Note: Numbers followed by the same letter indicate that they are not significantly different in the 5% DMRT test

Observing the number of leaves is very necessary to see how much the growth process is occurring, such as in the formation of plant biomass, the more leaves that appear on the explant, meaning the explant growth is better. Based on the results of the analysis of variance and further tests, DMRT 5% showed a real effect. Further analysis of 5% DMRT revealed that there were treatments that

showed significantly different leaf number values from treatment p₁ (control) as a comparison, namely p₂, p₄, p₆, and p₇.

Based on previous research, the DMRT test results were 5% when compared with observations of cultures that were one year old, there was a difference in the results of the treatment given to black orchid cultures as in p₁ in the previous study, the average number of leaves was 1.25 pieces, whereas in observations of cultures with age. one year the number of leaves increases by an average of 3.00 pieces, in p₂ of the previous study the increase in the number of leaves is an average of 1.00 pieces while in one year old cultures the increase in the number of leaves is an average of 4.80 pieces, in p₃ the previous study the increase the average number of leaves was 1.75 pieces and when the culture was one year old the number of leaves increased by an average of 3.60 pieces, in p₄ of the previous study the increase in the number of leaves was an average of 1.75 pieces and in the one year old culture the number of leaves increased an average of 6.20 pieces, in p₅ of the previous study the number of explant leaves increased by an average of 2.00 pieces, while in one year old cultures the number of leaves increased by an average of 4.00 pieces, in p₆ previous observations the number of leaves increased The average number of explants was 2.00 pieces, whereas in observations of one year old cultures the number of leaves increased by an average of 6.40 pieces, in p₇ of the previous study the number of explant leaves increased by an average of 2.00 leaves while the number of leaves in one year old cultures increased. The year had an average of 5.40 pieces, in p₈ previous studies the increase in the number of culture leaves was an average of 2.00 pieces, while in observations of one-year-old cultures, the increase in the number of leaves was an average of 2.40 pieces. This data comparison shows that there is a significant increase in the growth of one-year-old cultures on foliar fertilizer and organic material media.

3.3. Number of shoots

The results of further analysis using DMRT 5% of the number of shoots aged one year after inoculation were treatments that showed the value of the number of shoots was significantly different from the number of shoots in the p₂ treatment. These treatments are p₇ and p₈, while p₁, p₃, p₄, p₅, and p₆ are not significantly different. The highest data on the number of shoots was the p₇ treatment with an average value of 2.92 shoots, while the lowest data was the p₂ treatment with an average value of 1.18 shoots.

Table 3. Average number of black orchid shoots (shoots). Data were analyzed using the transformation results $\sqrt{(x+0.5)}$

Treatments	Replication					Average (shoots)
	1	2	3	4	5	
p ₁	3,81	2,55	1,22	2,74	0,71	2,21 ^{ab}
p ₂	0,71	1,22	2,55	0,71	0,71	1,18 ^a
p ₃	0,71	2,12	2,12	2,12	4,30	2,27 ^{ab}
p ₄	1,87	1,22	0,71	0,71	1,87	1,28 ^a
p ₅	1,58	1,87	0,71	2,55	2,74	1,89 ^{ab}
p ₆	2,35	0,71	0,71	2,55	2,55	1,77 ^{ab}
p ₇	3,24	2,55	2,55	3,54	2,74	2,92 ^b
p ₈	2,74	2,55	2,55	4,74	1,87	2,89 ^b

Note: Numbers followed by the same letter indicate that they are not significantly different in the 5% DMRT test

Observing the number of shoots is an important parameter in tissue culture, because the more shoots that are formed, the greater the opportunity to obtain seeds in large numbers. The results of the analysis of variance and further tests of DMRT 5% black orchid culture showed a real effect. Further analysis of 5% DMRT revealed that there were treatments that showed significantly different shoot number values from the p₂ treatment, namely in the p₇ and p₈ treatments.

Treatment p₇ (Growmore 1 g L⁻¹ + mung bean sprout extract 100 g L⁻¹ + sweet corn seed extract 100 g L⁻¹) was the media composition that produced the highest number of shoots. Mung bean sprouts and sweet corn seeds each contain auxin and cytokinin PGRs so they will stimulate shoot formation. Apart

from that, the natural ZPT content in the form of auxin groups such as IAA and IBA which are found in ambon bananas and mung bean sprouts, and cytokinin group ZPTs which are found in ambon bananas, mung bean sprouts, and corn seeds can influence the growth and development of explants, especially for shoot growth.

3.1.4. Number of roots

Based on the results of the analysis of various applications of Growmore fertilizer combined with several organic materials, it showed a significant effect on the number of roots at the age of one year. The average number of black orchid roots at the age of one MSI is presented in Table 4.

Table 4. Average number of black orchid roots (roots)

Treatments	Replication					Average (shoots)
	1	2	3	4	5	
P ₁	8,00	1,00	5,00	5,00	2,00	4,20 ^a
P ₂	7,00	7,00	14,00	9,00	5,00	8,40 ^{abc}
P ₃	15,00	14,00	10,00	20,00	6,00	13,00 ^c
P ₄	5,00	9,00	5,00	13,00	7,00	7,80 ^{abc}
P ₅	12,00	12,00	9,00	24,00	8,00	13,00 ^c
P ₆	15,00	8,00	9,00	10,00	6,00	9,60 ^{abc}
P ₇	10,00	5,00	14,00	19,00	10,00	11,60 ^{bc}
P ₈	7,00	9,00	3,00	8,00	8,00	7,00 ^{ab}

Note: Numbers followed by the same letter indicate that they are not significantly different in the 5% DMRT test

The results of further analysis using DMRT 5%, the number of roots aged one MSI after inoculation, and some treatments showed the number of shoots was significantly different from the number of shoots in treatment p₁ (control) as a comparison, namely p₃ and p₅, whereas with treatments p₂, p₄, p₆, p₇ and p₈ not significantly different. The highest number of root data was in the p₃ and p₅ treatments with an average value of 13.00 pieces, while the lowest data was in the p₁ treatment with an average value of 4.20 pieces.

Observing the number of plant roots indicates how wide the plant's reach is in absorbing nutrients, so the more roots there are, the wider the plant's reach and the more nutrients it can absorb. The results of the analysis of variance and further tests of DMRT 5% black orchid culture showed a real effect. Further analysis of 5% DMRT revealed that there were treatments that showed significantly different root number values from treatment p₁ (Control) as a comparison, namely p₃ and p₅.

Media composition p₃ (Growmore 1 g L⁻¹ + mung bean sprout extract 100 g L⁻¹) and p₅ (Growmore 1 g L⁻¹ + ambon banana extract 100 g L⁻¹ + sweet corn seed extract 100 g L⁻¹) produce the largest number of roots, meaning that the media and plants contained in the bottle can interact well so that they can induce growth in the number of roots produced. Banana extract, sweet corn seeds, and mung beans contain the growth regulators auxin and cytokinin which function as stimulants of tissue proliferation, facilitating metabolism and respiration. Apart from that, it also contains carbohydrates which are the basic ingredients for producing energy in the respiration process and materials for the formation of new cells (Bey Y., Syafii W., and Sutrisna. 2006)

3.5. Root length

Based on the results of the analysis of various applications of Growmore fertilizer combined with several organic materials, it showed no significant effect on root length at the age of one MSI. The average root length of black orchids at one MSI is presented in Table 5. The highest root length data is the p₅ treatment with an average value of 7.08 cm while the lowest data is the p₄ treatment with an average value of 3.48 cm

Table 5. Average root length (cm)

Treatments	Replication					Average (cm)
	1	2	3	4	5	
p ₁	3,90	3,30	5,00	7,80	2,60	4,52 ^b
p ₂	4,30	3,00	7,20	4,30	5,20	4,80 ^c
p ₃	7,40	10,00	3,90	7,80	3,80	6,58 ^e
p ₄	2,80	5,80	2,80	2,80	3,20	3,48 ^a
p ₅	10,40	7,60	4,50	8,90	4,00	7,08 ^f
p ₆	5,60	5,20	5,70	4,00	4,00	4,90 ^c
p ₇	4,20	4,60	7,40	2,70	3,20	4,42 ^b
p ₈	6,10	7,50	7,00	6,00	4,50	6,22 ^d

Note: Numbers followed by the same letter indicate that they are not significantly different in the 5% DMRT test

Based on the results of the analysis of variance, shows that there is no significant effect on the root length parameter. It is suspected that the composition of the media contained can change the ratio of exogenous and endogenous growth regulators to be unbalanced so that it can inhibit the growth of black orchid culture root length.

The results of the research showed that the longest root length was found in the p₅ treatment with an average value of 7.08 cm. Ambon banana extract and mung bean sprouts contain a lot of the hormone auxin which encourages plant root growth. Root length is the result of the extension of the cells behind the tip meristem (Dewi IR. 2007)

3.6. Wet Weight Plant

The results of further analysis using 5% DMRT of black orchid explants aged one MSI showed that the wet weight value was significantly different from treatment p₁ (control) as a comparison. The treatments are p₃ and p₅, while the treatments p₂, p₄, p₆, p₇ and p₈ are not significantly different. The heaviest wet weight data is treatment p₅ with an average value of 0.45 g, while the lowest data is treatment p₁ with an average value of 0.07 g.

Table 6. Average wet weight (g)

Treatment	Replication					Average (g)
	1	2	3	4	5	
p ₁	0,13	0,07	0,06	0,09	0,03	0,07 ^a
p ₂	0,10	0,08	0,28	0,11	0,07	0,13 ^{ab}
p ₃	0,34	0,41	0,17	0,28	0,26	0,29 ^{bc}
p ₄	0,27	0,15	0,05	0,12	0,11	0,14 ^{ab}
p ₅	0,71	0,36	0,09	0,92	0,16	0,45 ^c
p ₆	0,38	0,25	0,15	0,20	0,14	0,23 ^{abc}
p ₇	0,30	0,08	0,36	0,39	0,24	0,27 ^{abc}
p ₈	0,40	0,08	0,13	0,39	0,07	0,21 ^{ab}

Note: Numbers followed by the same letter indicate that they are not significantly different in the 5% DMRT test

Based on the results of the analysis of variance and further tests, 5% DMRT showed a real effect on the wet weight parameters. Further analysis of 5% DMRT revealed that there were treatments that showed significantly different leaf number values from treatment p₁ (Control) as a comparison, namely p₃ and p₅.

The p₅ treatment is one of the toughest treatments in this study, which is thought to affect the increase and cell division contained in the composition of the media. Physiologically wet weight consists of two contents, namely water and carbohydrates (Ruswaningsih, F. 2007)

4. CONCLUSION

Based on the research results and discussion, it can be concluded that:

1. The addition of ambon banana extract, mung bean sprouts, or sweet corn seeds to foliar fertilizer can improve the culture of black orchids using tissue culture. The addition of sweet corn seed extract to the media was able to produce the highest number of leaves obtained in the p₄ treatment (Growmore 1 g L⁻¹ + sweet corn seed extract 100 g L⁻¹) namely 6.20 leaves and the p₆ treatment (Growmore 1 g L⁻¹ + ambon banana extract 100 g L⁻¹ + sweet corn seed extract 100 g L⁻¹) produces 6.40 leaves. The addition of mung bean sprout extract produced the highest number of roots, namely in the p₃ treatment (Growmore 1 g L⁻¹ + mung bean sprout extract 100 g L⁻¹) and p₅ (Growmore 1 g L⁻¹ + Ambon banana extract 100 g L⁻¹ + extract mung bean sprouts 100 g L⁻¹) as many as 13.00 pieces and the longest root length in treatment p₅ (Growmore 1 g L⁻¹ + Ambon banana extract 100 g L⁻¹ + mung bean sprout extract 100 g L⁻¹) namely 7, 08 cm.
2. The best media was found in treatment p₅ (Growmore 1 g L⁻¹ + ambon banana extract 100 g L⁻¹ + mung bean sprout extract 100 g L⁻¹) which produced the highest plant height (5.02 cm), the highest number of roots (13.00 pieces), the longest root length (7.02 cm) and the highest wet weight (0.45 g).

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

- 1.
- 2.
- 3.

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