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IN VITRO INDUCTION OF CALLUS ON AGLAONEMA "BUTTERFLY" LEAVES WITH A COMBINATION OF 2,4-D AND BAP

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ABSTRACT

This research aims to determine the effect of variations and the concentration level of 2,4-D in a medium containing BAP on the callus formation of Aglaonema Butterfly. This research is an experimental study using 2,4-D (0 ppm; 0.5 ppm; 1 ppm; 2 ppm; 4 ppm) and 1.2 ppm BAP. Explants were taken from young leaves of the 2nd and 3rd Aglaonema Butterfly from the shoots, with the size of the explants being 1 x 2 cm. The parameters observed were callus emergence time, the percentage of explants forming callus, the average callus size, the percentage of live explants, and the level of explant browning. The variation of 2,4-D in a medium containing BAP affected the callus emergence time, the percentage of explants forming callus, and the average Aglaonema Butterfly callus size. The best treatment was at a concentration of 2 ppm 2,4-D + 1.2 ppm BAP concentration at 4 weeks after planting, with 50% of explants forming callus, the average callus size was 0.82 mm, live explants were 87.5%, and the lowest browning rates.

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1. INTRODUCTION

Cultivating ornamental plants has become a trend in society because ornamental plants have various beautiful shapes, patterns and colours so they can function to beautify home gardens. In Indonesia, one of the ornamental plants that people are very interested in is Aglaonema or Sri Rejeki.

Aglaonema is an ornamental plant with the title "Queen of Leaves" because the plant's main attraction is the variety of patterns and colours of its leaves. Because of its beauty, quite a few Aglaonema have high economic value and increasing market demand. One of the Aglaonema that is the favourite is the Butterfly type, which has leaves that are quite wide and lanceolate in shape and have beautiful patterns with a variety of color combinations such asgreen, red, and white. Generally, Aglaonema can be propagated through stem cuttings, shoot cuttings, and seeds, but the success of shoot growth is still low and takes a long time. Another problem that arises from conventional propagation is that a lot of plant material is needed, which can damage the parent plant.

Aglaonema can be propagated through tissue culture techniques by inducing callus formation. One of the benefits of doing tissue culture by inducing callus formation is to prepare methods for developing new, unique plant variants through mutation induction, transgenic technology, and genome editing; so that it can increase the selling value of the plant.

The medium used in in vitro culture has a major influence on the growth and development of explants. The growth regulators added to tissue culture growth medium vary greatly, depending on the desired direction of plant tissue growth. The use of growth regulators in tissue culture media functions to control organogenesis and morphogenesis in the formation and development of shoots, roots, and callus formation. Two groups of growth regulators are often used in tissue culture, namely cytokinins and auxins. Often a combination of the two PGRs is needed, depending on the comparison or ratio of cytokinin to auxin or vice versa (Lestari, 2011).

According to research conducted by Lin Zhong et al. (2016), the addition of 9.04 µM 2,4-dichlorophenoxyacetic acid (2,4-D) to MS medium was the highest rate of embryogenic callus formation on the flower axis of *Amorphophallus konjac* (Araceae). The growth regulator 2,4-D is a synthetic auxin that can stimulate callus formation, cell elongation or growth, root initiation and induction of somatic embryogenesis (Damayanti et al., 2005). The addition of BAP as a cytokinin hormone can increase cell division, tissue, and organogenesis, and induce shoot formation and proliferation of axillary shoots (Damayanti et al., 2005 and Siti et al., 2008).Based on this, it is necessary to carry out research regarding the effect of giving variations of 2,4-D in MS medium containing BAP, so that optimal concentrations are obtained for callus growth on Aglaonema Butterfly explants in vitro, to produce new plant variations so that they become plants. which is superior to the previous type.

2. RESEARCH METHODS

a. Types of research

This research is experimental, because there is treatment, controlled variables, and results testing. The research was structured using the basic pattern method of Completely Randomized Design (CRD) with 4 replications with a ratio of 2.4-D: BAP = 0 ppm: 0 ppm (P0); 0 ppm: 1.2 ppm (P1); 0.5 ppm: 1.2 ppm (P2); 1 ppm: 1.2 ppm (P3); 2 ppm: 1.2 ppm (P4); and 4 ppm: 1.2 ppm (P5).

b. Time and Place of Research

The research took place from April 2021 – to June 2021, carried out at the Tissue Culture Laboratory, Biology Education Department, FMIPA, UNY.

c. Research Target/Subject

The population in this study was one Aglaonema Butterfly plant provided at the Biological Garden of Yogyakarta State University. The research samples used were 48 explants from the 2nd and 3rd young leaves of the shoots of the Aglaonema Butterfly plant.

d. In vitro media

The medium used was MS with addition of 6 variations of plant growth regulators (2,4-D 0 ppm; 0.5ppm; 1 ppm; 2 ppm; 4 ppm and BAP 1.2 ppm) witheach treatment having 4 repetitions. So the number of experimental units was 24 experimental units, and each unit in one petri dish was planted with 2 Aglaonema Butterfly leaf explants, thus the total number of samples was 48 explants.

Also added to the medium was 20 gL-1 sucrose, 0.05% PPM, 1 gL-1 activated carbon, and 7 gL^{-1} agar. The medium was adjusted to have a pH of 5.8.

e. Explant Sterilization

The young leaves of Aglaonema Butterfly were washed in running water for 15 minutes, then soaked in solution of liquid soap for 5 minutes, again the leaves are rinsed in running water for 15 minutes. To reduce fungal and bacterial contamination from the external environment, the leaves were soaked with fungicide and bactericide for 20 minutes. After rinsing, the leaves were soaked in 20% Sodium hipochlorite solution for 7 minutes, then rinsed with sterile distilled water for 15 minutes 3 times.

f. Explant Planting

Planting is carried out under sterile conditions in Laminar Air Flow (LAF). The leaves were placed on a petri dish lined with sterile filter paper. The leaves were cut lengthwise with a size of 1 x 2 cm. Explants are planted in the medium with the abaxial part in contact with the medium, each petri dish contains 2 explants.

g. Data analysis

The data obtained included the time the callus appeared, the percentage of explants forming the callus, the average size of the callus, the rate of live explants, and the level of browning in the explants. Data was taken every week until the explants were 6 weeks after planting (WAP). The data obtained was then entered into a table and analyzed. Quantitative data analysis on the parameters measured, with visual observations analyzed using descriptive methods.

3. RESULTS AND DISCUSSION

a. Callus Appearance Time

Observations of the time when callus appeared on the explants were observed every week using a stereo microscope. The results of the analysis showed that the effect of adding a variety of 2,4-D to the medium containing BAP influenced the time of callus appearance (Table 1).

Table 1. Time of Aglaonema Butterfly Callus Appearance at 6 weeks after planting

Treatment	Callus formation time (wap)
P1 (0 ppm 2,4-D + 1.2 ppm BAP)	5
P2 (0.5 ppm 2,4-D + 1.2 ppm BAP)	5
P3 (1 ppm 2,4-D + 1.2 ppm BAP)	5.75
P4 (2 ppm 2,4-D + 1.2 ppm BAP)	4
P5 (4 ppm 2,4-D + 1.2 ppm BAP)	4.75

The appearance of callus on explants is an indicator of growth in in vitro culture. Giving variations of 2,4-D with different concentrations will have different effects on a target cell. Callus formation on Aglaonema Butterfly leaf explants was fastest in the treatment of 2 ppm 2,4-D + 1.2 ppm BAP (P4) which was able to induce callus with an average of 4 days after planting. Callus formation was slower in the treatment of 4 ppm 2,4-D + 1.2 ppm BAP (P5), with an average of 4.75 days after treatment. Treatments of 0 ppm 2,4-D + 1.2 ppm BAP (P1) and 0.5 ppm 2,4-D + 1.2 ppm BAP (P2) had the same average callus formation time, namely 5 WAP. The combination of 1 ppm 2,4-D + 1.2 ppm BAP (P3) was the longest treatment in inducing callus with an average of 5.75 weeks.

The addition of the 2,4-D plays a role in stimulating cell enlargement and division to form callus. Cell enlargement is caused by increased plasticity of the cell wall and the formation of the cellulase enzyme which is useful for dissolving cellulose in the cell wall, thereby making the cell wall membrane easier for oxygen, water, and mineral salts to pass through for the process of cell growth and enlargement (Wilkins, 1970).

Callus first appears at the edge of the explant incision which is in contact with the medium. Microscopically, callus looks like clear white tissue like water droplets that line the edge of the explant. This is shown by the results of Khaniyah & Yelnititis (2012), where the callus formation is characterized by swelling and the formation of white bumps that crowd the surface of the explant. The callus produced is the result of injury to the tissue and the response to the plant growth regulators (PGR) . Raghavan (1997) found facts that support these findings, that the presence of auxin can activate signal transduction so that cells can reprogram gene expression and induce cell division leading to callus growth. The appearance of this callus on the injured part is thought to be due to stimulation of the explant tissue to cover the wound.

Meristematic cells that can grow and divide are characteristics of parenchyma cells. This was also stated by Esau (1960), that parenchyma is adult tissue that is reversible so that the cells can become meristematic again and actively divide. The formation of callus tissue along the explant cut is an example of the resumption of cell division by the parenchyma.

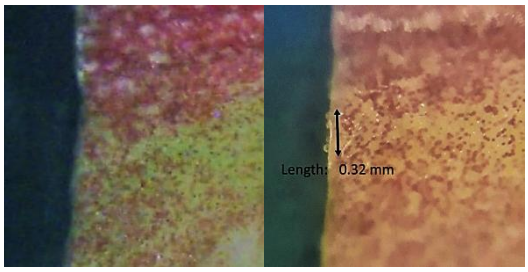

Figure 1. Callus formed in the explant wound area, (A): age 0 mst; (B): age 5 mst. K=Callus.

The use of auxin (2,4-D) in a medium containing cytokinin (BAP) was seen to be able to grow callus on Aglaonema Butterfly explants when compared with no addition of PGR. Asra et al. (2020) found facts that support these findings, that the use of 2,4-D can induce callus formation at low doses because 2,4-D has a higher activation effect. 2,4-D is the best synthetic auxin among various other synthetic auxins, because 2,4-D is more easily absorbed by plants, does not decompose easily, and plays a role in encouraging morphogenesis activity. The cytokinin used was BAP with a concentration of 1.2 ppm which was the optimum level in inducing callus in the Aglaonema sp within 8 weeks after culture (Dwi Kusuma Wahyuni et al.,2014). According to Gaspar (1996), BAP also works actively in the growth and reproduction of callus, causing BAP to be the most active cytokinin.

b. Explant Growth

Explant growth was observed based on the percentage of explants that formed the callus, the average size of the callus, and the rate of explants that were alive (Table 2).

The percentage of callus formation indicates the level of responsiveness of the explant to the treatment given. The analysis results in Table 2 show that variations of 2,4-D added to the medium containing BAP affect the percentage of explants that form callus and the average size of callus. The percentage of callus formation on explants showed that in the treatments of 0, 2, and 4 ppm 2,4-D +

1.2 ppm BAP had the highest percentage, namely 50% of each explant succeeded in forming callus. The percentage of explants forming callus decreased to 37.5% in the 0.5 and 1 ppm 2,4-D + 1.2 ppm BAP treatments.

Table 2. Growth of Aglaonema Butterfly Explants at 6 weeks after planting

Treatment	Number of Explants	Live explants (%)	Explants form callus (%)	Average callus size (mm)
P1	8	87.5	50.0	0.25
P2	8	25.0	37.5	0.55
P3	8	87.5	37.5	0.73
P4	8	87.5	50.0	0.82
P5	8	50.0	50.0	0.57

The callus that appears on the explants is caused by the endogenous hormones in each explant being able to suffice its growth, and coupled with the administration of exogenous hormones which can stimulate rapid growth of the explants, so that with the presence of endogenous and exogenous hormones at the right levels they can work optimally to induce the formation of new cells. This difference in concentration with the same percentage of callus formation is possible because the response of one explant tissue to another is different in inducing callus. From several replications, some explants could not produce callus. A callus that does not appear is possible because some explants that are planted physiologically do not have complete tissue so callus formation is hampered. This is by research by Aziz et al. (2014) that callus does not appear because the explant tissue does not have complete formation and physiological equipment as a result it cannot enter the cell division cycle.

The highest average callus size was observed at week 6 in the medium of 2 ppm 2,4-D + 1.2 ppm BAP reaching 0.82 mm, followed by 1 ppm 2,4-D + 1.2 ppm BAP around 0.73 mm. The 0.5 ppm and 4 ppm 2,4-D + 1.2 ppm BAP treatments had almost the same average callus size, namely around 0.56 mm. The treatment with the smallest callus size is at 0 ppm 2,4-D + 1.2 ppm BAP around 0.25 mm. If we look at the data on the time of callus appearance, the concentration of 2 ppm 2,4-D + 1.2 ppm BAP is more optimal when compared to other treatments, because at this concentration it can induce callus more quickly with a high percentage of explants forming callus, and has a larger average callus size compared to other concentrations.

c. Explant Morphology (browning level)

The response to explants when viewed from their morphology is the color of the explant fading to brownish, which is related to the percentage of living explants. However, not all explants experience this change. The response to explant color change in this study was different for each treatment.

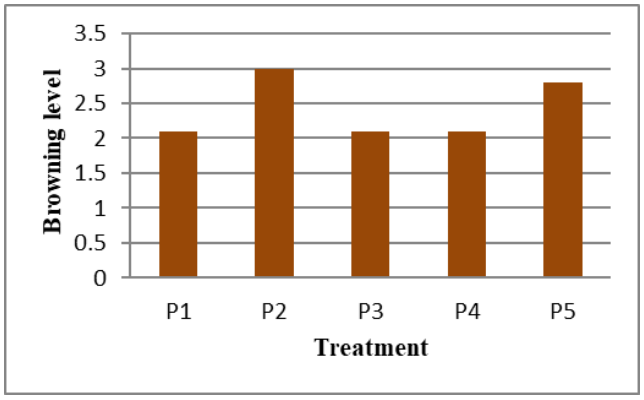

Figure 2. Level of Browning in Aglaonema Butterfly explants at 6 weeks after planting.

The percentage of live explants is presented in Table 2, which shows that the 0, 1, and 2 ppm 2,4-D + 1.2 ppm BAP treatments had a high percentage of live explants, namely 87.5%. Treatment of 4 ppm 2,4-D + 1.2 ppm BAP had a live explant percentage of 50%. The percentage of live explants decreased in the 0.5 ppm 2,4-D + 1.2 ppm BAP treatment, namely 25%.

The browning response was assessed using a score for each treatment and the results were in the form of a bar diagram presented in Figure 2. The highest level of browning was in the 0.5 ppm 2,4-D + 1.2 ppm BAP treatment of 3, then in the treatment 4 ppm 2,4-D + 1.2 ppm BAP browning level of 2.8. Treatments of 0, 1, and 2 ppm 2,4-D + 1.2 ppm BAP have the same browning level, namely 2.1. The results of this research show that on average the explants experience color fading leading to browning when itwas 6 wap. This response could be due to interactions that occur between the explant and the medium at different times.

The process of wounding the explant is thought to be a factor that influences the explant to brown. The change in color of the Aglaonema leaf explant to fade or turn brownish is thought to indicate that the explant has been damaged. The explant experiences color changes, starting from the color of the explant fading until it becomes brown on the entire surface of the explant.

Browning can occur due to oxidation of phenolic compounds released due to injury to the explant. Several types of tropical plants contain high levels of phenolic compounds and will oxidize when cells are injured or senescence occurs (George & Sherrington, 1984). Ozygit et al. (2008), stated that explants that have been cut cause the contents in the cytoplasm and vacuoles to mix and come out so that phenolic compounds can be oxidized by air. The process between the enzymes polyphenol oxidase (PPO) and peroxidase (POD) and the polyphenols that form quinones occurs enzymatically, then polymerizes to produce a brown color.

4. CONCLUSIONS AND SUGGESTIONS

a. Conclusion

Based on the research that has been carried out, the following conclusions are obtained:

1) The effect of variations in 2,4-D added to the medium containing 1.2 ppm BAP affected the time for the callus to appear, the percentage of explants forming the callus, and the average size of the Aglaonema Butterfly callus.
2) The best (optimum) treatment for Aglaonema Butterfly callus formation is at a concentration of 2 ppm 2,4-D which is added to a medium containing 1.2 ppm BAP at 4 WAP, with 50% of the explants forming callus, the average callus size is 0.82 mm, as many live explants 87.5%, and has the lowest browning rate.

b. Suggestion

1) Further research needs to be conducted regarding the use of various concentration levels of 2,4-D and BAP to induce callus in a short time and with a high percentage of callus.
2) There is a need for further preliminary research into the explant sterilization stage so that contamination can be handled optimally and prevent browning of the explants.
3) Further research is needed to induce callus formation and increase its regeneration capacity for plant propagation

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