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The Effect of BAP and NAA Combination on Callus Induction of *Aglaonema* Siam Aurora Leaf Explants in Vitro

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ABSTRACT

This research aims to determine the effect of Naphthalene Acetic Acid and Benzyl Amino Purine combination on callus growth of *Aglaonema* Siam Aurora leaf explants and to determine the combination level at the most optimal concentration on the callus growth of *Aglaonema* Siam Aurora leaf explants. Observation variables included callus emergence time, explants percentage of formed callus, callus length, and explant life percentage. The explants used were the first young leaves explants from shoots that were opened, with 2x2 cut size. Results show that the addition of a NAA and BAP combination had an effect on . Combination of 1.2 ppm BAP + 1 ppm NAA treatment is the most optimum treatment for callus growth with 87.5% explant showing callus growth, the callus start time at week 3, the average callus length is 0.77 mm and the percentage number of survived explants is 100%

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1. INTRODUCTION

Aglaonema is a tropical plant originating from Southeast Asia. In Indonesia, *the Aglaonema* plant, commonly known as sri refortune which belongs to the family Araceae or taro-talasan. *Aglaonema* got the title of queen of ornamental plants because of its elegance and relatively expensive price. *Aglaonema* Siam Aurora is the result of a cross between two parents who have the characteristics of a combination of green leaves in the middle, and bright red at the edges. *Aglaonema ornamental plants* are one of the horticultural commodities that have received great attention because of the beauty of their leaves which attract the public, especially ornamental plant lovers. The large number of enthusiasts makes an opportunity to add various types of ornamental plants bigger and wider.

Conventional propagation is the main problem in the cultivation of *Aglaonema* Siam Aurora plants. An alternative way to do plant propagation is tissue culture which can produce propagation of a large number of seedlings with the same quality as the parent. According to Yunita (2009), tissue culture through induction of somaclonal variation is the right way to obtain new varieties that are unique, resistant to disease, and resistant to extreme environments. This variation results in genetic changes caused by the process of crossing. The crossing process is not produced by segregation or recombinant genes, but due to genetic mutations in explants induced in *vitro*.

The success of tissue culture requires appropriate techniques, such as aseptic conditions, proper culture room temperature, controlled lighting and the provision of appropriate media to obtain the desired

variety. The use of tissue culture techniques can experience various obstacles such as contamination or *browning* on the explant. This needs to be overcome by adding ingredients such as activated charcoal in the media. The addition of activated charcoal to the medium is often used for various phases of tissue culture growth, because activated charcoal is able to absorb toxic compounds that inhibit the dedifferentiation process, as well as being able to spur root initiation and somatic embryogenesis (George and Sherrington, 1984).

Somatic embryogenesis induction and callus induction will produce callus, the more calluses that are formed and produced, the higher the chances of success to obtain many seeds (George and Sherrington, 1992). Callus is a source of planting material used to regenerate plants, because each plant has the ability to be able to produce new individuals. Callus can form if there is the addition of growth regulators (ZPT), the addition of ZPT will help explants leaf organs or stems differentiate to form calluses. ZPT commonly used in tissue culture can be in the form of auxin or cytokinin hormones. According to Hendaryono (1994), one type of synthetic auxin used in tissue culture is NAA (*Naphthalene Acetic Acid*) because it has more stable and more effective properties than IAA which is a natural auxin and is also more stable than 2,4D. Synthetic cytokinins used in tissue culture are BAP (*Benzyl Amino Purine*), because of its role that can spur the synthesis of RNA and protein in various tissues, so as to encourage cell division.

Each type of explant has a different response to the administration of growth regulators, so it is necessary to know which combination of groups is right for the combination of auxins and cytokinins to be able to induce callus growth in tissue culture techniques, where in this study the auxin and cytokinin growth regulators used are NAA and BAP.

2. RESEARCH METHOD

2.1 Types of Research

This research is an experimental study. The treatment in the study used BAP 1.2 ppm with variations in the combination of NAA 0 ppm, 0.5 ppm, 1 ppm, 1.5 ppm, 2 ppm with the object of research *Aglaonema* Siam Aurora plant leaves *in vitro* to induce callus growth.

2.2 Time and Place of Research

The research was conducted in April 2021-June 2021 at the Plant Tissue Culture Laboratory FMIPA UNY.

2.3 Research Materials and Tools

The basic medium of *in vitro* culture used is *Murashige &; Skoog* (MS). Each treatment was repeated 4 times consisting of 2 explants each repetition.

2.4 Procedure

Media creation

The basic media used is MS with the addition of activated charcoal. Synthetic ZPT addition treatment of 1.2 BAP with a combination of NAA variations. The medium is set to have a pH of 5 or 6. In the medium also added agarose as much as 1.05 gr. The addition of agarose aims to compact the medium.

Leaf Sterilization

The explant is washed, soaked in liquid soap, and washed again. After that, the leaves need to be soaked in a mixture of fungicide solution (dithane) 1mg / L and bactericidal (Agrept) 1 mg/L for 30 minutes. Then rinsed again under running water 15 minutes. Next, sterilization is carried out in LAF and continued sterilization using 20% clorox. Finally, the leaves are rinsed using sterile aqueous 3 times each 15 minutes.

Explant Planting

Planting is carried out in aseptic conditions in *Laminar air flow* (LAF). The leaf explants planted came from *the Aglaonema* Siam Aurora plant taken from the first to the second shoots that had opened. The explant is planted into a prepared medium petri dish. One treatment petri planted with 2 pieces of leaf explant.

2.5 Data, Instruments and Data Collection

The data obtained include data on the time of callus appearance, the percentage of explants that form calluses and the percentage of explant life 8 weeks after planting (WAP). The data obtained is then entered into a table and analyzed.

2.6 Technical Data Analysis

The data obtained are analyzed and presented descriptively.

3. RESULTS AND ANALYSIS

The results and discussion in this study discuss the results of *Aglaonema* Siam Aurora leaf explant culture by giving a combination treatment of BAP and NAA hormone concentrations to be able to form calluses, as well as to determine the most optimal level of hormone concentration combinations.

3.1 When cases arise

The appearance of callus is characterized by changes in the implanted explant, such as swelling of the explant and the appearance of clear or white grains around the leaf lesions due to slices. This is in accordance with research that has been done by Sopa Putri, *et al.* (2017), that callus is a mass of cells that are not organized.

Giving growth regulators to the right planting media will diffuse into plant tissue through the base of the edge of the explant injured by the incision, so that growth regulators will be absorbed and will stimulate cell division, especially cells that are at the edge or base around the explant wound .

Table 1. Time parameters appeared callus explant *Aglaonema* Siam Aurora with a combination of BAP and NAA concentrations in *vitro* (WAP)

Combination of Treatment Levels (ppm)	Number of explants	Callus Start Time (WAP)
BIN1	8	4,50
B1N2	8	5,25
B1N3	8	3,50
B1N4	8	5,00
B1N5	8	4,00

Average explants with B1N3 concentration treatment (BAP 1.2 ppm + NAA 1 ppm) had the fastest time in inducing callus growth in *Aglaonema* Siam Aurora explants (Table 1). Based on observations at these concentrations calluses appear on average the third week, at these concentrations the amount of auxin and cytokinins added is balanced. This finding is in accordance with the opinion of Hayati *et al.* (2010) that, callus induction is influenced by a balanced concentration of auxins and cytokinins, if the necessary combination of hormones is right then the explant is able to induce callus growth optimally. The same research results were found in the combination of NAA and BAP in callus induction of Artemisia *annua L. leaf explants* with the addition of balanced concentrations of BAP 0.5 mg/l and NAA 0.5 mg/l (Ragapadmi, 2011).

Based on observations, it shows that the combination treatment of NAA and BAP growth regulators has an effect in inducing callus growth. In this study, the resulting callus only achieved cell induction and division. The differentiation stage has not occurred because the callus formed does not show development and only forms in the area of the explant incision. Leaves and stems are the result of cell differentiation that makes up tissues to form organs. In this study, the isolation tissue used was leaf explants, where the explants were isolated on treated media and dedifferentiated so that calluses could form. Dedifferentiation can occur because plant cells, which are naturally autotrophs are then conditioned to become heterotrophs by adding nutrients that are quite complex in the culture medium, thus stimulating cells to undergo uncontrolled division to form a disorganized cell mass or callus. In his experiments, Haberlandt wound nucellus and ovule cells with a needle prick. The experiment obtained two embryos. It is suspected that the emergence of double embryos

comes from the injury of nucellus cells triggered by cell death so as to stimulate the release of necrotic hormones. The resulting compound is very likely to be an endogenous compound. If the endogenous compounds produced by plant cells are low, cell division will not occur and calluses do not form. The addition of exogenous growth regulators needs to be added to be able to help stimulate callus induction. Growth regulators BAP and NAA can generally stimulate dedifferentiation. Cell dedifferentiation will occur in cells that have initially been differentiated, then these cells will return to meristematic cells.

3.2 Percentage of Explants Forming a Callus

Observation on each explant that formed a callus of varying degrees of combined concentrations of growth regulator treatment. The callus has the form of small clear grains that will appear on the wound of the explant incision. According to Hendaryono & Wijayanti (1994), a mass of cells or callus will form on the entire surface of the explant slice.

Table 2. Average Size of Calluses Formed at Age 8 WAP

Combination Treatment Level (ppm)	Number of explants	Average Explants Formed by Callus (%)	Average Kalus Length (mm)
B0N0	8	~	~
B1N1	8	50%	0,28
B1N2	8	62,5 %	0,66
B1N3	8	87,5%	0,77
B1N4	8	87,5%	0,43
B1N5	8	87,5%	1.01

Description: ppm = part per million (mg/l), ~ = No callus, WAP = week after planting

Based on the observations, it can be seen that the combination of BAP and NAA growth regulators used, has an influence on the results of explants that have been planted. Explants grown on each medium with additional treatment can induce callus, but the callus formed is microscopic, so the observation must use a stereo microscope. According to Palei (2017), callus formation is influenced by the type of explant used, the composition of the culture media, and the content of endogenous auxin hormones. Media serves to supply nutrients and direct growth through growth regulators. Growth regulators function to stimulate explants to experience cell development and growth. Callus can form if the explant has meristematic tissue and interacts directly between plant tissue and the arrangement of growth regulators contained in the media. According to Gunawan (1998), callus formation requires a sufficient amount of growth regulators. Each plant contains different endogenous growth regulators. Furthermore, Karjadi and Buchory (2008), said that the need for exogenous hormones depends on the amount of endogenous hormones contained in the explant. The content of endogenous growth regulators owned by *Aglaonema* Siam Aurora leaf explants is very low which is proven that there is no callus growing in control treatment, so exogenous growth regulators from the auxin and cytokinin groups are needed to increase the concentration of endogenous ZPT in cells.

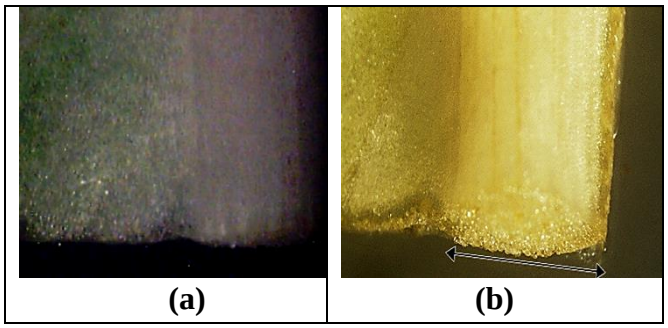

Figure 1. Callus formation at the combined concentration of B1N3, (a): Explant at 0 wap; (b): Explant at 3 wap

In the combination of B1N1 and B1N2 treatment concentrations, the optimal average data to form calluses of 50% and 62.5% and the average callus length of 0.28 mm and 0.66 mm. In this treatment, it is known that the amount of NAA auxin concentration is lower than the amount of BAP cytokinin concentration, so the number of explants that form calluses is still quite small when compared to explants treated with auxin with a balanced or higher concentration. B1N1 has data on the percentage of treatment that forms the lowest callus where in the treatment the media is only added cytokinin growth regulators. In general the addition of auxin at high concentrations can spur callus formation, but if the ratio of auxin and cytokinins in the media is lower, it will spur the growth of regenerating explants to form organs Thomy (2012). The high percentage of callus formation in each explant indicates that the composition of the hormone given is right. According to Hadipoentyani *et al*. (2008), the right combination and balance of growth regulators will affect the speed of callus formation.In B1N3 treatment, the average explant can form a callus and has an average callus length of 0.77 mm. It is known that the level of the two combinations of hormones has an almost balanced concentration. Hartman *et al.* (1990), suggested that the NAA auxin growth regulator added to the media can affect callus formation. That is because auxin is the primary hormone in producing callus, so that if the addition of auxin is at moderate to high levels it will be able to affect the explant to be able to form callus.

The success of an explant to induce callus in several treatments in the media, indicates that the combination of BAP and NAA concentrations can encourage cell division and elongation, so that the cell is able to induce callus. According to Winata (1987), there are two classes of growth regulators that are very important for use in tissue culture, namely auxin and cytokinin hormones.

3.3 Live Percentage of Explants

This parameter aims to see the number of explants that still survive in each treatment in the media. The average percentage of live explants is calculated at the age of 8 weeks after planting. An explant is declared alive if, the explant is not contaminated and *browned.* Hutami (2016) said, that activated charcoal has a strong absorption to absorb toxic compounds in chemical processes and minimize the inhibition of the dedifferentiation process in the explant.In addition, Tisserat (1979), also argued that activated charcoal can reduce browning in explants, and can spur explants to grow organogenesis. Giving activated charcoal is able to eliminate *browning* by absorbing and oxidizing phenols and activating peroxidase.

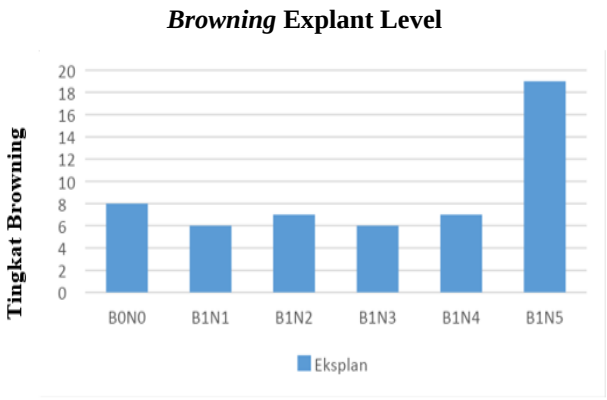

Figure 2. Browning chart on explants

The highest explant mortality rate was found in B1N5 treatment with the lowest survival percentage of 56.25%. Where in this treatment most of the explants experience browning compared to other treatments where the average explant only experiences dryness at the edge of the explant and has an explant life percentage ranging from 75% - 87.5%. The administration of activated charcoal has an influence on the explant in suppressing toxic compounds such as phenol compounds that cause *browning*. *Browning* can occur due to increased production of phenolic compounds followed by oxidation by polyphenol oxidase enzymes and their polymeration. In the B1N5 treatment, the explant initially has a fresh green color and is able to produce callus because the auxin growth regulator given in this treatment has the highest level, so that callus can form. This is in accordance with Hartman's (1990) statement, which revealed that auxin at moderate to high levels is the primary hormone in producing callus. In the sixth week, the explant begins to show discoloration to fade then brown, this indicates that the explant is dying. This is in accordance with the research data obtained, namely only the highest NAA treatment is browned. In other treatments, the explant only experienced dry at the edges of the leaves. This proves that the addition of activated charcoal in the media is able to suppress toxic compounds in the form of auxin compounds absorbed by explants too high.

4. CONCLUSION

Based on the results of research that has been done, it can be concluded that:

The addition of a combination of NAA and BAP growth regulators to MS media affects the percentage of callus explants, callus time, and the average length of callus in callus induction from leaf explants of *Aglaonema* Siam Aurora plants.

The combination of B1N3 treatment concentration (BAP 1.2 ppm + NAA 1 ppm) is the most optimal concentration combination for callus growth on the parameters of callus percentage, callus start time, mean callus length, and explant life percentage. The percentage of callus is 87.5%, with the start time of callus in the third week, the average callus length is 0.77 mm and the percentage of the number of living explants reaches 100%.

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