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Mass Propagation of Agarwood Producing Plant (*Aquilaria Malacensis* L.) with Application Auxin and Cytokinine Concentrations in Vitro Culture

Benni Satria, Rachmad Hersi Martinsyah, Warnita

Department of Agrotechnology, Agriculture Faculty, Andalas University, Padang, Indonesia

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Abstract— *The objective of study to obtain: the best concentration of thidiazuron in encouraging explants to form shoots and the best combination of concentrations of NAA and BAP to regenerate callus and shoots to form plantlets. This experiment was carried out in Plant Tissue Culture Laboratory, Faculty of Agriculture Andalas University from May to October 2021. This study consisted of two experimental stages. where the first stage to callus induction with 6 levels of Thidiazuron concentration: 0.00, 0.125, 0.250, 0.375, 0.50 and 0.625 ppm. The second stage of the experiment was the shoot and callus regeneration stage with a combination treatment of NAA + BAP concentration, with 7 levels of treatment: 0.0 ppm NAA + BAP 0.0 ppm; 0.0 ppm NAA + 1.00 ppm BAP; 0.0 ppm NAA + 2.0 ppm BAP; 0.0 ppm NAA + 3.0 ppm BAP; 0.50 ppm NAA + 1.0 ppm BAP; 0.5 ppm NAA + 2.0 ppm BAP and 0.50 ppm NAA + 3.0 ppm BAP. The study was completely randomized design (CRD) with 3 replications in a The data were analyzed by using the F test and followed by the Least Significant Difference test (LSD). The experiment was conducted with completely randomized design with 3 replications. The result show the highest percentage of live explants was 100%, the fastest time callus induction was 15 days and the percentage of explants formed callus of was 70% at treatment with a concentration of 0.25 ppm Thiduazuron. The callus structure of all treatment levels was compact and the color of the callus was white, yellowish white and whitish yellow. callus forming the fastest shoots was 11.67 days, the percentage of callus forming shoots was 50% and the number of callus forming shoots was 5 pieces obtained at a concentration of 0.5 ppm NAA + 3.0 ppm BAP.*

Keywords— ***Auxin, Cytokines, In vitro culture, Mass propagation***

I. INTRODUCTION

Propagation of Agarwood-producing plants is usually carried out generatively using seeds but in its development in nature there are obstacles because Agarwood-producing plants only flower and bear fruit at the age of 7-10 years, while at the age of 5 years, farmers/loggers have started to harvest this plant and if there are trees that has been fruitful, then the ripe fruit is likely to be eaten by birds so that some are flown by birds to other places, and some fall under the tree, besides that the germination of agarwood seeds is relatively low, only about 47%; while vegetatively with cuttings and grafts it takes a long time to produce large numbers of seedlings, seedling growth is not uniform, depending on the season, is not free from systemic disease, and the percentage of growth is only about 55%.

The in vitro culture technique is the first step towards breeding Agarwood-producing plants that are faster, uniform, in large quantities, of better quality, and most importantly can be used as a source of germplasm for Agarwood-producing plants compared to conventional methods using seeds or cuttings or grafts. Furthermore, research on the propagation of Agarwood-producing plants, especially Aquilaria malaccensis species in vitro in Indonesia, has not yet developed, especially in the area of West Sumatra, research on this has not yet been reported.

The propagation of agarwood-producing plants in vitro is largely determined by the material from which the explants came from, the media, growth regulators, and the growing environment. Planting material (explants) taken from the parent tree must have criteria, including: healthy, exposed to sunlight, meristematic parts. The planting media made is adjusted to its purpose, if we want to form callus then we should use liquid media, and if for ordinary propagation we use solid media, then if we cultivate woody plants such as Agarwood-producing plants, we recommend using WPM media (Satria, Gustian Swasti and Kasim 2008).

Growth regulators are very influential in the success of tissue culture techniques. Growth regulators used for the formation of shoots are cytokines, while for the formation of roots or callus auxin is used as growth regulators (Untung and Nursandi, 2001 and Lestari, 2011). One type of cytokines that is widely used in the formation of shoots is thidiazuron. Thidiazuron belongs to the group of strong cytokines, thidiazuron with low concentrations is able to show a response to plants (Harahap, 2012). According to Khawar et al (2003) thidiazuron is able to induce shoot propagation faster than other types of cytokines. Administration of thidiazuron (TDZ) at a concentration of less than 1 M can induce shoots in woody plants (Huetteman and Preece, 1993).

Based on research by Fernando (2017), it was shown that the concentration of TDZ 0.25 mg/L in MS medium was the best concentration in inducing shoots of female Andalas plants. The use of TDZ can stimulate the growth of female Andalas plant explants to form shoots in vitro. Research by Swandra et al (2012) showed that shoot multiplication of Andalas (Morus macroura Miq. Var. macroura) using thidiazuron and different explant sources in vitro can produce shoots, both plants without colchicine induction and colchicine induction results. According to the results of Yunita's research (2004), it was shown that administration of thidiazuron on MS media resulted in higher melinjo shoot multiplication, both using in vitro explants and explants taken from the field. Warnita *et al.* (2021) also use MS media at potato culture.

Callus and shoot regeneration is largely determined by the balance of growth regulators Auxin and Cytokines, which will affect the growth, morphogenesis and regeneration of callus and shoots to form plant plantlets in vitro. The use of growth regulators is adjusted to the desired direction of plant tissue growth. Regeneration of callus and shoots to form plantlets in mangosteen plants at a combination of 0.50 ppm NAA + 1.75 ppm BAP was able to encourage shootlet and plantlet formation (Satria, Dwipa and Jamsari, 1999).

In order for the agarwood propagation technique in vitro to be used standardly to obtain quality, uniform, and uniform Agarwood plantlets in a relatively short time, and as a source of germplasm, the best composition of growth regulators must first be found. This study aims to obtain: 1. the best concentration of thidiazuron in encouraging explants to form shoots and 2. the best combination of concentrations of NAA and BAP to regenerate callus and shoots to form plantlets.

II. RESEACH METHODS

This experiment was carried out at the Tissue Culture Laboratory, Faculty of Agriculture, Andalas University, Padang, from May to October 2021. The materials used were: explants of agarwood axillary shoots of Aquilaria malacensis originating in the Mentawai district of West Sumatra, agar, sucrose, nutrients that make up the media MS, WPM and B5, vitamins, ZPT Thidiazuron, NAA, alcohol, spriritus, aquades, Benlate, insulating plastic, streptomycin, 10% bayclin, tween 80, NaOH, HCI. The tools used include: analytical balance, beaker, measuring cup, measuring flask, filter paper, electric heater, autoclave, oven, culture bottle, pH meter, spray bottle, suction pipette, tweezers, scalpel, scissors, petridis, laminar air flow cabinet, aluminum foil and others.

The experiment consisted of two stages, where the first stage was the shoot induction stage on MS media with Thidiazuron concentration treatment with 5 levels of treatment, namely: 0, 0.125 ,0.250 p, 0.375 ,0.50 and 0.625 ppm Thidiazuron. The second stage of the experiment was the shoot and callus regeneration stage, where the shoots and callus formed were regenerated on WPM media with a combination treatment of NAA + BAP concentration, with 7 levels of treatment, namely: NAA 0.0 ppm + BAP 0.0 ppm, NAA 0.0 ppm + BAP 1.0 ppm NAA 0.0 ppm + BAP 2.0 ppm, NAA 0.0 ppm + BAP 3.0 ppm, NAA 0,5 ppm + BAP 1,0 ppm, NAA 0,5 ppm + BAP 2,0 ppm and NAA 0,50 ppm + BAP 3,0 ppm. The experiment was arranged based on a Completely Randomized Design (CRD) with 3 replications, so that in each experimental stage there were 7 x 3 = 21 experimental units. Each experimental unit consisted of 10 culture bottles, so that 210 culture bottles were obtained at each stage of the experiment. The data were analyzed by using the F test and followed by the Least Significant Difference test (LSD) 5 %.

Prior to making stock solutions and culture media, sterilization of the equipment that will be used as a medium and culture bottles was carried out to grow the explants. The culture bottles were washed with detergent and rinsed thoroughly, then sterilized in an autoclave with a pressure of 15 psi at 121 ºC for 60 minutes. The

sterilized bottles were stored in an oven at 80 ºC and the bottles were used as a medium. Plants such as petridish, scalpel, tweezers, scissors are also sterilized in an autoclave. Before putting these tools into the autoclave, they were first wrapped in parchment paper. Meanwhile, sterilizing the air flow cabinet is carried out with 70% alcohol and irradiating an ultra violet lamp for 30 minutes.

Preparation of media in stages 1 and 2 of the experiment: the nutritional ingredients were weighed (the nutritional composition of the MS (shoot induction) and WPM (shoot regeneration) then stock solution was made. After that the stock solutions were grouped into 6 groups (A, B.C, D, E, and F) and group I for vitamins (Myo inositol, Nyacin, Pyridoxin HO, and Thiamin HCI). Each nutrient group was placed in a container, namely a measuring flask with a size of 1000 ml, while the vitamins group was made into a separate container, namely a 100 ml volumetric flask. After the stock solution was prepared, it was put in each of the volumetric flasks, and stored in the refrigerator before use.

Preparation of culture media (according to treatment) is carried out by diluting the nutrient and vitamin stock solution according to the provisions. After the nutrient and vitamin solutions were well mixed, activated charcoal was given according to the dose, without PGR according to each treatment per liter of media. The volume of this mixed solution is made up to one liter by adding sterile distilled water. Furthermore, the pH was determined to be 5.8 by adding a solution of NaOH or HCL. Each mixture of media that had been made was labeled according to the treatment for both shoot induction and callus and shoot regeneration.

Furthermore, each treatment was heated with an electric heater while stirring continuously. Prior to reaching the boiling point, 7.0 grams of agar per liter of media was added in the third stage of the second series of experiments. After the solution became clear, the heating was stopped and immediately put into culture bottles as much as 15 ml per bottle with micro pinchers. Furthermore, the media in the culture bottle was sterilized in an autoclave for 20 minutes at a pressure of 15 psi with a temperature of 121 ºC. After sterilization, the vial containing the media was incubated for 1 (one) week in the transfer room before being used for explants.

The purpose of incubation is to determine whether the media in culture is completely sterile or uncontaminated. The media used for the third series of experiments in the second series (the stage of searching for sterile substances), the third series (the stage of finding the origin of explants), and the fourth series (the stage of finding the concentration of growth regulators) that were optimal were derived from the optimal culture media obtained from the first series of experiments. The procedure for making media for the second, third, and fourth series of experiments was the same as the first series of experiments.

The explants used in this experiment were shoots of Aquilaria macensis L.). The shoot explants were taken from the mother tree in the field, by cutting the shoots by 5-10 cm, then put into a 1 liter aqua bottle containing 0.05 grams of ascorbic acid solution and the bottle was closed. Furthermore, for the sterilization of explants, both explants measuring 0.50 cm were sterilized in a solution of: 0.05 grams of ascorbic acid per liter of aqua for 15 minutes; tween 80 2 drops per 300 ml of aqua for 1 minute; 70% alcohol for 1 minute; bayclin 20% for 5 minutes; and after each sterilization in the solution, the explants were rinsed three times with sterile distilled water, and all sterilization steps were carried out in a Laminar Air Flow Cabinet, but for the second series experiment the explants were also sterilized in various anti-fungal and bacterial solutions (according to the treatment). In the first stage of the experiment, the sterilized explants were immediately planted with 0.50 cm size explants in each culture bottle filled with 15 ml of MS media according to the treatment (Thiaduazuron concentration), then the weight of the culture was covered with plastic aluminum foil, and all the culture is done in Laminar Air Flow Cabinet. After that, the bottles containing the explants were stored in the culture room (incubation) at a temperature of 22 0C and the light intensity was regulated for approximately 12 hours. Furthermore, for the second stage of the experiment, explants in the form of callus and shoots formed in the first stage of the experiment were sub cultured (regenerated) on WPM media enriched with a combination of ZPT concentrations of NAA and BAP (according to treatment).

Culture space is always considered which includes; temperature of 22 0C, lighting of 40-watt TL lamp and humidity to prevent condensation in the culture bottle. If any planting material is contaminated by microorganisms (fungi and bacteria), it is immediately separated and removed from the culture room. The culture room was sprayed daily with 70% alcohol and once a month sterilized with formalin.

The variables observed in the shoot induction stage experiment, starting 1 (one) week to 10 weeks after explant culture, which include: Percentage of surviving explants, When explants form callus, Percentage of explants that form callus, Callus structure, Callus color, When callus explants form shootlets, Percentage of callus explants that form shootlets. The number of callus explants forming shoots.

III. RESULTS AND DISCUSSION

3.1. Percentage of live explants, when callus was formed and Percentage Explant forming callus

The concentration of Thiduazuron gave a significantly different effect on the percentage of live explants, when explants formed callus and Percentage Explant forming callus (Tabel 1).

Table 1. Percentage of live explants, when explant formed callus and Percentage Explant forming callus of Agarwood Producing Plant at the Concentration of Thidiazuron

Thiduazirone concentration (ppm)	Percentage callus %	time of callus formation (day)	Percentage Explant forming callus(%)
0	60,00c	25,00 c	20 d
0,125	80,00b	23,33 c	30 cd
0,250	100,00a	15,00 d	70 a
0,375	86,67 ab	30,00 b	50 b
0,500	80,00 b	32,00 b	40 bc
0,625	80,00 b	36,00 a	40 bc
KK(%)=	10,48	5,11	21,91

Note: The numbers in the same column, followed by the same lowercase letters according to the LSD test are significantly different at the 5% level.

Table 1 shows that the highest percentage of live explants was found at 100% and when explants formed the fastest callus were found in leaf petiole explants cultured on MS media enriched with 0.25 ppm Thidauzuron significantly different from other treatments (Figure 1). Response to changes in leaf petiole explants after being cultured on MS media it can be said to be quite fast. Initially, the explants changed from yellowish white to brown on the cut site and greenish on the uninjured area. On observation 1 week after culture, the explants swelled and the tips of the explants cracked, and 1 week later callus was formed. According to the research of Priyono et al. (2000), explants can form callus in a few weeks after sowing. Callus formation is caused by wound stimulation (Fowler, 1983). This stimulus causes the balance in the cell wall to change direction, some of the protoplasts flow outward so that callus begins to form.

For callus formation, depending on the type of explant used, the composition of the culture media, and the content of endogenous and exogenous auxin hormones, high auxin levels should be used (Suryowinoto, 1985 in Ambarwati, 1987). According to Priyono et al., (2000); Elliot (1982) and Widiastoety (1985) in tissue culture of ovule explants, where explants are able to regenerate without additional auxin from the outside, it is suspected that bananas contain sufficient endogenous auxin to mobilize cells to form new individuals.

The results of variance on the percentage of agarwood-producing explants formed callus due to the treatment of various culture media and types of plant explants Aquilaria malacensis L can be seen in Appendix 8c. The combination of explant types and culture media had a significantly different effect on the percentage of explants forming callus, after proceeding with the DMNRT test at a level of 5% which is presented in Table 1, and for more details on callus growth can be seen in Figure 1.

Table 1 shows that the highest percentage of explants forming callus was found in leaf petiole explants cultured on MS media enriched with Thiduazuron concentration and significantly different from petiole explants cultured on MS media, on leaf petiole explants. Leaf petiole explants cultured on MS media resulted in the highest percentage of explants forming callus, which was 50.00%. This shows that there is a strong growth response starting from day 2 of the leaf petiole in absorbing the nutrients present in the MS media and endogenous hormones found in the leaf petiole explants so as to stimulate tissue development which is characterized by elongation of the explants and callus begins to appear on average. on day 14. Callus formation started from the injured explant rim and then covered the explant surface. In cells damaged by injury, autolysis occurs, and from these damaged cells compounds are produced that stimulate cell division in the next layer to form callus.

In addition, the high callus formation in this type of explant is due to the fact that the petiole of the leaves has a lot of transport tissue that functions as a transport route for photosynthate so that it contains many nutrients and endogenous hormones. The cell wall against the protoplasm is reduced, this causes the protoplast to absorb water around the cell, so that the cell becomes long, especially the cells in the meristem. Besides, Auxin can also encourage the formation of a number of cells that are quite large but do not divide, this collection of cells is called a callus. Callus is formed due to the accumulation of cells that expand as a result of the entry of water, nutrients from the culture media and PGR into the cells, all of these materials cannot be spread throughout the plant body such as roots, stems and leaves, so they gather in one point. In accordance with the opinion of Wareing and Philips (1981); Wattimena (1988); Hendaryono and Wijayani, 1994 and; Suryowinoto, (1996) and Gunawan (1988) that only endogenous hormones present in explant tissue will

affect plant physiological and morphological processes. Besides, according to George and Sherrington (1984) that MS media is the type of media most widely used in tissue culture where the specialty of MS media is the high content of nitrate, potassium and ammonium (Kyte, 1990).

The low percentage of callus formation was due to disruption of the balance of endogenous hormones above the optimum limit, so that the cell proliferation process eventually became disrupted and consequently the number of explants that formed callus decreased. In general, the low percentage of callus formation was also due to the large effect of phenol released by all types of explants. From the research that has been done, it was found that the ability of agarwood shoot explants to form callus was lower than that of petiole leaf explants.

According to Gunawan (1988) the callus formation ability of the tissue depends, among others; physiological age and tissue when isolated, plant parts used as a source of explants and plant species. Callus formation occurs when the ratio between the concentrations of Auxin and Cytokines is in a balanced state, both endogenous and exogenous (Rao, Sin, Kothagoda, and Hutchinson, 1981; and Widiastoety, 1985)., Wattimena, and Gunawan (1991) that the ability of explants to form callus and its growth rate may differ between parts of the explant tissue. This also happened because the different explants formed callus, caused by the difference in the toti potency of the explants. This is in accordance with the experimental results of Masyudi (1993) where there are differences in the ability of explants to form callus and callus regeneration power between parts of the explant tissue.

Fig.1 : . Placement of culture bottles containing explants in incubation room

3.2. Callus Structure and Callus Color

Observation of callus structure and callus color was done visually and using tweezers. The callus structure produced at various concentrations of Thiduazuron in Agarwood-producing plants is shown in Table 2. From Table 2 it can be seen that the responses of various types of explants cultured on various culture media showed differences in callus structure and callus color of Agarwood-producing plants. Generally, the callus structure formed in this experiment is compact and has a different color.

Based on these various results, the callus structure obtained in this experiment was influenced by the genotype of the explants used and the composition of the culture media, the endogenous regulators used and environmental conditions. Wattimena (1988) stated that the formation of callus or organs in in vitro culture is more influenced by genotype, initiation of culture, growing environment and tissue physiology used. The shape, texture, color and morphogenetic ability as well as cell differentiation depend on the age and purity of the tissue used as explants. The differences that occur will be greater if the explants are composed of more than one cell type (George and Sherrington, 1984).

Table 2. Callus structure and color callus of Agarwood Producing Plant at the Concentration of Thidiazuron.

Concentration Thiduaziron (ppm)	Callus structure	Color callus
0,00	Compact	White
0,125	Compact	Whitish Yellow
0,250	Compact	Yellowish White
0,375	Compact	White
0,500	Compact	Yellowish White
0,625	Compact	Yellowish White

Callus from various species can differ in texture, viability and color and callus formation is characterized by changes in the explant texture to become rough and the surface shiny when reflected by light (Wetherell, 1982). Based on the experimental results above, it is shown that generally the callus structure is compact, so this type of callus is suitable for organogenesis. Triatminingsih, Karsinah and Wahyuni (2000) stated that the shape and color of the callus will determine the direction of further morphogenesis. The crumb callus is suitable for embryogenesis while the compact callus is suitable for organogenesis.

Meanwhile, according to Darmawati (2002) reported that the callus type with a callus structure is easier to produce single cells in liquid media that is always shaken and will divide more quickly, compared to the type of callus with a compact or solid structure, as well as the opinion of Thomas and Davey (1975); Widiastoety (!987) that crumb callus is also called embryonic callus, where it is able to differentiate to form plants.

The compact callus structure obtained in this experiment showed that the opportunity for callus to be developed and grown further into direct plantlets was greater. This can be an added value for plant breeding in an effort to overcome the scarcity of Agarwood-producing plants in vitro. From Table 1 it can be seen that the response of various explants to form callus (%) to the concentration of Thiduazuron showed differences in callus structure and callus color of Agarwood-producing plants (Ficture 2). Generally, the callus structure formed in this experiment is in the form of crumbs and compact and has a different color.

Based on the various results, the callus structure obtained in this experiment was influenced by the genotype of the explants used and the culture media used. Wattimena et al (1992); Wetherell, (1982) stated that the formation of callus or organs in in vitro culture is more influenced by genotype, initiation of culture, growing environment and tissue physiology used. The shape, texture, color and morphogenetic ability as well as cell differentiation depend on the age and purity of the tissue used as explants. The differences that occur will be greater if the explants are composed of more than one cell type (George and Sherrington, 1984).

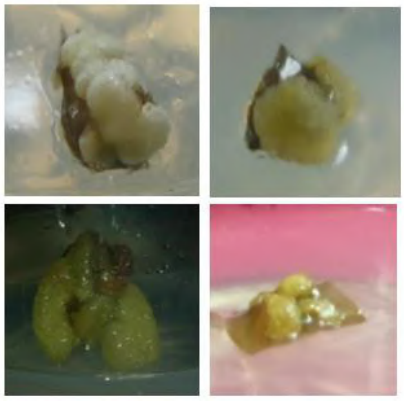

Fig.2: The forms of explants into plant callus agarwood producer

Triatminingsih *et al*, (2000) stated that the shape and color of the callus will determine the direction of further morphogenesis. The crumb callus is suitable for use for embryogenesis, while the compact callus is for organogenesis. The compact callus structure obtained in this study illustrates that the opportunity for callus to be developed and further grown into plantlets directly is greater. This can be an added value for plant breeding in an effort to overcome the scarcity of Agarwood-producing plants in vitro. Satria *et al (2017)* state that Discoloration occur can be caused by the pigmentation of the chlorophyll that undergoes degradation. The more the presence of chlorophyll, the greener the callus color. Wahyuni *et al. (2020)* report that colored callus yellow and compact struture has a chance used for organogenesis.

3.3. When callus form shootlet, Callus Percentage form Shootlet and Number of Callus form shootlet

The results of the variance on the time of shootlet formation and the percentage of callus regenerating to form shootlets (Figure 3), after the callus formed in the previous experiment was subcultured in the treatment of various combinations of NAA and BAP concentrations which gave significantly different effects in Appendix 1e and after being continued with the LSD test on level of 5% presented in the table. Table 5 shows that when callus formed shootlets the fastest was 11.67 days and the percentage of callus regenerated formed shootlets at a combined concentration of 050 ppm NAA + 3.0 ppm BAP. because various combinations of concentrations of 0.50 ppm NAA + 3.00 ppm BAP were able to encourage the fastest and highest growth and development of explants so that callus explants had the ability to live and have the ability to regenerate shootlets. Moore (1979); George and Sherrington (1984) reported that administration of growth regulators Auxins and Cytokines at low concentrations was able to stimulate the growth and development of explants and maintain the viability of explant tissues, but at high concentrations growth regulators could inhibit the development of morphogenesis explants.

Balance The concentration of Auxin and Cytokines growth regulators in explant tissue can increase the survival, growth and development of explant tissue (Satria, Dwipa, and Jamsari, 1999). Satria, Ferita, Dwipa and Jamsari, 1999b and Pierik, 1987 stated that due to endogenous PGR or exogenous Cytokines are able to stimulate cytokinesis, there is an increase in the number of cells. Cytokinesis is the process of cell division, in which cells that have absorbed more water, there is an addition of plasma and followed by these cells growing lengthwise, then the cells undergo differentiation which causes these cells to specialize in function.

Table 4. When Callus and Percentage of Explants Form Shootlet Agarwood-Producing Plants at Thidiazuron Concentration in Vitro at the age of 10 MST

Concentration NAA + BAP (ppm)	when explant callus form shootlet	percentage of callus forming shootlet	number of callus forming shootlet
0,0 + 0,0	33,00 a	10,00 c	1,0 c
0,0 + 1,0	29,67 ab	13,33 c	1,3 c
0,0 + 2,0	25,67 bc	16,33 c	1,6 c

0,0 + 3,0	22,67 c	33,33 b	3,3 b
0,5 + 1,0	20,33 cd	40,00 ab	4,0 **b**
0,5 + 2,0	16,33 d	43,33 ab	4,3 ab
0,5 + 3,0	11,67 e	50,00 a	5,0 a
KK(%) =	8,94	14,78	14,78

Fig.3 : . Callus explants regenerate to form shootlets

Furthermore, endogenous growth regulators are low in auxin and high in cytokines contained in explants, but in a balanced state encourage the growth of explants and the development of explants to form shootlets. Satria, Hervani and Gustian (2005) reported that high stockinins function in stimulating shoot formation, affecting cell metabolism and stimulating cells. Wiendi, et al (1991); and Wattimena 1988 reported that the growth and morphogenesis of plants forming shootlets and plantlets in vitro was controlled by the balance of growth regulators Auxins and Cytokines in explant tissues.

The addition of the number of shoots is one of the parameters that can be measured quantitatively, and is an indicator of the success of a tissue culture. Shoot growth is not only influenced by cytokines hormones and available nutrients, but each plant also has endogenous hormones that will affect shoot growth. The combination concentration of 0.50 ppm NAA + 3.00 ppm BAP, Cytokines was proven to be able to increase the number of shoots in agarwood-producing explants with an average value of 5.0 shoots (Figure 3).

From this research, it is known that the combination of auxin and cytokines growth regulators (NAA and BAP) has shown that with increasing concentrations of NAA and BAP in the media, the number of plant shoots tends to increase. This is in accordance with the statement of Bhojwani and Razdan (1983) that the higher the concentration of cytokines, the higher the number of shoots, but each of these shoots will be stunted. George and Sherrington (1984) also stated that BAP is best used to stimulate shoot formation. In contrast to research conducted by Rosdayanti (2007) research using a combination of cytokines (BAP and kinetin) at a level of 1 mg/L and 0.5 mg/L was able to produce 45 adventitious shoots in Aquilaria malaccensis. It is suspected that the combination of auxin and cytokines is effective in vertical multiplication (elongation) while the combination of cytokines is able to produce horizontal multiplication. Furthermore, according to Maulida (2005) stated that BAP stimulates shoot multiplication compared to kinetin. While kinetin has the effect of accelerating shoot induction. In addition, the suitability of the use of growth regulators is also a limiting factor for plant species (Wattimena 1992).

In the research conducted, the combination of growth regulators (NAA and BAP) on the number of shoots produced on average was only able to have 1 shoot in each explant (Figure 2). The small number of shoots formed was thought to be because the explants were less able to absorb the nutrients and hormones given to the media. In addition, during observations, it was seen that the explants that formed callus were getting bigger and then slowly covering all parts of the plantlet so that the plantlets did not experience the addition of new shoots at the nodes. The same thing was stated by Handayani (2003) that the addition of a growth regulator of NAA with a low dose followed by the administration of a high enough BAP could cause callus growth to be inhibited because the dose given was not balanced, as a result very little new callus was formed so that the shoots that grew relatively small or new callus did not produce a large number of shoots and did not even form buds at all until the end of the observation.

IV. CONCLUSIONS AND SUGGESTIONS

Based on the results of the research that has been carried out, it can be seen that the administration of growth regulator Thiaduazuron in the first stage of the study had an effect on the callus induction of Agarwood-producing plants (Aqulilaria malaccensis L.) in vitro. The callus formed in the first stage of the study was regenerated by giving a combination of NAA + BAP concentrations in the second stage of the study and showed an effect on callus regeneration to form shootlets.

Giving the concentration of ZPT Thiduazuron 0.250 ppm showed the highest percentage of live explant was 100%, when callus was formed the fastest (15 days) and the highest percentage of explants formed callus was 70%. Furthermore, the combination of 0.5 ppm NAA + 3.0 ppm BAP ZPT concentration showed that when callus explants formed the fastest shoots at 11.67 days, the highest percentage of callus explants formed shoots was 50% and the number of callus explants formed the most shoots was 5.0 shoots.

Based on the research activities that have been carried out, it is recommended to use explants with a larger size, and to update the sterilization technique used so that the percentage of explants that form callus can be greater. For the regeneration of callus explants, shoots should be carried out 14 days after the callus appears so that callus freshness is maintained.

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