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PENGARUH TYPE MEDIA DAN KONSENTRASI NAA PADA PERKEMBANGAN TUNAS GAHARU (*Aquilaria malaccensis* Lamk.) DALAM KULTUR *IN VITRO*

Abstrak

Tanaman gaharu (*Aquilaria malaccensis* Lamk.) merupakan salah satu komoditas hasil hutan yang mempunyai nilai ekonomi tinggi dan ketersediaannya di alam semakin menipis. Dalam rangka membantu upaya konservasi dan budidaya gaharu, diperlukan bibit berkualitas tinggi dalam jumlah yang memadai. Melalui kultur *in vitro* dapat diproduksi bibit tanaman gaharu dalam jumlah banyak dalam waktu yang relatif lebih singkat. Penelitian ini dilakukan dengan tujuan untuk mempelajari pengaruh interaksi antara tipe media dan konsentrasi NAA pada perkembangan tunas gaharu; dan menentukan tipe media dan konsentrasi NAA terbaik perkembangan tunas gaharu. Penelitian dilakukan dalam dua tahapan yaitu induksi dan perkembangan tunas. Pada tahap induksi tunas, digunakan rancangan acak lengkap (RAL) pola faktorial dengan 2 faktor. Faktor pertama adalah tipe media yang terdiri atas 3 taraf yaitu medium MS dengan pemadat phytagel 2,5 $g.l^{-1}$; medium MS cair dengan *filter paper bridge*; medium MS cair dengan spons. Faktor kedua adalah konsentrasi NAA dengan 5 taraf yaitu 0 µM; 2 µM; 4µM; 6 µM; 8 µM. Pada tahap kedua, dilakukan subkultur tunas hasil tahap pertama pada media MS dengan tipe media yang sama namun tanpa penambahan NAA. Variabel yang diamati adalah perkembangan tunas gaharu, dengan parameter meliputi jumlah tunas, tinggi tunas, dan jumlah daun. Hasil penelitian menunjukkan bahwa perkembangan tunas gaharu (Aquilaria malaccensis Lamk.) dipengaruhi oleh type media yang digunakan. Medium MS cair dengan penyangga jembatan kertas saring menghasilkan rataan jumlah tunas terbanyak (4.93 & 5,87 tunas/eksplan) dan rataan panjang tunas terpanjang (3.25 & 3.64 cm/eksplan) pada tahap induksi tunas dan tahap perkembangan tunas.

Kata kunci : *Jembatan kertas saring, medium cair, phytagel, spons,*

THE EFFECT OF MEDIA TYPES AND NAA CONCENTRATIONS ON AGARWOOD (*Aquilaria malaccensis* Lamk.) SHOOT DEVELOPMENT IN *IN VITRO* CULTURE

Abstract

Agarwood (*Aquilaria malaccensis* Lamk.) is a forest product commodity that has a high economic value, but its availability in nature is decreasing. With a view to help both conservation efforts and production, the availability of high quality seed is a necessitate. A large number of high quality seedling can be produced via *in vitro* culture in much sorter time. This study has been carried out to study the effect of the interaction between media types and NAA concentrations on the development of agarwood shoots; as well as to determine the best medium type and NAA concentration to stimulate the development of agarwood shoots. This research consited of two stages, shoot induction and development stages. Research on shoot induction stage has been conducted experimentally with a completely randomized design (CRD) on a factorial treatment pattern of 2 factors. The first factor was the types of media which consisted of MS medium solidified with 2.5 $g.l^{-1}$ phytagel; liquid MS medium supported with filter paper bridge; and liquid MS medium supported with viscous sponge. The second factor was NAA concentration consisted of 5 levels i.e. 0 µM; 2 µM; 4 µM; 6 µM; 8 µM. The shoots produced from the first stage were culture on MS media with the same media type but without any NAA addition. The variables observed in both stages were the development of agarwood shoots, with the parameters measured included the number of shoots, number of leaves, and plant heights. The research results showed that the growth of agarwood shoots

(*Aquilaria malaccensis* Lamk.) was influenced by the type of medium used. Liquid MS medium supported with filter paper bridge produced the highest average number of shoots formed (4.93 & 5,87 shoots/explants) and shoot length (3.25 & 3.64 cm/explant) in shoot induction and shoot development stage, respectively.

Keywords **:** *Filter paper bridge, liquid medium, phytagel, viscous sponge*

INTRODUCTION

Agarwood (Aquilaria malaccensis Lamk.) is a forest product commodity with a very high economic value. Agarwood has a fragrant wood which contains resin known as agarwood, aloeswood, or oudh. Agarwood is widely used as raw material for perfume, soap, incense, cosmetics, medicines (Azwin, 2016; Gultom et al., 2012; Kosmiatin et al., 2016). The prospect of the agarwood market is increasing along with the development of both society and industry. Between 1996-2015 Indonesia's agarwood export reached more than 2.5 million kilograms (CITES, 2004). The increase in agarwood demand has resulted in the exploitation of agarwood plants, leading to the decrease of agarwood population in nature. Therefore, since 1994, agarwood producing plants including *A. malaccenensis* Lamk. had been listed on APENDIX II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Arhvitasari et al., 2019).

Efforts to propagate agarwood plants to meet increasing market demand while conserving them in nature can be done conventionally, but it takes a longer time and has a low success rate. Plant *in vitro* culture technique can be used to produce large numbers of agarwood plants, which are free from pathogens and diseases, as well as economically efficient (George et al., 2008; Pierik, 1982; Trigiano & Gray, 2004). The explant growth and differentiation in *in vitro* culture is controlled by many factor including the plant genetic make up, nutrient, physical growth factors, and the use of plant growth regulators (Pierik, 1982). Shoot development is an essential step in *plantlet* production. Good shoot will ease up the rooting processes leading to the formation of good *plantlet*. Plantlet with good rooting system can increase the success of the acclimatization processes. Shoot development is influenced by several factors, including growth regulators and the type of medium used. 1-Naphthaleneacetic acid (NAA) is a synthetic auxin commonly used for root induction in plant *in vitro* culture. NAA can accelerate seedling growth, accelerate root formation and extension from fibrous roots, and encourage shoot cell elongation (Mahadi et al., 2015). NAA is also not easily oxidized and is more stable when compared to natural auxins (George et al., 2008; Pierik, 1982).

The types of medium commonly used in *in vitro* culture are solid, semi-solid, and liquid medium. The physical properties of the medium significantly affect the growth and differentiation of explants. The medium type affects the osmolarity of the solution in the medium and the availability of oxygen for the growth of cultured explants (Basri, 2016). Liquid medium is considered a better medium type because it has a high water potential, so water transportation from the medium to plants will be easier and faster (George et al., 2008). The use of explant supporting materials is also very important so that the explants get enough oxygen for their metabolic processes and growth (Marlin, 2009).

Several studies related to *in vitro* culture of agarwood plants have been carried out, among others, by Borpuzari & Kachari (2018); Julianti et al. (2013); Nadeak et al. (2012); Saikia & Shrivastava (2015); Wardatutthoyyibah et al. (2015). However, these previous research had not able to produced good agarwood plantlets. This research was conducted to analyze the effect of the medium types and NAA concentrations on agarwood shoot development, as well as to determain the best medium type and NAA concentrations for shoot development of agarwood. This is for the first time the effects of medium types and NAA concentrations on agarwood shoot development is studied. The expected benefits of this research are to accelerate and increase the production of agarwood seedlings that are free from diseases as well as to support the conservation efforts of agarwood plants.

MATERIALS AND METHODS

Plant material

The plant materials used were microshoots produced from a study by Tamyiz et al., 2022. The microshoots were maintained on Murashige-Skoog medium (MS, Sigma-Aldrich M5519) supplemented with 15 µM BAP (Sigma-Aldrich B3408) and 20 gL^{-1} sucrose and solidified with 0.25% phytagel (Sigma-Aldrich P8169). Before being used for this study, agarwood explants were subcultured on MS medium containing 20 gL^{-1} sucrose, supplemented with 5 µM BAP, and solidified with 0.25% phytagel for 12 days to obtain uniform explants.

Shoot induction

This study consisted of two stages i.e. shoot induction and shoot development. In the first stage, the effect of the medium type and various concentrations of NAA (Sigma-Aldrich N0640) on shoot induction were studied. This stage was conducted on an MS medium supplemented with 20 gL^{-1} sucrose. This research has been carried out experimentally using a completely randomized design on a factorial treatment pattern with three replications. The first

factor was the types of medium consisting MS medium supplemented with 2.5 gL^{-1} phytagel; liquid MS medium with filter paper bridge; and liquid MS medium with viscose sponge. The second factor was the concentration of NAA consisted of 5 levels: 0 µM; 2 µM; 4 µM; 6 µM; and 8 µM. The NAA concentrations used were a modification of the results reported by Prasetyo et al. (2020); Tamyiz et al. (2022); Wardatutthoyyibah et al. (2015). The medium pH was adjusted and set to 5.8. The microshoots were planted, 1 explant/bottle, and incubated at 24°C under continuous light for 12 weeks. The variable observed was the induction of agarwood shoots, with the parameters measured including the number of shoots, number of leaves, and shoot length.

Shoot development

In the second stage of the experiment, the shoots from induction stage were sub-cultured onto MS basal medium supplemented with 20 5 gL^{-1} sucrose, with three types of supporting material (phytagel, filter paper bridge, and viscose sponge) but no NAA was added. This stage was aimed to stimulate shoot development. The same variable was observed i.e. the induction of agarwood shoots, with the parameters measured including the number of shoots, number of leaves, and shoot length. This stage had been carried out for 8 weeks, under continuous light at 24°C.

Data Analysis

The data obtained were analyzed using an Analysis of Variance (ANOVA) followed with Duncan Multiple Range Test (DMRT) at 95% level of confidence.

RESULTS AND DISCUSSION

After 12 weeks of culture, it was found that the growth of agarwood explants was very diverse, as shown in the increase number of shoots and leaves formed, as well as shoot length measured. It was also found that although NAA was used, but no proper roots were formed. The fact that no proper roots were formed might be related to BAP accumulation in the explant from the previous culture stage. Exposure to BAP for a long time will result in the increase of BAP concentration in the explants. Such phenomena had also been reported by Tamyiz et al. (2022). BAP is a PGR that is easily absorbed, and translocated (Blakesley et al., 1991; Feng et al., 2017; Reinert & Yeoman, 1982; Schaller et al., 2014, 2015) and subsequently conjugated with glucose to become the storage form of BAP (Friml, 2003; Sauer et al., 2013).

Analysis of variance results of the effect of medium types, NAA concentration, and their interaction on agarwood shoot induction (Table 1) showed that media type controlled the number of shoots and shoot length. The appearance of shoot in different media type but at the same NAA concentration are shown in Figure 1. Data on Table 1 showed that NAA concentrations and their interaction with media type showed no effect on the parameters measured. These conditions might have been caused by the presence of sufficient endogenous growth regulators in the explants, such as BAP accumulated from previous culture. BAP is a cytokinin that plays a role in stimulating shoot growth (Erawati et al., 2020), but at a high concentrations it can be toxic and inhibit plant growth (Gethami & Sayed, 2020). The addition of exogenous growth regulators which did not significantly affect the growth of explants has also been reported by Muliati et al. (2017). Therefore, it is necessary to balance the concentrations of auxin and cytokinin. The interaction between auxin and cytokinin at optimal concentrations can regulate shoot and root growth (Mahadi et al., 2015).

Table 1. Analysis of variance results of the effect of medium types, NAA concentration, and their interaction on agarwood shoot induction

Source of variance	Number of Shoots	Shoot length	Number of Leaves
Media type	6,502*	6,630*	0,584
NAA concertation	0,342	1,750	0,162
Interaction M x N	1,311	0,630	0,354

Description: (*) has a significant effect

Futhermore, DMRT test results showed that liquid MS supported with filter paper bridge produced the highest average number of shoots formed (4.93 shoots/explants) and shoot length (3.25 cm/explant) which were significantly different to those produced by explant culture on Liquid MS supported with viscose sponge and MS medium solidified with 0,25 % Phytagel (Table 2). These results indicated that liquid MS medium with filter paper bridge supported the growth of explants by providing the necessary but not excessive nutrients and water (waterlogged). The same result was reported by Grzegorczyk-Karolak et al. (2017); Marlin (2009); Nuryadin et al. (2017). The growth of explants in liquid medium with a paper bridge filter showed the best results might be due to the optimal flow of nutrients from the medium to the explants (Marlin, 2009). According to Grzegorczyk-Karolak et al. (2017), the nutrients in liquid medium will be more evenly distributed so that the explants can absorb nutrients in the medium easily. In addition, George et al. (2008) stated that liquid medium could accelerate water and nutrients transport to plant tissues because it has high water potential.

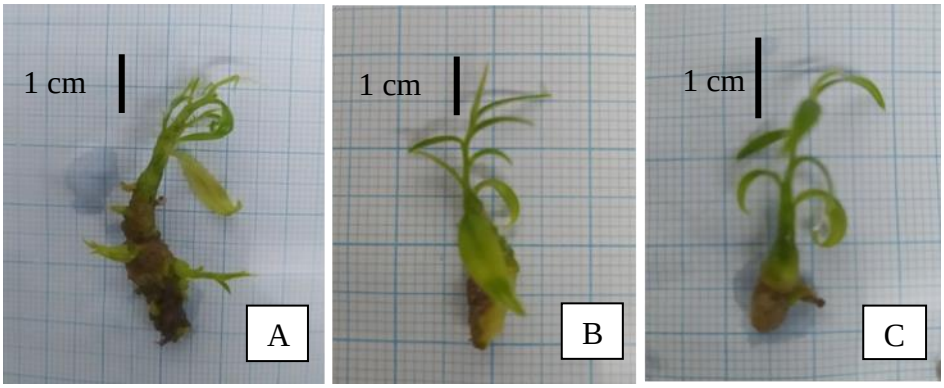

Figure 1. Agarwood shoot appearance at shoot induction state on different media type. (A) Shoot grown on liquid MS supported with filter paper bridge and supplemented with 2 µM NAA; (B) Agarwood shoot grown on MS medium solidified with 0,25 % Phytagel and supplemented 2 µM NAA; (C) Agarwood shoot grown on liquid MS supported with viscose sponge and supplemented with 2 µM NAA.

Table 2. DMRT test result on the effect of medium type on the number of shoots and shoot length (n=15)

Treatment	Number of Shoots	Shoot Length
Liquid MS supported with viscose sponge	2,13[a]	2,33[a]
MS medium solidified with 0,25 % Phytagel	3,13[a]	2,71[a]
Liquid MS supported with filter paper bridge	4,93[b]	3,25[b]

Note: Numbers followed by the same letter are not significantly different at DMRT 5%

On the other hand the use of liquid medium and high concentrations of endogenous cytokinins can cause hyperhydration on the explants (Mazri, 2015). Hyperhydration occurs because the explants absorb excess water. Hyperhidration can be caused by several factors such as ammonium levels, the concentration of plant growth regulators, and the use of liquid medium (Mazri, 2015). Hyperhydration in *in vitro* culture can be reduced by adding solidifying agents, reducing the concentration of cytokinins and nitrogen ions, as well as using buffers in liquid culture medium (Grzegorczyk-Karolak et al., 2017; Ivanova & Van Staden, 2011).

With a view to stimulate shoot development, all shoots from induction stage were subsequently subcultured on the same type of medium but without any growth regulators. After 8 weeks of culture, it can be seen that the shoot grew nicely as seen in the increase number of shoot and length (Figure 2). The analysis of variance results of the effect of medium types,

NAA concentration, and their interaction on agarwood shoot development (Table 3) also showed the same results as the treatment with NAA, the number of shoots and shoot length. In addition, DMRT test results also showed that liquid MS supported with filter paper bridge produced the highest average number of shoots formed (5,87 shoots/explants) and shoot length (3.64 cm/explant) which were significantly different to those produced by explant culture on Liquid MS supported with viscose sponge and MS medium solidified with 0,25 % Phytagel (Table 4).

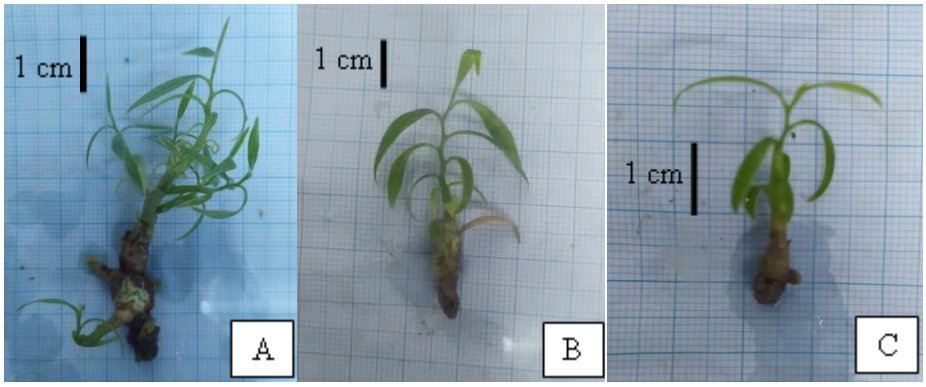

Figure 2. The appearance of agarwood shoot, derived from previous culture on 2 µM NAA, in shoot development state with no growth regulators application. (A) Shoot grown on liquid MS supported with filter paper bridge; (B) Shoot grown on MS medium solidified with 0,25 % Phytagel; (C) Shoot grown on liquid MS supported with viscose sponge.

Table 3. Analysis of variance results of the effect of medium types, NAA concentration, and their interaction on agarwood shoot development

Data	Number of Shoots	Shoot length	Number of Leaves
Media type	6,194*	4,227*	1,170
NAA concertation	0,241	1,022	0,460
Interaction M x N	1,501	0,854	0,898

Description: (*) has a significant effect

Table 4. DMRT test result on the effect of medium type on the number of shoots and shoot length in media without any growth regulator (n=15)

Treatment	Number of Shoots	Shoot Length
Liquid MS supported with viscose sponge	3,27[a]	2,65[a]
MS medium solidified with 0,25 % Phytagel	4,13[a]	2,93[a]
Liquid MS supported with filter paper bridge	5,87[b]	3,64[b]

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

It was also observed that there were two explants, derived from culture on solid medium supplemented with 6 µM NAA (Figure 3. A) and on liquid medium with supported with viscose sponge and supplemented with 4 µM NAA (Figure 3. B) formed roots after being subcultured on shoot development media for 8 weeks, although they were limited in number (1 root per explant) and small in size (0.6 cm). From the available data, statistical analysis cannot be carried out. Rooting is controlled by physiological, biochemical, and genetic factors, medium composition, physical growth factors, and plant growth regulators especially auxin (Arab et al., 2018; Li et al., 2021). The fact that rooting could not be induce by NAA was contradictory with results reported by Tamyiz et al. (2022). Tamyiz et al. (2022) reported that NAA was the best auxin type to induce rooting. NAA is a synthetic auxin which is more stable (Gunawan, 1992; Zaerr & Mapes, 1982), and it has a slow translocation and low activity (Kumlay, 2014; Zaerr & Mapes, 1982).

Figure 3. The appearance of agarwood root on media without growth regulator: (A) on an explant derived from culture on solid medium supplemented with 6 µM NAA; and (B) on an explant derived from liquid medium supported with viscose sponge and supplemented with 4 µM NAA.

CONCLUSIONS

It can be concluded that the development of agarwood shoots (Aquilaria malaccensis Lamk.) was controlled by the type of medium used. Liquid MS medium supported with filter paper bridge produced the highest average number of shoots formed (4.93 & 5,87 shoots/explants) and shoot length (3.25 & 3.64 cm/explant) in shoot induction and shoot development stages, respectively.

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