Effect of monochromatic led illumination on the in vitro growth of gaharu (*Aquilaria malaccensis*) on in vitro conditions

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Abstract. This study aimed to evaluate the influence of culture media supplemented with plant growth regulators and different monochromatic LED light spectra on the morphological growth of agarwood (*Aquilaria malaccensis*) under in vitro conditions. The experiment was conducted using half-strength Murashige and Skoog (MS ½) basal medium supplemented with 3 mg/L indole-3-butyric acid (IBA) and 1 g/L activated charcoal, combined with five monochromatic LED light spectra: red, yellow, blue, green, and white as control. The culture conditions were maintained under a photoperiod of 16 h light and 8 h dark, at an incubation temperature of 25 ± 2 °C, with the medium pH adjusted to 5.8 prior to sterilization. Growth parameters observed included plant height, number of leaves, number of roots, root length, and fresh weight. Data were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT). The result indicated blue light was the most effective treatment for stimulating root development (number and length) and enhancing biomass accumulation (fresh weight), while white light was optimal for promoting plant height. These findings suggest that the combination of MS ½ medium supplemented with IBA and activated charcoal, together with the use of specific LED spectra, particularly blue and white, can optimize in vitro propagation techniques of agarwood and improve the efficiency of high-quality seedling production.

1 Introduction

Gaharu (*Aquilaria malaccensis* Lamk.) is a forest tree with high economic value because its wood contains a fragrant resin. Internationally, it is known as agarwood, aloeswood, or oudh. Gaharu has been used for centuries in religious practices and is also an ingredient in perfumes, soaps, medicines, and shampoos [12]. Its economic potential is very high, with gaharu oil valued between US $20,000 and US $50,000 per liter. The global market for gaharu is estimated at US $6–8 billion and continues to grow [1].

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In Indonesia, gaharu has mostly been harvested directly from the wild, leading to a drastic decline in natural populations. Since 1994, CITES (Convention on International Trade in Endangered Species) has listed gaharu-producing trees in Appendix II, which restricts trade to cultivated plants only [4]. The main challenges for cultivation are overexploitation and the lack of efficient propagation technologies. Conventional propagation methods, both generative and vegetative, are slow and often have low success rates. Moreover, gaharu trees need around 15 years before they can produce resinous wood, making alternative propagation techniques essential. One promising method is plant tissue culture [9].

Tissue culture involves growing isolated plant cells or tissues under sterile conditions until they regenerate into complete plants. This method offers several benefits, such as producing uniform planting material, supporting continuous production of valuable secondary metabolites, and providing controlled growth conditions. It can also be combined with plant breeding techniques, including mutation induction, somaclonal variation, genetic transformation, and anther culture [7].

Among environmental factors that influence plant growth, light is particularly important. It plays a key role in photosynthesis, metabolism, chlorophyll formation, and biomass accumulation . Artificial lighting can be used to manipulate these processes, especially with LED (light-emitting diode) technology. Chlorophyll mainly absorbs red (600–700 nm) and blue (400–500 nm) light. Blue light generally supports vegetative growth, while red light promotes generative development [11].

LEDs are increasingly used in plant research because they are durable, energy-efficient, produce little heat, and can emit specific wavelengths. Many studies have shown the positive effects of monochromatic LEDs on plant growth, but research on gaharu is still very limited. Since gaharu may have specific light requirements, understanding the influence of different wavelengths is important for its cultivation. Previous studies have shown that light type can significantly affect plant growth and physiology. For example, in vitro cultures of *Tagetes* sp., *Oncidium* sp., *Arabidopsis thaliana*, *Brassica* sp., and *Lactuca sativa* showed improvements in height, leaf and root length, sugar content, and root activity under specific light conditions. Blue light, in particular, has been linked to increased leaf number, chlorophyll synthesis, protein levels, and enzyme activity [10]. Red light is also widely used, as it supports photosynthesis, stem elongation, and carbohydrate synthesis [5].

Research has also explored the effects of green and yellow light, although findings are less consistent. Some studies suggest that removing green light improves plant growth, while others report mixed effects depending on the proportion used . Yellow light, in some cases, has been found to inhibit plant growth, though its role remains less clear. The utilization of LED technology in agriculture offers great potential, not only for gaharu but also for other crops that benefit from controlled lighting. It allows growers to create optimal conditions for plant growth and improve productivity [5].

Several attempts at in vitro gaharu propagation have been reported, though with limited success. Most studies used MS medium with combinations of growth regulators such as BAP, TDZ, NAA, or IAA. While shoot induction and multiplication were achieved, rooting remained limited, indicating that further optimization of MS-based protocols is still required for successful gaharu tissue culture [8].

2 Material and Methods

2.1 Time and Place

This study was conducted from March 2025 to August 2025. The tissue culture of gaharu under LED light treatments and the observation of morphological (fresh weight, plant height,

number of leaves, number of roots, and root length) and biochemical parameters (chlorophyll, glucose, sucrose, and fructose) were carried out at the Chemistry Laboratory, PT. Bukit Asam Tbk.

2.2 Media Preparation, Sterilization, and Inoculation

Murashige & Skoog (MS) medium was used: half-strength Murashige & Skoog (MS). The media were supplemented with sucrose (30 g/L), agar (7 g/L), indole-3-butyric acid (IBA, 3 mg/L), and activated charcoal (1 g/L). The pH was adjusted to 5.8 utilizing 1 N NaOH, and the media were heated on a hot plate until fully homogenized. Each medium was dispensed into culture bottles (20 mL per bottle). Sterilization was carried out utilizing an autoclave at 121 °C for 20 minutes. The media were then placed on culture racks and left for three days before utilize.

2.3 Plant Material

The explants used were one-month-old gaharu plantlets (about 2 cm in length). Each explant was inoculated into MS medium [2]. Each treatment consisted of one explant per bottle, with three replicates for each medium.

2.4 Treatments and Culture Conditions

After inoculation, the explants were kept under fluorescent light for 7 days, then transferred to culture racks under LED lighting with different wavelengths: white, red (660 nm), blue (450 nm), green (525 nm), and yellow (580 nm), with a photoperiod of 14 h/day. Cultures were maintained for 100 days at a relative humidity of 75 ± 5% and a temperature of 25 ± 2 °C [2].

2.5 Measurement of Plant Growth Parameters

Growth parameters were recorded weekly, including plant height and number of leaves. At day 90, additional parameters were measured: fresh weight, plant height, number of leaves, number of roots, and root length, using standard measurement scales. Proliferation was assessed by counting the number of newly developed leaves and shoots.

2.6 Statistical analysis

Processing and analysis of the experimental data were performed using Microsoft Excel 2016 and IBM SPSS ver.18. Results were tested using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at the significance level of 0.05.

3 Results and Discussion

The number of leaves of *Aquilaria malaccensis* increased progressively under all LED light treatments during the 12-week culture period (Figure 1). Differences among treatments became more pronounced after week 4. Explants exposed to yellow and blue LEDs exhibited the highest leaf proliferation, reaching more than eight leaves by week 9 and continuing to increase until the end of the experiment. Red and green LEDs promoted moderate leaf formation, while white light consistently resulted in the lowest leaf number throughout the culture period.

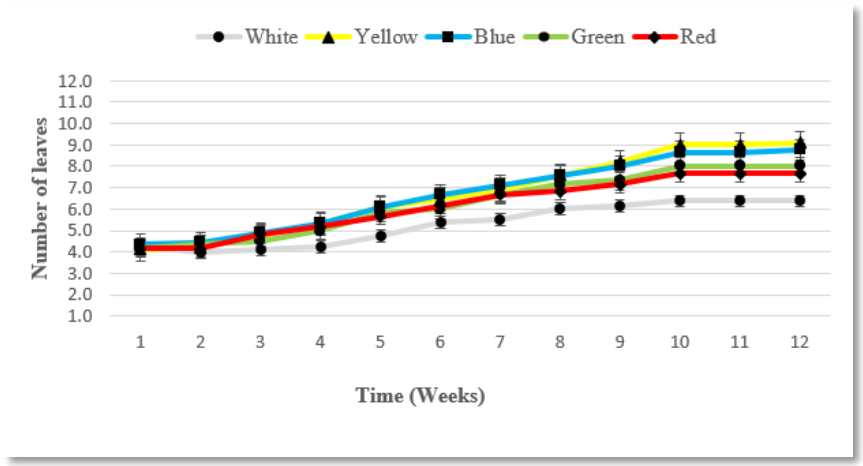

Fig. 1. The effect of light treatment on the number of leaves of Aquilaria malaccensis.

These results indicate that light quality significantly influences leaf morphogenesis in *A. malaccensis*. Previous studies have shown that blue and red wavelengths are the most effective for promoting leaf development, as they regulate photosynthetic efficiency, chlorophyll synthesis, and hormone signaling. Blue light, in particular, has been reported to enhance stomatal opening and leaf expansion, while red light is associated with biomass accumulation and cell division. Yellow light, although less frequently studied, has been shown to stimulate leaf proliferation in certain woody species by improving photosynthetic responses and carbohydrate allocation [11]. In contrast, white light often results in lower growth responses because its broad spectrum dilutes the specific effects of red and blue wavelengths critical for morphogenesis. Overall, the findings suggest that specific light spectra, particularly yellow and blue LEDs, can enhance leaf formation and improve the efficiency of in vitro propagation in gaharu.

Plantlet height of *Aquilaria malaccensis* increased gradually under all LED light treatments throughout the 12-week culture period, although the extent of elongation varied depending on the wavelength applied (Figure 2). Among the treatments, white LED light consistently promoted the greatest plantlet height. Yellow and blue LEDs supported moderate elongation, while green and red LEDs resulted in the shortest plantlets.

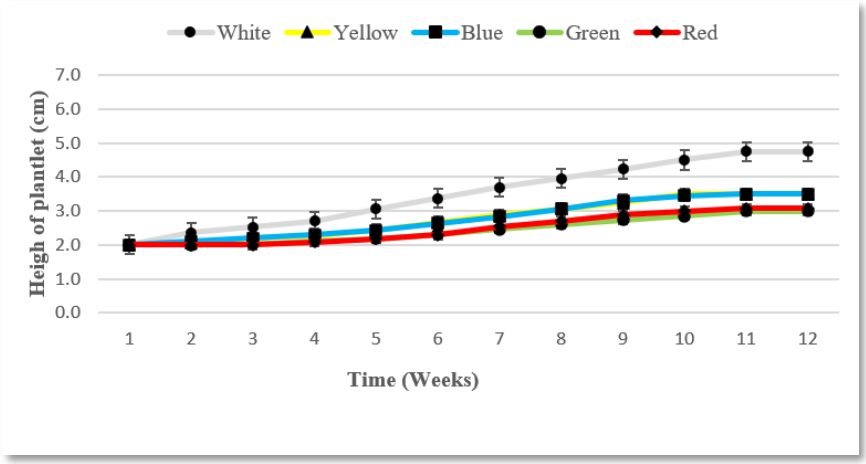

Fig. 2. The effect of light treatment on the height of plantlet of *Aquilaria malaccensis*

The superior performance of white LED light in promoting stem elongation can be attributed to its broad spectral range that closely resembles natural sunlight, providing a balanced composition of red, blue, and green wavelengths. Muslihatin et al. [13,14] reported that *Curculigo latifolia* plantlets grown under white LED light exhibited the greatest plant height, highest leaf number, extensive root development, and the highest chlorophyll content compared to other light treatments. This suggests that the full-spectrum nature of white light supports coordinated shoot and root growth through enhanced photosynthetic activity and chlorophyll biosynthesis. In contrast, blue light, while beneficial for leaf proliferation and chlorophyll formation, promoted only moderate elongation, resulting in more compact plantlets. Yellow light showed a stimulatory effect on leaf development but was less effective in promoting vertical growth, indicating that it contributes more to photosynthetic efficiency and biomass accumulation than to stem elongation. Overall, the findings demonstrate that white LED light provides the most favorable spectral environment for balanced plantlet growth and vigor, while blue and yellow lights exert more specific physiological influences on Aquilaria malaccensis, consistent with the light responses observed in *Curculigo latifolia* [12].

Red and green light treatments resulted in the lowest plantlet height. Red light alone often promotes stem elongation when combined with blue light, but in the absence of blue wavelengths it can cause reduced elongation or abnormal morphology. Green light, on the other hand, is poorly absorbed by chlorophyll and is generally less effective in stimulating elongation growth, although it may penetrate deeper into leaf tissues to complement photosynthesis. The reduced height observed under these treatments suggests that both wavelengths are insufficient on their own to drive optimal shoot elongation in *A. malaccensis*.

Overall, these findings indicate that light quality strongly influences shoot elongation in gaharu plantlets. White LED light proved most effective, suggesting that a broad spectral composition is necessary for balanced growth. This contrasts with the leaf number results, where yellow and blue light were more effective, highlighting that different developmental parameters in *A. malaccensis* respond to distinct light spectra. For practical applications, alternating or combining white light (to promote elongation) with blue/yellow light (to enhance leaf production) could provide an optimized strategy for in vitro propagation of gaharu.

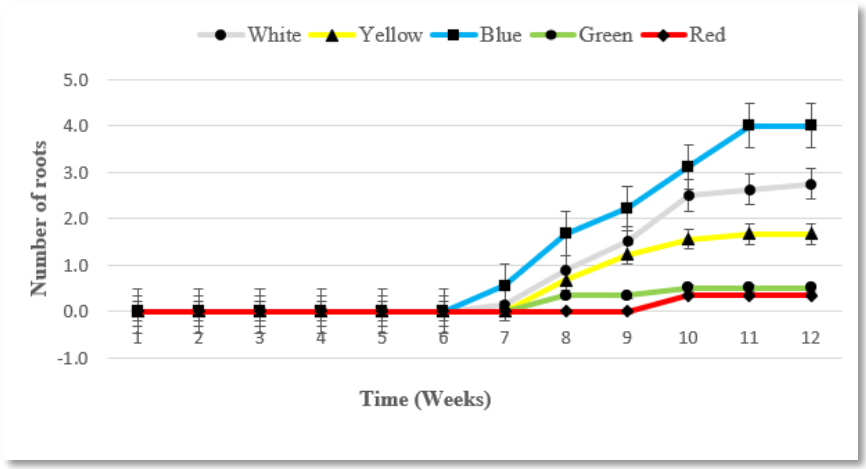

Fig. 3. The effect of light treatment on the number of roots of Aquilaria malaccensis

Root initiation of *Aquilaria malaccensis* began after week 6, with clear differences among light treatments (Figure 3). White and yellow LEDs produced the highest number of roots (up to four roots per plantlet by week 12), while blue and green induced moderate rooting

(2–3 roots). Red light was the least effective, with almost no root development. The superior rooting under white and yellow light may result from their broad or intermediate spectra, which provide a balance of wavelengths supporting photosynthesis and auxin-related root induction. Blue light moderately promoted rooting, consistent with its role in regulating auxin transport [6], while green light had limited effects due to low photosynthetic efficiency [5]. Red light suppressed rooting, likely due to hormonal imbalance under monochromatic exposure. Overall, these results show that rooting in *A. malaccensis* is highly influenced by light quality, with white and yellow LEDs being most effective.

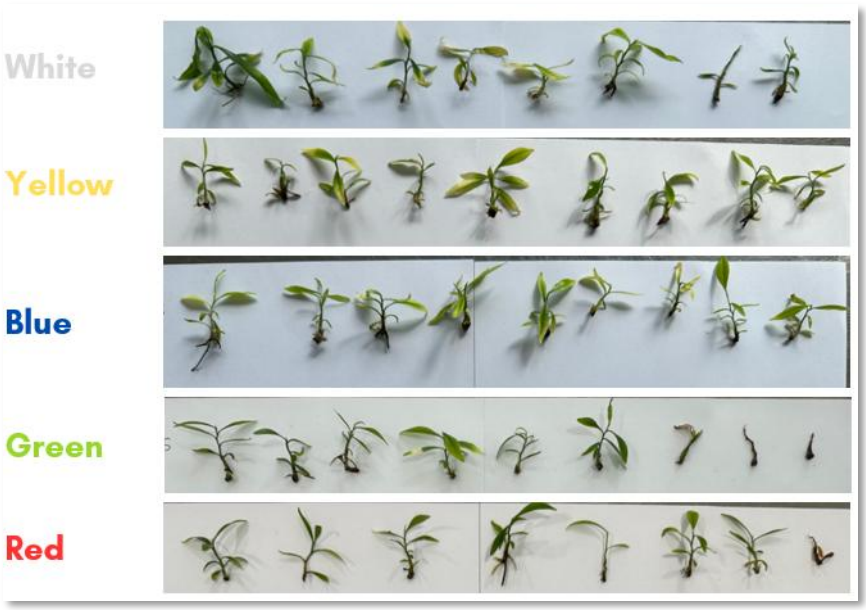

Fig. 4. The effect of light on growth of in vitro plantlet of *Aquilaria malaccensis.*

The Figure 4 show clear variations in plantlet appearance among treatments, including differences in shoot height, leaf development, root formation, and overall vigor. These visual observations support the quantitative data presented in Table 1, demonstrating that blue and yellow light tended to enhance leaf proliferation, white light promoted shoot elongation, and blue light strongly stimulated rooting and biomass accumulation. The figure provides a complementary visual representation of how specific light wavelengths influence plantlet performance under controlled culture conditions.

Table 1. Effect of light on growth of in vitro plantlet of *Aquilaria malaccensis.*

Light Condition	Number of leaves	Height of plantlet	Number of roots	Root length	Fresh weight
White	6.375 ± 0.843	4.750 c ± 0.412	2.750 bc ± 0.491	0.625 ± 0.250	0.074 bc ± 0.011
Yellow	9.000 ± 0.534	3.313 ab ± 0.162	1.750 ab ± 0.526	0.375 ± 0.420	0.072 bc ± 0.010
Red	6.000 ± 1.195	2.875 ab ± 0.157	0.250 a ± 0.250	0.250 ± 0.313	0.040 a ± 0.009
Blue	9.000 ± 0.500	3.438 b ± 0.148	4.125 c ± 0.990	1.000 ± 0.000	0.082 c ± 0.010
Green	6.000 ± 1.363	2.500 a ± 0.390	0.375 a ± 0.375	0.313 ± 0.375	0.048 ab ± 0.009

Values represent mean ± SE. Different letters within each column indicate significant differences at $p < 0.05$ according to Duncan's Multiple Range Test (DMRT).

The analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) revealed significant effects of light spectra on all measured parameters of *Aquilaria*

malaccensis plantlets (Table 1). For number of leaves, yellow and blue light produced the highest values (9.0 leaves), significantly greater than white, red, and green light. This indicates that specific spectra stimulate enhanced photosynthetic activity and cell division in leaf primordia. Previous reports showed that blue and yellow wavelengths are particularly effective in enhancing leaf proliferation in tissue culture due to their influence on chlorophyll biosynthesis and photosynthetic efficiency. Shoot height was significantly influenced by light treatment, with white light producing the tallest plantlets (4.75 cm), forming a distinct group compared to other treatments. The broad-spectrum nature of white light provides a balanced range of wavelengths, supporting both photosynthesis and elongation processes. By contrast, green and red light treatments resulted in shorter shoots, which may be linked to limited spectral energy absorption and inefficient photomorphogenic signaling. This is consistent with previous studies on chrysanthemum and lettuce showing that white or mixed spectra enhance elongation [12].

Root formation was strongly stimulated by blue light (4.125 roots), followed by white light (2.75), while red and green treatments induced minimal rooting (<0.5 roots). Blue light also resulted in the longest roots (1.0 cm), indicating its dual role in root initiation and elongation. The promotive effect of blue light on rooting may be attributed to its regulation of auxin transport and root meristem activity [15]. In contrast, red light has been widely reported to suppress rooting when applied alone, which was also observed in this study.

Fresh weight accumulation was highest under white LED light (0.082 g), followed by blue light (0.074 g) and yellow light (0.072 g), while red and green lights showed the lowest biomass. These findings are in agreement with [13,14], who reported that Curculigo latifolia plantlets grown under white and blue LED lights exhibited superior growth performance, including greater plant height, higher leaf number, enhanced root formation, and increased chlorophyll content compared to those exposed to red light. The higher biomass observed under white and blue light is likely associated with enhanced photosynthetic efficiency, chlorophyll biosynthesis, and improved physiological adaptation to the in vitro environment. Taken together, these results indicate that light quality plays a crucial role in the growth and development of A. malaccensis plantlets. Blue and yellow lights were optimal for leaf development, white light promoted stem elongation, and blue light strongly enhanced rooting, root elongation, and biomass accumulation. The combination or alternation of these light spectra could therefore be a promising strategy to improve micropropagation efficiency and plantlet quality for large-scale gaharu (A. malaccensis) production, consistent with the positive light responses reported in C. Latifolia [13,14].

4 Conclusion

The present study demonstrated that light spectra significantly affected the *in vitro* growth of *Aquilaria malaccensis* plantlets. Yellow and blue lights were most effective for leaf production, white light promoted shoot elongation, and blue light strongly enhanced root formation. Duncan's Multiple Range Test (DMRT) confirmed significant differences among treatments, with each light spectrum showing distinct effects on plantlet development. These findings suggest that optimizing light quality, particularly through the application of blue and yellow spectra in combination with white light, could improve the efficiency of *A. malaccensis* micropropagation systems.

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