The effect of medium formulation and BAP concentration of growth and development of *Aquilaria malaccensis* Lam. shoot *in vitro*

B W Hapsari*, Rudiyanto, A F Martin and T M Ermayanti

Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI)  
Jalan Raya Bogor Km. 46, Cibinong 16911, West Java, Indonesia

*Email: betalini_widhi@yahoo.com

**Abstract.** Tissue culture of agarwood tree (*Aquilaria malaccensis* Lam) was done to produce a large number of transplants in the laboratory. The compositions of medium culture are commonly modified to obtain the best growth of explants to regenerate into plantlets. Our study aimed to evaluate and compare different medium formulations supplemented with BAP on agarwood shoot culture growth. In this study we used MS, NN, B5, AR and WP medium containing 0 (control treatment), 0.25, 0.5, 1.0 and 2.0 mg/L BAP. The shoot tips were cultured for 16 weeks in the treatment media. Height of shoots, number of leaves, number of adventive shoots, and number of roots was recorded every week to determine the growth. The results showed that after 16 weeks of culture, the best medium for shoot height was NN medium with BAP fortification (3.54 cm) for number of leaves and adventive shoots proliferation was Gamborg B5 medium fortified with 0.5 mg/L BAP (21.63 leaves and 8,38 shoots), while NN medium without BAP was best for rooting.

1. **Introduction**

Indonesian forest is extraordinarily rich in plant diversity from which various kinds of commodities have been derived. One non-timber forest product commodity that brings many benefits and has a high economic value is the resin-producing agarwood/sapwood from trees of *Aquilaria malaccensis* Lam. and other species and some other genus belonging to the family Thymelaeaceae. Agarwood has been recognized for its numerous medicinal values, widely used in perfumery and incense in some religious events [1].

The high demand for the resinous agarwood has endangered the agarwood trees in the wild and mandated its international trade regulation. Agarwood-producing tree species have been listed in Appendix II CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora); therefore, trade in the world is regulated and restricted by quota [2,3]. Amid trade regulation, agarwood industries have been mandated to plant agarwood trees. The interest in the cultivation of agarwood trees has also been growing together with the development of inoculation techniques to induce resinous agarwood [4,5]. Agarwood plantation on an international scale has also been advertised on social media. These growing interests in cultivating agarwood trees require suitable planting materials, either from seeds or vegetatively propagated materials.
Aquilaria malaccensis is generally propagated through seeds, which have a brief dormancy period. Consequently, the seeds can germinate readily after maturity; however, their germination rate sharply reduces with the increase in the period of storage or decrease in its moisture contents. The maximum percent germination (90%) occurs within 20 days for fresh seeds and seven days for stored seeds with very less germination percentage [6]. Conventional vegetative propagation of agarwood trees has been reported to be difficult [7,8]. Until now, there is no validated success of vegetative propagation technique due to complex and lengthy processes by which farmers usually take 3 to 4 years for the plant to mature [9]. Alternative techniques such as tissue culture might help resolve this problem.

Some studies of in vitro agarwood propagation have been reported [9] with limited success. Most of those works used MS base medium with the addition of BAP and TDZ [10], BAP and NAA [11,12], IAA (in seeds) [13]. An experiment on BAP on several media has also been reported [14]. One of the research that was using medium other than MS was reported by Minh [15] using Aquilaria crassa (agarwood), a Vietnamese forest tree. The result showed that Woody Plant Medium (WPM) was a better basal medium than Murashige and Skoog, and for initial shoot induction, BA at 1mg/L and coconut water at 10% were used. For subcultures, BA at 0.1mg/L, NAA 0.1mg/L, and coconut water at 10% gave highest shoot multiplication. A low level of rooting was obtained using either IBA or NAA at 0.3 mg/L.

Various ready-to-use basal medium are widely offered in the market. In general, most of the existing studies used MS as a primarily medium containing plant growth regulator like cytokinin and or auxin, but rarely has reported the use of other basal medium. The aim of this work was to evaluate and compare different medium formulations supplemented with BAP on the growth of Agarwood shoot culture.

2. Materials and Methods

2.1 Plant materials

The explants used in this study was shoot culture stock obtained from previous study [14]. The four month old shoot culture; single shoot with 2.5cm height and had more than 5 leaves; were cultured on MS [16] medium supplemented with 30 g/l sucrose and 8 g/l agar and incubated in culture room at 25 ± 2ºC under 1000 lux continuous light regime for 24 hours.

2.2 Methods

The shoot tip inocula with approximately 1.1-1.5cm in length and all leaves removed, were excised from the shoot culture stock, and planted into treatment media, leaving ca. 0.5 cm of shoot tip. The treatment media consisted of combination of media formulation and concentration of Benzyl Amino Purine (BAP), a cytokinin growth regulator. The media included five formulations of Murashige & Skoog (MS) [16], Nitsch & Nitsch (NN) [17], Gamborg B5 [18], Anderson Rhododendron (AR) [19] and Woody Plant Medium (WP) [20] composed of macro salts, micro salts and organic addenda (table 1). While BAP concentrations covered 0, 0.25, 0.5, 1.0 and 2.0 mg/L. All treatment media were supplemented with 30 g/l sugar, pH adjusted at 5.7-5.8, solidified with 8 g/l Agar and sterilized by autoclaving at 121ºC, 1 atm, for 15 min. The treatment media were dispensed in 25 ml aliquot into glass vessels with volume of 250 ml and capped with plastic caps. There was a total of eight replicates of inocula for each treatment media in two culture vessels. The cultures were maintained in a culture room at 25 ± 2ºC under continuous 1000 lux light regime for 24 hours. The growth variables such as height of shoot, number of leaves, number of adventitious shoots, and number of roots were recorded every two weeks for 16 weeks of culture. The height of shoot was observed by measuring the height of the highest shoot, and number of leaves was recorded by calculating the total of open leaves, young curled leaves were not recorded.

2.3 Statistical Analysis

All generated data were analyzed by Analysis of Variance (ANOVA) (results of this analysis was not presented). Average values of variables followed by their standard errors were presented as graphs or...
Mean separation was carried out using Duncan’s Multiple Range (DMRT) post hoc test using R statistical software [21] with Agricolae pack [22].

Table 1. Compositions of basal medium formulation tested for the experiment.

| Composition               | MS\(^1\) (mg/L) | NN\(^2\) (mg/L) | B5\(^3\) (mg/L) | AR\(^4\) (mg/L) | WPM\(^5\) (mg/L) |
|---------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Macronutrients:           |                 |                 |                 |                 |                 |
| NH\(_4\)NO\(_3\)         | 1650            | 720             | 0               | 400             | 400             |
| (NH\(_4\))\(_2\)SO\(_4\) | 0               | 0               | 134             | 0               | 0               |
| KNO\(_3\)                 | 1900            | 950             | 2500            | 480             | 0               |
| KH\(_2\)PO\(_4\)         | 170             | 68              | 0               | 0               | 0               |
| Na\(_2\)HPO\(_4\).H\(_2\)O | 0               | 0               | 150.08          | 330.6           | 0               |
| CaCl\(_2\)                | 332.2           | 166             | 113.24          | 332.2           | 72.47           |
| Ca(NO\(_3\))\(_2\).4H\(_2\)O | 0               | 0               | 0               | 0               | 556             |
| MgSO\(_4\)               | 180.7           | 90.37           | 122.09          | 180.7           | 180.7           |
| K\(_2\)SO\(_4\)          | 0               | 0               | 0               | 0               | 990             |
| Micronutrients:           |                 |                 |                 |                 |                 |
| CoCl\(_2\).6H\(_2\)O      | 0.025           | 0               | 0.025           | 0.025           | 0               |
| CuSO\(_4\).5H\(_2\)O     | 0.025           | 0.03            | 0.025           | 0.025           | 0               |
| CuSO\(_4\)               | 0               | 0               | 0               | 0.16            | 0               |
| C\(_{10}\)H\(_8\)N\(_2\)O\(_5\)S | 0             | 0.05            | 0               | 0               | 0               |
| H\(_3\)BO\(_3\) (Boric Acid) | 6.2             | 10              | 3               | 6.2             | 6.2             |
| MnSO\(_4\).H\(_2\)O      | 16.9            | 18.9            | 10              | 16.9            | 22.3            |
| Na\(_2\)MoO\(_4\).2H\(_2\)O | 0.25           | 0.25            | 0.25            | 0.25            | 0.25            |
| KI                        | 0.83            | 0               | 0.75            | 0.3             | 0               |
| ZnSO\(_4\).7H\(_2\)O     | 8.6             | 10              | 2               | 8.6             | 8.6             |
| Iron:                     |                 |                 |                 |                 |                 |
| C\(_{10}\)H\(_8\)N\(_2\)Na\(_2\)O\(_8\).2H\(_2\)O | 0             | 37.26           | 0               | 74.5            | 0               |
| C\(_{10}\)H\(_2\)N\(_2\)NaFeO\(_8\).3H\(_2\)O (EDTA) | 37.26          | 0               | 36.7            | 0               | 37.3            |
| FeSO\(_4\).7H\(_2\)O      | 27.8            | 0               | 0               | 55.7            | 27.85           |
| Vitamin and other supplements: |               |                 |                 |                 |                 |
| C\(_6\)H\(_12\)O\(_6\) (Myo – Inositol) | 100         | 100             | 100             | 1886            | 100             |
| C\(_6\)H\(_5\)NO\(_2\) (Nicotinic Acid) | 0.5          | 5               | 1               | 0               | 0.5             |
| C\(_{12}\)H\(_7\)ClN\(_4\)O\(_3\)HCl (Thiamine) | 100         | 0.5             | 10              | 0.4             | 1               |
| C\(_6\)H\(_11\)NO\(_3\).HCl (Pyridoxine) | 0.5          | 0.5             | 1               | 0               | 0.5             |
| C\(_3\)H\(_3\)NO\(_2\) (Glycine) | 0            | 2               | 0               | 0               | 2               |
| C\(_{10}\)H\(_10\)N\(_2\)O\(_6\) (Folic Acid) | 0            | 0.5             | 0               | 0               | 0               |

Description: 'Murashige and Skoog [16], 2Nitch and Nitch [17], 3Gamborg B5 [18], 4Anderson’s Rhododendron [19], 5Woody Plant Medium [20].
3. Results and Discussion

3.1. The effect of medium type and BAP concentration on adventitious shoot number.

The proliferation of shoots until the end of observation period showed different rate dependent upon the treatment medium (figure 1). Some treatments resulted in the maximum adventive shoot number as early as week 8-10, while some other treatment allowed adventive shoot number to continue increasing until week 16. Shoot proliferation was more effected by BAP concentration than the media formulation as indicated by difference between lowest and highest average shoot number which is among BAP concentrations reached eight, while the difference among medium formulation were only four.

![Figure 1](image1.png)

**Figure 1.** Growth curve of number of adventive shoot of *A. malaccensis* for 16 weeks cultured on five basal media formulations containing BAP at concentration of 0 (A), 0.25 (B), 0.5 (C), 1.0 (D) and 2.0 mg/L BAP (E).

The BAP concentration of 0 mg/L induced only minimal shoot proliferation of 1-2 shoots on medium MS, but in some other medium formulations, such as WPM and NN, not all the adventive shoots did even grow. However, with BAP addition at concentration to 0.25-1 mg/L, the adventive shoot number increases and then decreases at 2 mg/L. The shoot number response to BAP concentrations was affected by medium formulation in two categories, highly responsive and moderately responsive. Medium formulation such as NN and Gamborg B5 that allowed adventive shoot number difference among BAP concentration up to 4-5 points could be considered highly responsive, while the medium formulation of standard MS, AR and WPM only allowed difference in number of adventive shoot as many as 2-3 points could be considered moderately responsive.
Medium formulation NN allowed shoot number to reach average of 6-8 at the end of 16 weeks with BAP concentration of 0.25-2 mg/L. However, high average shoot number of 7 were reached by medium formulation of Gamborg B5 with 0.5 mg/L BAP only after 8 weeks of culture, which is 4 weeks faster than that of NN medium with BAP 0.25-2 mg/L.

3.2 The effect of medium formulations and BAP concentrations on shoot length
The growth curve of shoot length of *A. malaccensis* shoot in culture showed that the shoots grew slowly, and after 16 weeks, the longest shoot reached less than 3 cm (figure 2). Even after 16 weeks, the shoot length still tended to increase except only for a few treatment media. The shoot lengths apparently were affected by both medium formulations and BAP concentrations.

![Figure 2](image)

**Figure 2.** Growth curve of shoot length of *A. malaccensis* for 16 weeks cultured on five basal media formulations containing BAP at concentration of 0 (A), 0.25 (B), 0.5 (C), 1.0 (D) and 2.0 mg/L BAP (E).

The effect of medium formulation on shoot length were notable when there were no BAP in the medium; in which the medium formulation of NN allowed the highest shoot length followed by MS and the rest of B5, AR and WPM clustered in a lower shoot length (figure 2 A). Addition of BAP at lower concentration of 0.25 and 0.5 mg/L tend to suppress shoot length except for some medium formulation (figure 2 B, C), and the suppression of shoot length were even more severe at BAP concentration of 1-2 mg/L for all media formulation (figure 2 D, E). It worth to note that medium formulation of Gamborg B5, allowed the shoot length remained long even at BAP concentration of 0.25 and 0.5 mg/L and even longer than that of 0 mg/L BAP (figure 2).
3.3 The effect of medium type and BAP concentration on number of leaves

The growth curve (figure 3) showed that average number of leaves developing from the shoot cultures were affected by both BAP concentrations in the medium and medium formulations. The effect of the treatment media started to show differences at week 10 and continue to the end of observation period of 16 weeks. The medium formulation did not affect leave number at BAP concentration of 0 mg/L (figure 3A). Increasing concentration of BAP from 0.25 to 0.5 mg/L ameliorate the effect of medium formulation on the number of leaves (figure 3B, C). At higher concentration of 1-2 mg/L BAP suppressed the number of leaves (figure 3D, E) to a level similar to 0 mg/L. It is clear that the response of number of leaves to increasing concentration of BAP are parabolic.

![Figure 3](image)

**Figure 3.** Leaf numbers of *Aquilaria malaccensis* shoots at 0-16 weeks cultured on MS, NN, B5, AR and WP medium containing 0 (A), 0.25 (B), 0.5 (C), 1.0 (D) and 2.0 mg/L BAP (E).

The highest number of leaves of 20 were obtained at treatments with medium formulation of B5 combined with BAP concentrations of 0.25-5 mg/L, while the standard medium MS and the rest of media tested combined with the same BAP concentration reached maximum average of 15 leaves (figure 3B, C).

To summarize the effect of medium formulation and BAP concentration of the growth of *A. malaccensis* shoot cultures, the observation of week 16 were tabulated (table 2). The highest number of shoots as a measure of proliferation was obtained by treatment of B5 combined with 0.5 mg/L was 7 shoots. At this treatment the shoot length was not the highest but could still be considered long as it was more than 2 cm and gave high number of leaves as well. Another treatment that allowed high number
of shoots were NN medium in combination with 0.25 mg/L BAP. However, this high shoot number of 8 was accompanied with significantly shorter shoot of 1.5 cm and significantly few numbers of leaves of 10. Another treatment medium that allowed reasonably high shoot number with long shoot was medium AR with 0.5 mg/L BA. It is clear that the interaction between media formulation and the concentration of growth regulator cytokinin BAP were very notable.

Table 2. The effect of media formulations and BAP concentrations on the growth variables of A. malaccensis shoots culture after 16 weeks.

| Formulation | Medium | BAP (mg/L) | Height of shoots | Number of leaves | Number of adventitious shoots | Number of roots |
|-------------|--------|------------|-----------------|-----------------|-------------------------------|----------------|
| MS          | 0      | 2.28 ± 0.11<sup>abc</sup> | 9.50 ± 1.07<sup>c-f</sup> | 1.50 ± 0.46<sup>bcd</sup> | 0.13 ± 0.13<sup>c</sup> |
| NN          | 0      | 2.84 ± 0.21<sup>a</sup> | 10.00 ± 0.57<sup>c-f</sup> | 0.13 ± 0.13<sup>d</sup> | 1.25 ± 0.16<sup>a</sup> |
| WPM         | 0      | 1.64 ± 0.14<sup>c-f</sup> | 11.13 ± 1.52<sup>b-f</sup> | 0.00 ± 0.00<sup>f</sup> | 1.13 ± 0.52<sup>ab</sup> |
| B5          | 0      | 1.78 ± 0.20<sup>b-f</sup> | 8.75 ± 0.90<sup>c-f</sup> | 0.88 ± 0.23<sup>d</sup> | 0.63 ± 0.26<sup>abc</sup> |
| AR          | 0      | 1.41 ± 0.19<sup>b-f</sup> | 6.75 ± 0.65<sup>def</sup> | 1.00 ± 0.46<sup>d</sup> | 0.50 ± 0.27<sup>bc</sup> |
| MS          | 0.25   | 1.65 ± 0.40<sup>c-f</sup> | 14.63 ± 3.78<sup>ab</sup> | 5.88 ± 1.86<sup>abc</sup> | 0.00 ± 0.00<sup>c</sup> |
| NN          | 0.25   | 1.56 ± 0.19<sup>c-f</sup> | 10.50 ± 1.91<sup>c-f</sup> | 8.38 ± 1.96<sup>a</sup> | 0.00 ± 0.00<sup>c</sup> |
| WPM         | 0.25   | 1.39 ± 0.06<sup>def</sup> | 14.25 ± 1.73<sup>ab</sup> | 6.25 ± 2.05<sup>abc</sup> | 0.00 ± 0.00<sup>c</sup> |
| B5          | 0.25   | 2.24 ± 0.08<sup>c-d</sup> | 20.00 ± 1.18<sup>b</sup> | 4.63 ± 0.68<sup>ab</sup> | 0.00 ± 0.00<sup>c</sup> |
| AR          | 0.25   | 1.76 ± 0.13<sup>bc-f</sup> | 11.63 ± 1.74<sup>b-f</sup> | 3.75 ± 0.67<sup>d</sup> | 0.00 ± 0.00<sup>c</sup> |
| MS          | 0.5    | 1.48 ± 0.03<sup>c-f</sup> | 16.38 ± 3.01<sup>abc</sup> | 5.25 ± 0.59<sup>abcd</sup> | 0.00 ± 0.00<sup>c</sup> |
| NN          | 0.5    | 1.43 ± 0.18<sup>c-f</sup> | 7.38 ± 0.94<sup>c-f</sup> | 5.88 ± 0.69<sup>abc</sup> | 0.00 ± 0.00<sup>c</sup> |
| WPM         | 0.5    | 1.29 ± 0.08<sup>ef</sup> | 13.63 ± 3.29<sup>ab-e</sup> | 6.75 ± 1.80<sup>ab</sup> | 0.00 ± 0.00<sup>c</sup> |
| B5          | 0.5    | 2.58 ± 0.28<sup>ab</sup> | 21.63 ± 2.68<sup>a</sup> | 7.13 ± 1.79<sup>ab</sup> | 0.00 ± 0.00<sup>c</sup> |
| AR          | 0.5    | 2.10 ± 0.12<sup>ab-e</sup> | 14.13 ± 0.69<sup>ab-e</sup> | 6.50 ± 1.56<sup>ab</sup> | 0.00 ± 0.00<sup>c</sup> |
| MS          | 1      | 1.43 ± 0.20<sup>c-f</sup> | 6.25 ± 1.71<sup>def</sup> | 4.88 ± 0.67<sup>abcd</sup> | 0.00 ± 0.00<sup>c</sup> |
| NN          | 1      | 1.34 ± 0.06<sup>ef</sup> | 7.88 ± 1.64<sup>c-f</sup> | 7.00 ± 1.34<sup>ab</sup> | 0.00 ± 0.00<sup>c</sup> |
| WPM         | 1      | 0.95 ± 0.07<sup>f</sup> | 7.00 ± 1.22<sup>def</sup> | 6.00 ± 0.93<sup>abc</sup> | 0.00 ± 0.00<sup>c</sup> |
| B5          | 1      | 1.25 ± 0.12<sup>ef</sup> | 5.38 ± 0.92<sup>def</sup> | 3.38 ± 0.56<sup>abcd</sup> | 0.00 ± 0.00<sup>c</sup> |
| AR          | 1      | 1.54 ± 0.08<sup>c-f</sup> | 7.38 ± 1.44<sup>c-f</sup> | 4.75 ± 0.96<sup>abcd</sup> | 0.00 ± 0.00<sup>c</sup> |
| MS          | 2      | 1.65 ± 0.25<sup>c-f</sup> | 9.50 ± 2.33<sup>c-f</sup> | 4.00 ± 0.38<sup>abcd</sup> | 0.00 ± 0.00<sup>c</sup> |
| NN          | 2      | 1.13 ± 0.04<sup>f</sup> | 3.50 ± 0.19<sup>ef</sup> | 3.13 ± 0.87<sup>abcd</sup> | 0.00 ± 0.00<sup>c</sup> |
| WPM         | 2      | 0.91 ± 0.10<sup>f</sup> | 4.88 ± 0.61<sup>ef</sup> | 3.88 ± 0.40<sup>abcd</sup> | 0.00 ± 0.00<sup>c</sup> |
| B5          | 2      | 1.61 ± 0.15<sup>c-f</sup> | 5.75 ± 1.08<sup>def</sup> | 3.38 ± 0.50<sup>abcd</sup> | 0.00 ± 0.00<sup>c</sup> |
| AR          | 2      | 1.33 ± 0.08<sup>ef</sup> | 3.88 ± 0.44<sup>c</sup> | 5.13 ± 0.95<sup>abcd</sup> | 0.00 ± 0.00<sup>c</sup> |

Description: Mean ± s.e. followed by the same letter in the same column are not significantly different by Duncan's multiple range test at α = 5%.

3.4 The effect of medium type and BAP concentration on root formation
The shoot cultures of A. malaccensis that grew on the medium formulation supplemented with all concentration of BAP did not develop roots, but roots developed in the medium formulation devoid of BAP (Fig 4). The root grow responded differently with different media formulation. Root developed as early as 6 weeks after subculture in NN medium and as late as 12 weeks after culture. Root numbers were higher in medium NN and WP followed by B5 and AR and the lowest number of roots developed in MS medium.
Figure 4. Root numbers of A. malaccensis shoots at 0-16 weeks cultured on MS, NN, B5, AR and WP medium devoid of BAP.

Several studies on agarwood plant tissue culture have reported the use of BAP for inducing shoot proliferation. The highest number of shoots obtained at 0.5 mg/L BAP (6.11 shoots), but then it decreased with increasing of BAP concentration [10]. Tiwari et al. [23] and Joshi et al. [24] suggest that high cytokinin concentrations could cause reduction of total of shoots, and high BAP concentrations could cause stunted shoots or canopy. Our results confirmed these studies and we have also obtained the best shoot growth of A. malaccensis with 0.5 mg/L BAP. Moreover, Tiengtum [25] reported that BAP was the most effective plant growth regulator to stimulate multiplication of shoots in vitro of agarwood (A. crassna and A. malaccensis). One of the most important factors in plant tissue culture especially in proliferation stage is cytokinin. It is well known that cytokinin play multiple roles in the plant development such as promotion of cell division and cell expansion, in plant protein synthesis stimulation and in the activation of some enzymes [26]. The cytokinin BAP is an inexpensive synthetic cytokinin and has been the mostly used growth regulator in plant tissue culture [27].

Several studies in A. malaccensis in vitro have used MS based medium with the addition of cytokinin and or auxin. For example, the agarwood explants grown in vitro in MS basal media containing BAP 0.5 mg/L or TDZ 0.25 mg/L produced the highest number of shoots and leaves of agarwood plantlets, as well as its plantlet shoot length [10]. Comparatively, several studies [11,12] also showed that the interaction between auxin (NAA) and Cytokinin (BAP) in MS medium has significantly influenced the growth of Aquilaria shoots during subculture, i.e. the best combination for shoot proliferation was 0.1 mg/L NAA and 2.5 mg/L BAP which resulted in proliferation of 11-12 shoots.

Our results also showed that the composition and concentration of nutrients contained in various medium formulation affect the growth and development of inocula, such as shoot proliferation, shoot elongation and leaf development. The best shoot growth was obtained in B5 medium whereas the most widely used MS medium did not support the best shoot growth, indicating that Aquilaria culture did not need nitrogen-rich medium such as MS medium.

Medium culture was made by considering the needs of the plants to grow and developed based on the purpose of cultured, therefore the composition of the media suitable for one plant can differ from other plants. Gamborg B5 is low in N total and low in NH₄⁺, but high in NO₃⁻ [18]. Woody plant medium (WPM) was customized for Kalmia [20], while Anderson and Rhododendron medium for Rhododendron plant species [19], and Nitch and Nitch medium was developed for anther rice culture [17].

The compositions and concentrations of media affect the response and growth of the plant. For examples, NH₄⁺ has been known to be inhibitory to trees such as avocado [28], reducing NH₄⁺ compared to NO₃⁻ was beneficial to avocado shoot cultures [29]. Other studies that modifying of macro nutrient in
MS medium by reducing the nitrogen and increasing the phosphor compound in medium in combination with sucrose concentration in *Tacca leontopentaloides* showed significant effect on shoot height, number of leaves and number of roots but not significant on the number of tubers [30].

Every medium in this experiment was supplemented with organic addenda such as myo-inositol, nicotinic acid, thiamin and pyridoxine, except AR medium which was supplemented with thiamine and high concentration myo-inositol of about 18 times higher than that of other media. Concentration of thiamine in Gamborg B5 medium was high (10 mg/L) and in MS medium in this experiment was very high (100 mg/L), much higher compared to the standard MS medium which is 0.1 mg/L. This thiamine concentration was raised to 0.4 mg/L in Linsmaier & Skoog medium [31]. In many plants, increase in vitamin concentrations enhance growth of callus, somatic cells, root formation and embryo development [32]. Vitamins are catalyst in the plant [33] and cofactors for carboxylase reaction and biosynthesis of amino acids [34]. Besides that, vitamins also affect growth and development of roots, and involve as antioxidant in the osmotic stress mechanism [35].

The root development of the shoots in this experiment occurred in medium devoid of BAP with different quantity dependent upon the medium formulation. The inhibition of root formation due to cytokinin in the medium is well known [36]. The medium formulation affects the number and the speed of root initiation. In *A. filaria*, the best medium for rooting were MS medium with addition of auxin – IBA (1-2 mg/L) or NAA (0.2–2 mg/L). For *A. agalocha*, 96.7% of shoots immersed in 5 μM NAA for 48h followed by cultured on half strength MS medium grew roots in two weeks later [37]. However, stimulation of root regeneration from *A. crassna* shoot showed that IBA was more effective than NAA, while no root growth showed in *A. malaccensis* shoot culture [25].

4. Conclusion

For slowly growing *A. malaccensis* shoot culture, shoot proliferation and growth require the present of low concentration of a cytokinin BAP in the range of 0.25–0.5 mg/L combined with low nitrogen content in medium such as B5 medium. The standard MS medium known for its high nitrogen content did not support the best growth. Other medium formulation such as NN, WPM and AR that were customized for specific species did not support the best growth of *A. malaccensis* shoot culture either. Rooting of the shoot formation were better in medium containing low nutrient concentrations devoid of BAP which
indicated the inhibitory effect of a cytokinin. Better rooting could be induced in low nutrient medium supplemented with auxin.

Acknowledgements
The authors would like to thank Dr. Hartati as the research coordinator in the Research Center for Biotechnology, Erwin Al Hafiizh, Evan Maulana, Lutvinda Ismanjani, and Destiana who had supported and assisted the work in the laboratory. This research was funded by DIPA CITES 2018 from Research Center for Biology LIPI.

References
[1] Naef R 2011 Flavour and Fragrance Journal 26 73-87
[2] CITES 2017 Convention on International Trade in Endangered Species of Wild Fauna and Flora: Amandements to Appendices I and II of CITES
[3] Barden A, Awang Anak N, Mulliken TA, Song M 2000 Heart of the matter: agarwood use and trade and CITES implementation for Aquilaria malaccensis. TRAFFIC International
[4] Liu Y, Chen H, Yang Y, Zhang Z, Wei J, Meng H, Chen W, Feng J, Gan B, Chen X, Gao Z, Huang J, Chen B and Chen H 2013 Molecules 18(3) 3086-106
[5] Tan C S, Isa N M, Ismail I and Zainal Z 2019 Frontiers in Plant Science 10 122-122
[6] Ahmed M and Gogoi P 2000 Proceeding of Seminar on Scope & Dimension of Agar (Aquilaria spp.) Plantation in N.E. region Khanapara, Gwahati 28-33
[7] Jayusman 2005 Jurnal Penelitian Hutan Tanaman 2(3)117-124
[8] Kosmiatin M, Husni A and Marisa I 2005 Jurnal AgroBiogen 1(2) 62-67
[9] Chiu SJSH 2016 In vitro cultures of Aquilaria malaccensis for agarwood production. University of Nottingham
[10] Azwin, Siregar I Z and Supriyanto 2006 Media Konservasi 11(3) 98-104
[11] Juliandi, Wulandari R S and Darwati H 2013 Jurnal Hutan Lestari 1(3) 327-335
[12] Karlianda N, Wulandari R S and Mariani Y 2013 Jurnal Hutan Lestari 1(1) 1-8
[13] Gultom M S, Anna N and Siregar E. BM 2012 Feronema Forestry Science Journal 1(1) 1-6
[14] Hapsari B W, Rudyianto and Ermayanti T M 2019 Seminar Nasional Konservasi dan Pemanfaatan Tumbuhan dan Sawa Liar Bogor 297-309
[15] Van Minh T 2005 37-42
[16] Murashige T and Skoog F 1962 Physiologia Plantarum 15 473-497
[17] Nitsch JP and Nitsch C 1969 Science 163(3862) 85-87
[18] Gamborg O L, Miller R A and Ojima K 1968 Exp Cell Res 50(1) 151-158
[19] Anderson W C 1984 Journal of the American Society for Horticultural Science 109(3) 343-347
[20] Lloyd G and McCown B H 1981 Proceeding of the International Plant Propagation Society 30 421-427
[21] R Core Team 2019 R: A language and environment for statistical computing. 3.5.3 edition ed. R Foundation for Statistical Computing, Vienna, Austria
[22] de Mendiburu F 2019 agricolae: Statistical Procedures for Agricultural Research, R package version 1.3-1 ed
[23] Tiwari V, Tiwari K N and Singh BD 2001 Plant Cell, Tissue and Organ Culture 66(1) 9-16.
[24] Joshi M and Dhar U 2003 Plant Cell Reports 21(10) 933-939
[25] Tiengtum P 1995 Kasetsart Univ 68-75
[26] Arab M M, Yadollahi A, Shojaeiyan A, Shokri S and Ghojah S M 2014 Journal of Genetic Engineering and Biotechnology 12 81-87
[27] George E F, Hall M A and Klerk G J D 2008. Plant Growth Regulators II: Cytokinins, their Analogues and Antagonists In: George, E.F., Hall, M.A., Klerk, G.-J.D., editors. Plant Propagation by Tissue Culture: Volume 1. The Background. Springer Netherlands: Dordrecht, p. 205-226
[28] Witjaksono, Litz R E and Grosser J W 1998 Plant Cell Reports 18(3) 235-242
[29] Witjaksono and Litz RE 1999 *Plant Cell, Tissue and Organ Culture* **58**(2) 141-148
[30] Rudiyanto, Hapsari B W and Ermayanti TM *Jurnal Biologi Indonesia* **14**(1) 11-21
[31] Linsmaier E M, Skoog F 1965 *Physiologia Plantarum* **18**(1) 100-127
[32] Tomar R S, Khamba S, Kaushik S and Mishra R K 2018 *International Journal for Research in Applied Science and Engineering Technology* **6**(3) 423-426
[33] George E F, Hall M A and Klerk G J D 2007 *Plant Propagation by Tissue Culture*. Springer Netherlands
[34] Abrahamian P and Kantharajah A 2011 *American Journal of Plant Sciences* **2**(5) 669-674
[35] Chen H and Xiong L 2005 *The Plant Journal* **44**(3) 396-408
[36] Stenlid G 1982 *Physiologia Plantarum* **56**(4) 500-506
[37] He M L, Qi S Y and Hu L J 2005 *Journal of Zhejiang University. Science. B* **6**(8) 849-852