In vitro propagation of white mahang (*Macaranga hypoleuca* (Reichb.f.et Zoll.) Mull Arg.)

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**Abstract.** White mahang (*Macaranga hypoleuca* Mull.Arg) is considered as an alternative species for light construction and traditional medicine. *In vitro* propagation, it has been attempted through series of *in vitro* propagation protocols, i.e., explant sterilization, shoots multiplication, root induction, and acclimatization using terminal and axillary buds explants. WPM basal medium supplemented by plant growth regulators BAP (cytokinin group), NAA and IBA (auxin group) were used for shoot regeneration studies. Various concentration of BAP (0, 0.5, 1 mg l$^{-1}$), IBA (0, 0.1, 0.5, 1 mg l$^{-1}$) and NAA (0, 0.01, 0.05, 0.1 ml l$^{-1}$) were tested for shoot induction and elongation. Interaction between BAP concentration and explant types, both those taken from apex- and axillary- buds showed significant differences for shoot induction and elongation. The highest number of shoots were resulted from the WPM media containing BAP (0.5 mg l$^{-1}$), while the addition of IBA (0.5-1.0 mg l$^{-1}$) and NAA (0.01-0.1 mg l$^{-1}$) combined with BAP (0-1.0 mg l$^{-1}$) produced a high percentage of callus (> 60%). In vitro, rooting was induced on half-strength MS media supplemented with IBA (1 mg l$^{-1}$). The regenerated shoots with developed root systems were successfully acclimatized and established in pots containing sand and compost (3:1).

1. Introduction

In general, the species of genera Macaranga – as pioneer trees, have essential roles in forest succession from degraded land or abandoned fields areas [1]. The Macaranga species are also well-known as multi-purposes plant species for the local people as these trees can be used for general housing, frames, packing, musical instrument, matches, and particle-board. It is also used for traditional medicine because it contains phytochemical ingredients of the leaves and bark, which containing tannin compounds, flavonoids, macarangin, etc. [1, 2]. For example, *Macaranga hypoleuca* or white mahang is widely known has antiplasmodial-, expectorant- and febrifuge- effects [2]. Despite its ecological roles and utilization to support human needs, the silvicultural techniques – including its propagation, are have not yet studied comprehensively.

Mahang tress can be propagated through generative- and vegetative- methods. Vegetative propagation can be conducted either by conventional techniques such as grafting and cuttings, or more advanced technique using plant tissue culture method. The cutting technique provides a different survival rate depending on the seedling nurturing conditions and cutting source. The cutting technique, which followed by nurturing in a greenhouse with a fog-cooling system, resulted in a 74% survival
rate after three months [3], while the survival rate of the cutting originated from stump sprouting was only 6.67 – 35% [4]. These conditions are not sufficient to meet the needs of white mahang seedlings. In order to obtain large amounts of plant material, it is, therefore, necessary to develop a more prospective propagation technique, i.e., micropropagation using tissue culture method. The technique offers certain advantages over the traditional method, viz. its ability to produce genetic-identical seedlings with the mother tree in large quantities without depending on the season.

The application of plant tissue culture to regenerate tree species has been commonly used in the past few years. Tissue culture techniques have been applied for mass production of Eucalyptus pellita clones in plantations industries in Indonesia. Several forest trees, such as Eucalyptus sp. [5, 6], Terminalia catappa [7], Araucaria excelsa [8], Pinus pinaster [9], Pterocarpus santalinus [10], Enterolobium cyclocarpum [11], teak [12], Styrax benzoin [13], also have been successfully propagated through tissue culture method.

Plant tissue culture, which depends on the concept of plant cell’s totipotency in a controlled environment, is determined by several factors, i.e., type of explants, culture media, plant growth regulators (PGR), culture pH and the physical environment [14]. Thousands of plants can be produced from small pieces of tissue (which known as ‘explant’) using the technique, which consists of several stages. Those stages are: (a) Explant sterilization and shoots induction – a serial procedure to obtain aseptic explants that will be grown in an aseptic- and axenic- in vitro condition; (b) Shoots multiplication – a step to increase the numbers of shoots by repeated subcultures into new media containing certain PGR, such as BAP (benzyl amino purine), BA (benzyl adenine), kinetin, thidiazuron, 2i-P (2-isopentenyl adenine/6g,g-dimethylallyl amino purine, zeatin, PBA (6-benzyl amino)-9(2-tetrahydropranyl) purine -9H), until the planned numbers of plants are attained; (c) Root formation which is carried out by sub-culturing it into fresh media containing PGR from auxins group that can stimulate root growth, such as NAA (naphtalene acetic acid), IBA (indole butyric acid), IAA (indole acetic acid), 2,4-D (2,4 dichlorophenoxy acetic acid), CPA (chloropenoxy acetic acid); (d) Acclimatization – an adaptation phase of rooted shoots (plantlets) on soil media in a nursery before they were planted in the field [19].

This research aims to study white mahang propagation using plant tissue culture techniques. It is conducted in order to address the need for a large number of white mahang (M. hypoleuca) seedlings that cannot be supplied by generative- and traditional vegetative- regenerations.

2. Material and methods
2.1. Explants collection
The seed of white mahang was obtained from Muara Takus, West Pasaman, West Sumatera. Seeds were sown on the germinating media in the greenhouse until approximately 40cm-juvenile shoots were grown. Explants were taken from apex- and axillary- parts of the selected shoots. Three days before explants collection, the shoots were watered with a solution contains 0.2% fungicide and 0.2% bactericide.

2.2. Culture media
Wood Plant Medium (WPM) basal media supplemented with various concentrations of auxins and cytokinin were used for shoots initiation and multiplication. While WPM enriched by 20 g l \(^{-1}\) sugar without PGR was used for shoots extension. The MS (Murashige and Skoog) medium supplemented with different concentration IBA was used for root induction [14].

2.3. Explant sterilization and establishment of aseptic conditions
Surface sterilization of the explants was conducted through a series of washing steps, viz. 0.2% fungicide solution for 60 min, 0.2% bactericide for 60 min, distilled water to remove the remained fungicide and bactericide, 10% NaOCl for 10 min, 15% NaOCl for 15 min, 70% alcohol and five times washed in sterile distilled-water to removes the trace elements.
The sterilized explants (approx. 1 - 1.5 cm length) were put onto WPM basal medium without growth regulator as a precondition media. The cultures were incubated in a room equipped irradiation for at least 12 hours/day and maintained at a maximum temperature of 24 °C and humidity level of 70 - 80%. The observation was conducted in 14 days to assess whether the explants are keeping alive in aseptic conditions, being contaminated or dead.

2.4. Shoot multiplication
The alive & aseptic shoot explants were then transferred from precondition medium onto multiplication media, namely WPM supplemented by various plant growth regulators (cytokinin and auxin). Different concentration of BAP (0, 0.5, 1 mg l	extsuperscript{-1}), IBA (0, 0.1, 0.5, 1 mg l	extsuperscript{-1}) and NAA (0, 0.01, 0.05, 0.1 mg l	extsuperscript{-1}) were used for shoots initiation. The number of shoots per explant and percentage of callus was recorded after six weeks of culture. For shoot multiplication and long-term establishment, the regenerated tissues were then sub-cultured onto fresh WPM medium supplemented with 20 gr l	extsuperscript{-1} sugar.

2.5. Root induction and acclimatization
The regenerated shoots (2 – 3 cm length) were excised and treated in rooting media composed of half-strength MS medium supplemented with IBA (0, 1,2,3, 4 g l	extsuperscript{-1}). The numbers of shoots with root- or callus- formation were recorded. Rooted shoots (plantlets) were then removed from the culture medium and washed gently under running tap water to remove any adherent gel from the roots and transferred to the cup containing a sterilized mixture of sand and compost (3:1). In order to maintain the high humidity (80%) and minimizing shocked environmental effects to the plantlets during acclimatization, the cups were covered with transparent plastic bags and regularly watered. The plastic covers were removed after two weeks in order to adapt the plants to the outdoor conditions and maintained them in a greenhouse under normal day length conditions. The survival plantlets were recorded after four weeks.

3. Result and discussion
3.1. Shoot sterilization
In theory, all plant organs or tissues are totipotent, so that can be used as explants sources. In fact, different parts of plants result in different success levels of in vitro regeneration. Juvenile shoots were selected as explants because they were fresh tissue, vigor, and containing more meristematic cells. In this experiment, the used shoots were collected from the apex and axillary buds.

Different surface sterilization methods were applied for apex and axillary shoots. Sterilization with 10% NaOCl	extsubscript{2} for 10 minutes was better for apex shoots. The application of this concentration for axillary buds resulted in heavy bacterial contamination. The best concentration for sterilizing axillary shoots, which resulted in less contamination, was 15% NaOCl	extsubscript{2} for 15 minutes. However, when this method was applied to sterilize the apex shoots, it causing browned tissues, which forced to the death of explants. This indicates that the sterilization of apex shoots should use a lower concentration and short immersion because the apex shoots were consist of more juvenile cells compared to the axillary shoots.

The efficacy of shoot sterilization methods was determined by visual observation. The alive and un-contaminated explants were indicated from its fresh-green tissues that thrived. After 14-days observation, the trials showed that 76% of apex shoot explants were kept in aseptic conditions, and the rest was contaminated. While the explants taken from axillary shoots showed that 70% was in aseptic condition, 24% was contaminated, and 6% was browning.

3.2. Induction and multiplication of shoots
The effects of plant growth regulators (auxins and cytokinin) on shoot regeneration of white mahang explants were presented in Table 1.
Table 1. Effect of growth regulator on shoot regeneration from apex shoot and axillary shoot explants of *M. hypoleuca* on the WPM medium.

| Auxin- Cytokinin (mg/l) | Apex shoot | Axillary shoot |
|-------------------------|------------|----------------|
|                         | Shoot number | Callus percentage | Shoot number | Callus percentage |
| IBA-BAP                 |             |                 |               |                 |
| 0-0                     | 1           | 10              | 1             | 10              |
| 0-0.5                   | 7           | 21              | 5             | 10              |
| 0-1                     | 6           | 10              | 5             | 10              |
| 0-1-0                   | 1           | 60              | 2             | 60              |
| 0-1-0.5                 | 1           | 80              | 2             | 80              |
| 0.1-5                   | 1           | 40              | 0             | 60              |
| 0.5-0                   | 1           | 60              | 0             | 60              |
| 0.5-0.5                 | 1           | 80              | 1             | 80              |
| 0.5-1                   | 4           | 40              | 2             | 80              |
| 1-0                     | 1           | 80              | 1             | 100             |
| 1-0.5                   | 1           | 80              | 1             | 80              |
| 1-1                     | 0           | 80              | 1             | 80              |
| NAA-BAP                 |             |                 |               |                 |
| 0-0                     | 1           | 10              | 1             | 10              |
| 0-0.5                   | 6           | 20              | 5             | 10              |
| 0-1                     | 4           | 10              | 5             | 10              |
| 0.01-0                  | 1           | 40              | 1             | 40              |
| 0.01-0.5                | 1           | 80              | 1             | 80              |
| 0.01-1                  | 1           | 80              | 2             | 80              |
| 0.05-0                  | 1           | 80              | 1             | 80              |
| 0.05-0.5                | 1           | 60              | 1             | 60              |
| 0.05-1                  | 1           | 80              | 0.25          | 80              |
| 0.1-0                   | 1           | 80              | 1             | 80              |
| 0.1-0.5                 | 1           | 80              | 0.25          | 80              |
| 0.1-1                   | 1           | 80              | 0             | 80              |

Source: Bogidarmanti and Nuroniah (2012)[15]

Multiplication of shoots was observed in 10 to 14 days after planting. Explants from the apex shoots showed better multiplication results compared to those from the axillary shoots (Table 1; Figure 1.b and 1.c). Addition 0.5 - 1 mg l\(^{-1}\) BAP into the WPM medium stimulates the highest shoot-multiplication to compare to other concentrations, which resulted in 5 and 6 - 7 shoots from axillary and apex shoot explants, respectively (Table 1).

3.2.1. Effects of IBA and BAP to induce shoot regeneration of white mahang. Analysis of variants of the effect of IBA and BAP on the shoots induction of white mahang explants was presented in Table 2.

Table 2. Analysis of variance the effect of IBA and BAP growth regulators on shoot induction of white mahang explants.

| Source | DF | Sum of squares | Mean squares | F      | Pr > F |
|--------|----|----------------|--------------|--------|--------|
| IBA    | 3  | 167.333 | 55.7777 | 133.8667 | < 0.0001* |
Table 3. Duncan's further tests of IBA*BAP*Explant combination treatment on shoot induction of white mahang explant.

| Category           | Mean (Number of shoots) | Groups |
|--------------------|-------------------------|--------|
| 1*2*Apex           | 7.0000                  | A      |
| 1*3*Apex           | 6.0000                  | B      |
| 1*2*Axillar        | 5.0000                  | C      |
| 1*3*Axillar        | 5.0000                  | C      |
| 3*3*Apex           | 4.0000                  | D      |
| 2*2*Axillar        | 2.0000                  | D      |
| 3*3*Axillar        | 2.0000                  | E      |
| 2*1*Axillar        | 2.0000                  | E      |
| 2*2*Apex           | 1.0000                  | E      |
| 1*1*Apex           | 1.0000                  | F      |
| 4*2*Axillar        | 1.0000                  | E      |
| 3*2*Apex           | 1.0000                  | F      |
| 3*1*Apex           | 1.0000                  | F      |
| 3*2*Axillar        | 1.0000                  | F      |
| 2*1*Apex           | 1.0000                  | E      |
| 2*3*Apex           | 1.0000                  | F      |
| 4*1*Axillar        | 1.0000                  | F      |
| 4*1*Apex           | 1.0000                  | E      |
| 4*3*Axillar        | 1.0000                  | F      |
| 4*3*Axep           | 1.0000                  | F      |
| 1*1*Axillar        | 1.0000                  | E      |
| 2*3*Axillar        | 0.0000                  | F      |
| 4*2*Apex           | 0.0000                  | F      |
| 3*1*Axillar        | 0.0000                  | F      |

Remarks: IBA 1 (0 mg l⁻¹), IBA 2 (0.1 mg l⁻¹), IBA 3 (0.5 mg l⁻¹), IBA 4 (1.0 mg l⁻¹) BAP 1 (0 mg l⁻¹), BAP 1 (0.5 mg l⁻¹), BAP 3 (1.0 mg l⁻¹)

Table 2 showed that the influence of IBA, BAP, their interaction, and the source of explants provide a significant difference in the number of shoots grown from the white mahang explants. Duncan's further tests were carried out to determine which treatments make a difference, and the results were listed in Table 3.
Combination of IBA (0 mg l\(^{-1}\)), BAP (0.5 mg l\(^{-1}\) or 1.0 mg l\(^{-1}\)) and the explants types (both apex- and axillary buds) were different from other combination treatments (Table 3). The dosages combination of these plant-growth regulators applied to either apex or axillary buds can produce the highest number of shoots of white *mahang*.

### 3.2.2. Effects IBA and BAP to induce the callus-mass formation of white mahang.

Analysis of variance of the effect of IBA and BAP growth regulator on callus-mass of white *mahang* explant was presented in Table 4.

**Table 4.** Analysis of variance the effect of IBA and BAP growth regulator on callus mass of white *mahang* explant.

| Source          | DF  | Sum of squares | Mean squares | F               | Pr > F       |
|-----------------|-----|----------------|--------------|-----------------|--------------|
| IBA             | 3   | 72.204.1667    | 24.068.0556  | 1.732.9000      | < 0.0001*)   |
| BAP             | 2   | 2.308.3333     | 1.154.1667   | 83.1000         | < 0.0001*)   |
| Explant         | 1   | 816.6667       | 816.6667     | 58.8000         | < 0.0001*)   |
| IBA*BAP         | 6   | 3.033.3333     | 505.5556     | 36.4000         | < 0.0001*)   |
| IBA*Explant     | 3   | 558.3333       | 186.1111     | 13.4000         | < 0.0001*)   |
| BAP*Explant     | 2   | 808.3333       | 404.1667     | 29.1000         | < 0.0001*)   |
| IBA*BAP*Explant | 6   | 1.566.6667     | 261.1111     | 18.8000         | < 0.0001*)   |

*Remarks: *) significantly different at 5% level

Table 4 showed that the influence of IBA and BAP, their interaction, and the source of explants provide a significant difference in callus formation of white *mahang* explants. Duncan's further tests were carried out to determine response differences amongst the treatments (Table 5).

**Table 5.** Duncan's further tests of IBA * BAP * Explant combination on callus mass of white *mahang* explants.

| Category | Mean of callus percentage (%) | Groups |
|----------|--------------------------------|--------|
| 4*1*Axillar | 92.5000                      | A      |
| 3*3*Axillar | 80.0000                      | B      |
| 2*2*Apex   | 80.0000                      | B      |
| 4*1*Apex   | 80.0000                      | B      |
| 4*2*Axillar | 80.0000                      | B      |
| 4*2*Apex   | 80.0000                      | B      |
| 4*3*Axillar | 80.0000                      | B      |
| 4*3*Apex   | 80.0000                      | B      |
| 3*2*Apex   | 80.0000                      | B      |
| 2*2*Axillar | 80.0000                      | B      |
| 3*2*Axillar | 80.0000                      | B      |
| 3*1*Axillar | 65.0000                      | C      |
| 3*1*Apex   | 62.5000                      | C      |
| 2*3*Axillar | 60.0000                      | C      |
| 2*1*Axillar | 60.0000                      | C      |
| 2*1*Apex   | 60.0000                      | C      |
In comparison to other treatments, the application of 1 mg l\(^{-1}\) IBA (without BAP) resulted in the highest callus percentage derived from axillary explants (92.5%). The application of IBA (0.1 – 0.5 mg l\(^{-1}\)) and BAP (0.5 – 1.0 mg l\(^{-1}\)) resulted in callus percentage ranging from 65 – 80%. While the lowest callus percentage (10%) was observed both from axillary- and apex- buds explants which grown on the media added with BAP (without IBA), concentrations were 0, 0.5, 1.0 mg l\(^{-1}\).

### 3.2.3. Effects of NAA and BAP to induce shoots regeneration of white mahang.

Analysis variance of the effects of NAA and BAP to induce shoot regenerations of white mahang explants was presented in Table 6.

Table 6. Analysis of variance of the influence of BAP and NAA growth regulator on shoots induction of white mahang.

| Source          | DF  | Sum of squares | Mean squares | F          | Pr > F  |
|-----------------|-----|----------------|--------------|------------|---------|
| NAA             | 3   | 138.7083       | 46.2361      | 154.8372   | < 0.0001* |
| BAP             | 2   | 2.2708         | 1.1354       | 3.8023     | 0.0269*  |
| Explant         | 1   | 0.3750         | 0.3750       | 1.2558     | 0.2662 ns |
| NAA*BAP         | 6   | 10.2292        | 1.7049       | 5.7093     | < 0.0001* |
| NAA*Explant     | 3   | 2.7083         | 0.9028       | 3.0233     | 0.0351*  |
| BAP*Explant     | 2   | 17.4375        | 8.7188       | 29.1977    | < 0.0001* |
| NAA*BAP*Explant | 6   | 69.7292        | 11.6215      | 38.9186    | < 0.0001* |

Remarks: *) significant at level 5%; ns is not significant at 5% level

Table 6 showed that the explant types (either apex- or axillary- shoots) did not significantly affect the in vitro regeneration of white mahang. The combination of NAA and BAP and their interactions showed more noticeable differences compared to other treatments. Duncan's further tests were conducted to find out which treatments made the difference (Tables 7).

Table 7. Duncan test analysis for NAA and BAP growth regulator for inducing shoots on white mahang explant.

| Category | Mean of shoot number | Groups |
|----------|----------------------|--------|
| 1*2      | 4.5000               | A      |
| 1*1      | 3.5000               | B      |
| 1*3      | 3.0000               | B      |
Based on Duncan's further test, the combination of NAA (0 mg l\(^{-1}\)) + BAP (0.5 mg l\(^{-1}\)) significantly different from other treatments and produced the highest number of shoots. This also showed that the increase of NAA and BAP concentrations tend to reduce the number of induced shoots.

### 3.2.4. Effects of NAA and BAP to induce the callus-mass formation of white mahang

The addition of NAA and BAP into basal media will induce various growth and development responses of the white mahang explants. Apart from shoots regeneration, this trial also showed that the growth of callus-mass was observed. The results of the analysis variance of the trial were presented in Table 8.

**Table 8.** Analysis of variants of the influence of BAP and NAA on callus mass formation of white mahang explants.

| Source       | DF  | Sum of squares | Mean squares | F          | Pr > F       |
|--------------|-----|----------------|--------------|------------|--------------|
| NAA          | 3   | 70,583.3333    | 23,527.7778  | 2,823.3333 | < 0.0001*    |
| BAP          | 2   | 1,633.3333     | 816.6667     | 98.0000    | < 0.0001*    |
| Explant      | 1   | 16.6667        | 16.6667      | 2.0000     | 0.1616 ns    |
| NAA*BAP      | 6   | 9,166.6667     | 1,527.7778   | 183.3333   | < 0.0001*    |
| NAA*Explant  | 3   | 50.0000        | 16.6667      | 2.0000     | 0.1216 ns    |
| BAP*Explant  | 2   | 33.3333        | 16.6667      | 2.0000     | 0.1428 ns    |
| NAA*BAP*Explant | 6 | 100.0000 | 16.6667 | 2.0000 | 0.0768 ns |

Remarks: *) is significantly different at 5% level; ns) is not significant at 5% level

Table 8 showed that concentrations of NAA, BAP, and the interaction among these two factors have a significant effect on the explant's response on callus-mass formation. Duncan's further tests were conducted to determine which treatment can make a difference. The results are listed in Table 9.

**Table 9.** Duncan's further tests of the treatment combination of the NAA and BAP in inducing callus of white mahang explant.

| Category | Mean of callus percentage (%) | Groups |
|----------|--------------------------------|--------|
| 2*2      | 80.0000                        | A      |
| 2*3      | 80.0000                        | A      |
| 3*1      | 80.0000                        | A      |
Table 9 showed that the higher NAA concentration being applied, the higher the percentage of callus-mass formation was recorded. While the application BAP without the addition of NAA yielded less callus-mass formation (10 - 15%).

The response of explants to form shoots or callus is determined by several factors, i.e., media, plant growth regulators, and the species itself. The effect of type and various concentrations of plant growth regulator was examined in this study. The role of cytokinins such as kinetin, 2iP, and BAP in shoot multiplication has been widely reported. This has been resulted in a significant effect in inducing adventitious shoots.

Another study showed that the optimum level of cytokinin BAP without auxin was in a range of 1 mg/l in Formosan sweet gum [16] and 0.5 mg l⁻¹ in Ceratonia siliqua [17]. Even though the optimum concentration in this study was the highest cytokinin-BAP (1 mg/l), but the addition of cytokinin concentration does not mean increase bud formation, as observed in Formosan sweet gum [16], Terminalia catappa [7] and Cassia siamea [18].

From these results, it appears that the multiplication of shoots affected by each plant growth regulator but not influenced by the interaction between cytokinin and auxin. Auxin inhibits the multiplication of shoots by inducing the elongation of shoots. On the contrary, cytokinin (BAP) stimulates the growth of new shoots. The use of cytokinin without auxin formed better shoots multiplication in the process of in vitro propagation of white mahang; as well as similar results were founded in other studies [15, 16]. This is due to the function of cytokinin BAP to stimulate cell division and suppress apical dominancy. Meanwhile, buds tend to multiply with short nodus [17]. BAP mainly reported more effective than other cytokines to propagate woody plants [19].

Callus formation mainly observed in media supplemented with auxin (IBA, NAA). The emergence of callus was negatively correlated with the number of shoots produced. Similar results were also described by other studies and assume that the callus is an inhibitor of the formation of shoots and roots [17, 18].

3.3. Rooting induction and acclimatization

Multiplied shoots were sub-cultured in the WPM medium enriched with sugar (20 g l⁻¹). Subculture was intended to extend the formed buds. Shoots with 2-3 cm height were achieved after four weeks of culture. These shoots were then transferred to rooting medium MS medium plus IBA concentrations (0, 1, 2, 3, 4 g l⁻¹). IBA is more effective than other auxins (NAA, IAA) for rooting induction [18, 20].

Rooting has been observed after two weeks of culture. The addition of plant growth regulators in rooting media is critical on the growth of roots, as rooting medium without plant growth regulator resulted in un-rooted plants (Table 11). The best root growth without callus formation obtained using MS medium supplemented of IBA (1 mg l⁻¹). More addition of IBA (started 2 mg l⁻¹) resulted in roots with callus formation, which is something that is avoided, as callus will reduce the quality of the root growth. Better root growth at low IBA concentrations was also reported in the tissue culture of
Pterocarpus santalinus [10] and Cassia siamea [18]. High concentrations of IBA formed callus prior to the induction of root.

**Table 10.** Effect of IBA on rooting of white mahang on half-strength MS medium.

| IBA concentration (mg/l) | Apex shoot | Axillary shoot |
|--------------------------|------------|----------------|
|                          | Root number | Callus percentage | Root number | Callus percentage |
| 0                        | 0          | 0               | 0           | 0               |
| 1                        | 3          | 10              | 2           | 10              |
| 2                        | 2          | 40              | 2           | 40              |
| 3                        | 2          | 70              | 1           | 60              |
| 4                        | 2          | 70              | 1           | 70              |

One of the crucial stages during *in vitro* propagation is acclimatization. This process removes the propagated plants (plantlets) from the culture to soil medium and fits for planting in the field conditions. Plantlets with well-developed roots were transferred to the mixed media sand and fine compost and maintained in the greenhouse in order to control the environment to 80% relative humidity and temperature of about 25°C- the survival rate of white mahang plantlets in greenhouse presented in Table 11.

**Table 11.** The average number of leaves and the survival rate of white mahang plantlets in the acclimatization process.

| No. | Treatment | Repetition | Research Unit | The average number of leaves | Survival rate (%) |
|-----|-----------|------------|---------------|-----------------------------|-------------------|
| 1.  | IBA 1 mg/l| 1          | 10            | 6                           | 100               |
|     |           | 2          | 10            | 6                           | 100               |
|     |           | 3          | 10            | 8                           | 100               |
| 2.  | IBA 2 mg/l| 1          | 10            | 4                           | 100               |
|     |           | 2          | 10            | 6                           | 100               |
|     |           | 3          | 10            | 6                           | 100               |

Based on Table 11 above, all plantlets that had been treated with IBA 1 mg l\(^{-1}\) or 2 mg l\(^{-1}\) had a survival rate of 100%. The average number of leaves formed after four weeks in the acclimatization process in greenhouse vary from 4 – 8 leaves.
Figure 1. Developmental stages of *in vitro* regeneration *M. hypoleuca* from explants to plantlets. 

a. aseptic apex shoot after 14 days of culture.

b. shoot multiplication of apex shoot on WPM+BAP (0.5 mg l\(^{-1}\)) after 6 weeks of culture.

c. rooting of shoots on MS +IBA (1 mg l\(^{-1}\)).

d. aseptic axillary shoot after 14 days of culture.

e. shoot multiplication of axillary shoot on WPM+BAP (0.5 mg l\(^{-1}\)) after 6 weeks of culture.

f. rooting of shoots on MS+IBA (1 mg l\(^{-1}\)).

g. acclimatization step in.hood.

h. Plantlet after acclimatization for 4 weeks.

4. Conclusion

In conclusion, an effective protocol for white *mahang* (*Macaranga hypoleuca* Mull.Arg) micropropagation was developed. The addition of cytokinin influenced shoot induction. The highest percentage of shoot formation was obtained using WPM medium + BAP (0.5 mg l\(^{-1}\)). The optimum rooting medium obtained using half-strength MS + IBA (1 mg l\(^{-1}\)). Acclimatization was conducted in four weeks in a controlled environment at 80% relative humidity and temperature under 25 °C.

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