The Formation of Shoots in Mangosteen (Garcinia Mangostana L.) Nodules From Bengkalis, Riau in Murashige Skoog and Woody Plant Medium

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Abstract. One of the mangosteen producers in Indonesia is Riau Province, Bengkalis regency to be exact. This mangosteen has some superiority, such as able to grow in peat swamp1). Nodules are cells that develop to become shoots only in proper nutrients and conditions. This study aimed to discover the response of mangosteen nodules (Garcinia mangostana L.) on MS and WPM media by adding BAP. This study used Completely Randomized Design using various concentrations of BAP and different media. The results of this study indicate that the best response on MS medium is the percentage of nodule formation at 1 mg / l BAP of 49.99%. The fastest callus appears on control and 5 mg/l BAP 17.25 and 6.75 days after planting (dap). The most significant number of nodules at 1 mg/l of BAP is 1.50 nodules. The callus texture is compact and crumbs with white and brown colour. The best response on WPM media is the percentage of formation of a nodule at 1 mg/l of BAP resulting 33.33%. The fastest shoots emerge at 1 mg / l BAP 13.25 dap. The highest number of nodules at 1 mg/l of BAP is 1.00 nodule, the highest leaf amount at 3 mg/l BAP was 6.75 sheets, and the highest shoot length at 5 mg / l BAP is 1.32 cm. The texture callus compact and crumb with white colour.

1. Introduction
Mangosteen (Garcinia mangostana L.), is an original fruit from Indonesia that has high economy value. Mangosteen have some specialities and excellence taste therefore this fruit called as queen of fruit and finest in the world. Mangosteen also rich with chemical compounds such as xanthone, (alfamangosteen and gamma mangosteen) anthocyanin and many phenolic compounds that play a great role in pharmacy and medicine.

The agribusiness prospect of mangosteen has a bright future and the chances to develop mangosteen is widely opened. The demand of mangosteen from foreign country keep increase until 10% per year. Mangosteen production in Indonesia able to achieve 20.29 ton at 2012. However, escalation in export in Indonesia both of international and local market still facing many obstacles [1]. Demand of mangosteen in global market keep increase because mangosteen is one of the most favorites fruits from tropical fruits. One of the highest consumer of mangosteen from Indonesia is Taiwan. One of the biggest producer of mangosteen in Indonesia is Riau province, Bengkalis regency to be exact. Mangosteen production in Riau at 2012 able to achieve 2.618 ton. Mangosteen originates from Bengkalis has some superiority such as able to growth in swamps, tolerant to acid soil (peat), faster time to flowering started at aged 7 year compared to another region. Harvesting time take place from July- September while other region from November to March [2, 3]
Propagation of mangosteen by vegetative has less benefits because seeds of mangosteen is recalcitrant and does not have a dormant period. Propagation vegetatively such as transplantation and grafting has low rate of success [4]. As a consequence, another vegetative propagation is hugely required to produce many superior seeds by tissue culture technique or in vitro[5]. That rate of success of in vitro method was affected by the selection of explant, growth regulator and types of medium [6]. Basic medium that frequently used for in vitro culture for mangosteen plant is Murashige Skoog (MS) and Woody Plant Media (WPM).

Murashige Skoog medium is a basic medium that contain nitrate, potassium, and high ammonium. conduct an in vitro research from banana jackfruit and shows that wounding in the explant surface will develop to formed into shoots [7]. The mangosteen seeds explant will producing nodules at first that intended to become shoots at MS medium [8]. The another medium except MS for in vitro culture is WPM medium. WPM medium is a good medium for mangosteen propagation by in vitro [9]. WPM is the most frequently used medium for woody plants because this medium has low ion total, but high concentration of sulfate. Macro element that contain in this media is high magnesium in which will supported tissue development [10].

Selection of explant is one of the most crucial factors in optimize in vitro culture. Explant use for culture including cell, organ, callus, protoplasm, embryo and nodules [11] Nodules used in vitro as an explant is a group of cells at certain place in callus that resembling cambium therefore this condition possible for cell division. The kinds of medium, type of explant, another growth regulator substance is extremely required to determine successful of in vitro culture [12]. Growth regulator substance used for nodules induction is BAP (Benzyl amino-purine). BAP is growth regulator substance from cytokine that the most frequently used in due to its high activity. BAP is kind of cytokinin and the most effective one in stimulating shoots and nodules[13].

MS medium by adding 3 mg/L BAP will produce the highest amount of shoots for mangosteen seeds originates from Bengkalis that halved producing 1.55 shoots [14]. The treatment 5 mg/l BAP and 7 mg/l BAP able to induce highest amount of nodules at MS and WPM medium. At MS medium with 7 mg/l BAP producing 28.0 nodules and WPM medium 5 mg/l BAP producing 23.4 nodules 40 dap [15]. The emerged nodules at mangosteen explant is expected to become shoots. However, to regenerate shoots and shoots elongation required another medium formula [16]. Therefore another research about response to nodules explant of mangosteen at MS and WPM medium by adding BAP is urgently required. This research aimed to determine best BAP concentration in responses mangosteen explant at MS and WPM medium.

2. Methodology

2.1 The materials of culture in vitro
Nodules explant used in this research originates from Integrated Laboratory of Department of Biology, Riau University that aged three months. Explant implantation was conducted in laminar airflow cabinet (LAFC) by planted sterile explant at culture medium. Nodules explant from the bottle were taken and placed at Petri dish. Planted explant at MS and WPM medium planted three nodules. Culture bottle was sprayed using alcohol every two days to prevent contamination. Incubation temperature was 23°C-25°C and illuminated using a lamp.

2.2. The Research Parameter
Observed parameter entailing the percentage of living explant (%), percentage of shoots formation (%), nodules formation (%), time of shoots appear(dap), time of callus appear (dap), number of nodules at(dap), number of shoots(dap), number of leaves, long of shoots(cm), and callus morphology.

2.3. Data Analysis
This research using complete random design using BAP concentration (0; 1; 3; 5; 7 mg/l) and different
medium. Each treatment was repeated five times until the resulting 50 test unit. Each of test unit (bottle) consisted of three mangosteen nodules. Based on observation using analysis of variance (ANOVA) using the F test at 5%. If there is a significant different followed by Duncan’s multiple range test at 5 % using SPSS.

3. Results and Discussion

3.1. Percentage of Living Explant, Shoots and Nodules Formation at MS and WPM medium

The Based of Analysis of Variance that addition BAP on MS and WPM medium not real effect towards the percentage of living explant in contrast towards than the percentage of nodules formulation (Table 1). All treatments were producing a percentage of living explant for 100%. It is suspected that a high percentage of living explant because of nutrition that still exists in growth medium until 70 days after planting. Giving BAP at medium also affected living explant in which MS and WPM medium supplemented with BAP able to induced intersected callus. All treatments both control and given BAP in this researachable to induce meristem cells division. Mango steen seeds that halved in length and producing living explants for 100% at WPM medium. The percentage of living explant were affected by some factors such as the physiological condition of explant, aged of explant and size of explant.

The rates percentage of shoots formation at MS medium about 50.00-66.66%. The highest rates of shoots found in 5 mg/l BAP for 66.66%. It indicates that the concentration is useful to stimulate shoots growth. At WPM medium the lowest rates percentage of shoots formation from nodules explant mangosteen found in control 0.00 cannot form shoots. It suspected that the endogen hormone at explant does not seem enough to increase the percentage of shoots formation. Given different BAP produce different shoots formation. The higher BAP concentration, a lower percentage of shoots formation at WPM medium.

3.2. Time Appears Shoots and Callus on MS and WPM

The giving different BAP on MS and WPM is significantly different towards a time of callus and shoots emerge (Table 2). At MS medium giving 1 mg/l BAP is significantly different with control and 5 mg/l, however, not significantly different with 3 mg/l dan 7 mg/l BAP. Rates of shoots emerged is 17.25 dap. It is suspected that nutrition at MS medium able to optimize explant that potentially to for a callus that callus formation occurs when plant tissue intersected. The interaction between auxin and cytokinin added to the medium with appropriate combination would cause the explant induced callus. However, the longest time to emerged occurs at the control at 12.25 dap. This condition occurs because of the balance ratio between endogen and exogen cytokinin at explant given BAP at MS medium able to produce callus for every treatment. While the longest time at control treatment 0.00 because the control is unable to form shoots, therefore, cytokinin is hugely required to add. An appropriate growth regulator able to increase cell division activity in morphogenesis and organogenesis

The given different BAP at WPM medium is significantly different towards the time for shoots and callus to emerge for 70 daps. Given BAP at WPM medium is significantly different towards the time of shoots to emerge. The fastest emerge shoots found at 1 mg/l BAP for 13.25 dap. It is suspected that appropriate BAP concentration at WPM medium able to stimulate cells division. Given BAP at WPM medium have a significant difference towards time for callus to emerge. Giving 1 mg/l BAP 3 mg/l BAP and 5 mg/l BAP is significantly different contrary with 7 mg/l BAP. The fastest time callus emerged found in given 1 mg/l BAP resulting in 7.75 daps. It suspected that given BAP concentration able to optimize callus formation. The balanced ratio between auxin and cytokinin able to stimulate cell division to form a callus. However, control treatment and 7 mg/l BAP did not form a callus resulting only 0.00 dap. This condition probably because unbalance of cytokinin and auxin. As a result, the callus did not form.
3.3. Nodules, Amount of Shoots and Shoots Length at MS and WPM

Giving different BAP on MS medium is significantly different towards the number of nodules contrary to the number of shoots, leaves and shoots length. Adding different BAP on WPM medium is significantly different towards the number of nodules, leaves and shoots length otherwise not considerably different the number of shoots (Table 3). The Process of shoots explant growth with giving BAP on MS and WPM Medium by in vitro (Figure 1). Based on the results giving BAP at MS medium significantly different towards the rates of nodules. Giving 1 mg/l BAP is significantly different at 5 mg/l BAP and 7 mg/l BAP otherwise not significantly different with control and 3 mg/l BAP. The highest rates of formed nodules found in 1 mg/l BAP for 1.50 after 70 daps. It is the possibility that this is an optimal concentration to form a nodule. It is giving BAP at MS media able to trigger organogenesis and producing nodules that will change into shoots.

The based on ANOVA, addition BAP at WPM medium is significantly different towards of number nodule. Giving 1 mg/l BAP is significantly different with control, 3 mg/l BAP, 5 mg/l BAP dan 7 mg/l BAP. The highest number of nodules formed found in given 1 mg/l BAP resulting in 100 nodules (dap). The possibility that is giving BAP at suitable medium able to stimulate organogenesis while the lowest nodules found in 3 mg/l BAP and 5 mg/l BAP resulting 0,00 nodule.

The giving BAP at MS and WPM medium did not show any significant difference towards the number of formed shoots. MS medium has the highest amount of shoot at 5 mg/l BAP for 2,75 because given BAP able to interact with endogen hormone that contained at medium and the explant able to optimise given BAP. While at WPM medium the percentage of the highest number of shoots at 1 mg/l BAP dan 3 mg/l BAP for 2.25 per explant. The possibility addition BAP at WPM medium able to interact with endogen hormone at the medium.

The shows that given BAP at MS medium did not show a significant difference towards the number of leaves at WPM medium. The highest the number of leaves found at control for 6.25 sheet per explant. Based on this research, the number of shoots determines the number of leaves formed in which fewer shoots formed more leaves is produced. The highest number of leaves found in 3 mg/l BAP for 6.75 sheets per explant. Amount of leaves were suspected as a response of BAP at WPM medium that interacts to endogen hormone that exists in the explant.

At MS medium, lengths of shoots are not significantly different. The highest rates of shoots lengths found in control for 0.87cm. It was predicted that in the most upper control treatment because of the existence of auxin in explant able to trigger shoots length. That planted explant in culture medium in control treatment ready to response length of shoots because the explant contains enough endogen auxin that leads to shoots elongation. The lowest rates of shoots found in 7 mg/l BAP producing 0.50 cm. It is the possibility that a given concentration of BAP was too high. As a result, it inhibits shoots elongation. Giving BAP at WPM medium show a significant difference towards shoots length. The highest rates of shoots found in 5 mg/l BAP for 1.32 cm this circumstance able to stimulate shoots elongation.

3.4. Responses Towards Callus Morphology at MS ad WPM Medium

The results that cultured on MS and WPM medium resulting in a compact texture and white callus in Table 4. Giving BAP at MS medium able to produce callus. Based on the results given 0, 1 mg/l BAP, 3 mg/l BAP, 5 mg/l BAP, 7 mg/l BAP generally producing a white callus that has a crumb texture. While morphology responses from nodules explant on WPM medium have compact crumb callus texture and clearly (Figure 2). Callus texture seems compact it The possibility that there is an effect from cytokinin therefore stiffening occurs towards the callus. Callus colour obtained in this research is white and brown. However, the vast majority of them are white. The best callus has white colour, in line with this stated that the bright colour of callus indicates that the callus is in good condition.

The callus morphology observed in this research is the texture and callus colour. The response towards forming callus was started by wounding in the explant surface. Wounding found explant is the beginning process towards growth regulator BAP given . while callus texture is a sign used to assess the quality of a callus. The balanced ratio between auxin and cytokinin able to stimulate cells divide to
form a callus. Giving BAP at WPM medium producing callus at 1 mg/l BAP, 3 mg/l BAP and 5 mg/l BAP. However, at 0 and 7 mg/l BAP did not produce callus. Callus formation occurs because of the interaction between given exogen BAP with auxin endogen hormone. The embryogenic callus has a crumb texture and has a yellowish-white colour. Callus colour obtained in this research is white.

**Table 1.** Percentage of living explant, percentage of shoots formation and percentage of nodules formation for 70 days at MS and WPM Medium.

| BAP Treatment (mg/l) | Percentage of Living Explant (%) | Percentage of Shoots Formation (%) | Percentage of nodules Formation (%) |
|---------------------|---------------------------------|------------------------------------|-----------------------------------|
| MS 1                | 100                             | 58,33                              | 49,99c                            |
| MS 3                | 100                             | 50,00                              | 41,66bc                           |
| MS 5                | 100                             | 66,66                              | 0,00a                             |
| MS 7                | 100                             | 50,00                              | 24,99b                            |
| WPM 0               | 100                             | 0,00                               | 0,00a                             |
| WPM 1               | 100                             | 75,00                              | 33,33b                            |
| WPM 3               | 100                             | 75,00                              | 0,00a                             |
| WPM 5               | 100                             | 66,66                              | 0,00a                             |
| WPM 7               | 100                             | 41,66                              | 8,33a                             |

Note: Data followed by different letters at same column are significantly different by Duncan Multiple Range Test at P>0,05.

**Table 2.** Time Rates of Callus and Shoots Emerge 70 days after planted (dap).

| BAP Treatment (mg/l) | Time of Shoots emerge (dap) | Time of Callus emerge (dap) |
|---------------------|-----------------------------|-----------------------------|
| MS 3                | 49,25<sup>b</sup>           | 7,00<sup>a</sup>            |
| MS 5                | 22,50<sup>a</sup>           | 6,75<sup>a</sup>            |
| MS 7                | 54,75<sup>b</sup>           | 9,00<sup>ab</sup>           |
| WPM 0               | 0,00<sup>a</sup>            | 0,00<sup>a</sup>            |
| WPM 1               | 13,25<sup>ab</sup>          | 7,75<sup>b</sup>            |
| WPM 3               | 29,75<sup>b</sup>           | 11,00<sup>bc</sup>          |
| WPM 5               | 35,00<sup>b</sup>           | 11,00<sup>bc</sup>          |
| WPM 7               | 18,50<sup>ab</sup>          | 0,00<sup>a</sup>            |

Note: Data followed by different letters at same column are significantly different by Duncan Multiple Range Test at P>0,05.
### Table 3. The rates number of Nodules, Shoots, leaves, and Shoots length for 70 days after planting at MS and WPM Medium.

| BAP Treatment (mg/1) | Number of nodules | Number of Shoots | Number of leaves (blade) | Shoots length (cm) |
|----------------------|-------------------|------------------|-------------------------|--------------------|
| MS 0                 | 1,00<sup>bc</sup> | 1,75             | 6,25                    | 0,87               |
| MS 1                 | 1,50<sup>c</sup>  | 1,75             | 6,00                    | 0,67               |
| MS 3                 | 1,25<sup>bc</sup>| 1,50             | 3,50                    | 0,60               |
| MS 5                 | 0,00<sup>a</sup>  | 2,75             | 4,75                    | 0,72               |
| MS 7                 | 0,75<sup>b</sup>  | 1,50             | 3,50                    | 0,50               |
| WPM 0                | 0,00<sup>a</sup>  | 0,00             | 0,00<sup>a</sup>        | 0,00<sup>a</sup>   |
| WPM 1                | 1,00<sup>b</sup>  | 2,25             | 6,00<sup>b</sup>        | 0,92<sup>b</sup>   |
| WPM 3                | 0,00<sup>a</sup>  | 2,25             | 6,75<sup>b</sup>        | 1,00<sup>b</sup>   |
| WPM 5                | 0,00<sup>a</sup>  | 2,00             | 5,50<sup>b</sup>        | 1,32<sup>b</sup>   |
| WPM 7                | 0,25<sup>a</sup>  | 1,25             | 3,00<sup>ab</sup>       | 0,62<sup>ab</sup>  |

Note: Data followed by different letters at same column are significantly different by Duncan Multiple Range Test at P>0.05.

### Table 4. The Responses Towards Callus Morphology for 70 Days After Planted (dap) at MS and WPM Medium.

| BAP Treatment (mg/1) | Callus Texture | Callus Color |
|----------------------|----------------|--------------|
|                      | Compact        | Crumb        |
| MS 0                 | ✓              | -            | Brown         |
| MS 1                 | ✓              | -            | White         |
| MS 3                 | ✓              | ✓            | White         |
| MS 5                 | ✓              | -            | White         |
| MS 7                 | ✓              | -            | White         |
| WPM 0                | -              | -            | -             |
| WPM 1                | ✓              | -            | White         |
| WPM 3                | -              | ✓            | White         |
| WPM 5                | ✓              | -            | White         |
| WPM 7                | -              | -            | -             |
Figure 1. Shoots Explant length of Mangosteen nodules at MS and WPM Medium at 70 days after planted (a) treatment 1 mg/l BAP (MS) (b) treatment 1 mg/l BAP (WPM) (c) treatment 3 mg/l BAP(MS) (d) treatment 3mg/l (WPM). Note: Arrow shows the highest shoots in every treatment.

Figure 2. Callus morphology towards mangosteen explant nodules at 70 dap observation. (a) White compact callus (b) White crumb callus Note: arrow shows white crumb callus.

4. Conclusion
The best BAP concentration in responses mangosteen nodules towards MS medium is 1 mg/l BAP resulting 49.99%. The fastest time for shoots to emerge found in control 17.25 dap (the days after planted). The fastest time for callus to appear is 5 mg/l BAP at 6.75 daps. The highest amount of nodule found in 1 mg/l BAP producing 1,50 nodules. Best callus texture is crumb texture with brown and white colour. Best BAP concentration in responses mangosteen nodules towards WPM medium is the percentage in nodule formation at 1 mg/l BAP resulting 33.33%. The fastest time shoots emerge at 1 mg/l BAP resulting in 13.25 dap. The fastest time for callus to emerge found in 1 mg/l BAP at 7.75 daps. The highest amount of nodules found in 1 mg/l BAP resulting in 1,00, the highest number of leaves found in 3 mg/l BAP resulting in 6.75 sheets. The best length shoots found in 5 mg/l BAP producing 1.32 cm. Callus texture is compact and crumbs with white colour.

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