Effect of 6-Benzylaminopurine and Honey for in Vitro Shoot Initiation of Mangosteen Seed Explant From Riau, Indonesia

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Abstract. Mangosteen (Garcinia mangostana L.) is a tropical plant that has export value because it has a rich of antioxidant xanthone that can be used for medicine as an anti-cancer agent. Mangosteen (Garcinia mangostana L.) from Riau, Indonesia has many superiorities such as grow in the marshes and tolerant to acid soil (peat soil). One of the alternative methods to produce large number and uniform mangosteen seedlings in a short period can be done using in vitro culture technique. The purpose of this research was to determine the optimal concentration of BAP and honey in shoot initiation of mangosteen explant from seed that had been divided into four parts and also to determine the best combination for initiation mangosteen shoots using in vitro method. This research used a Completely Randomized Design (CRD) with the concentration of BAP (3; 7 mg/l) and honey (3; 6; 9 ml/l) or in combination on MS medium with five replications. The result showed that the highest number of shoots (14.60) formed on MS medium supplement with 7 mg/l BAP + 3 ml/l honey. The combination between 3 mg/l BAP + 3 ml/l honey produced the tallest shoots (5.10 cm) and the highest number of leaves (3.20 leaves) on MS medium supplement with 3 mg/l BAP + 9 ml/l honey. In this study, we have successfully presented the effect of 6-benzyl amino purine and honey for in vitro shoots initiation from mangosteen seed explant (Garcinia mangostana L.) which taken from Riau Indonesia.

1. Introduction
Mangosteen (Garcinia mangostana L.) is one of the popular tropical fruit nicknamed as Queen of the Tropical Fruit because it has the beauty of the fruit skin and flesh, white and clean. Furthermore, mangosteen also contain secondary metabolites that is important for health. Antioxidant compounds such as xanthin in its pericarp layer is used as an anti-cancer agent that has the potential to be developed as a business in agribusiness [1]. Opportunities for agribusiness development is wide open in mangosteen because its request number reached 10% per year. Mangosteen production in Riau in 2012 reached 2,618 tons, while the volume of Indonesian mangosteen reached 20.29 tons [2]. However, the increase in exports of mangosteen in Indonesia for the international and local markets are still experiencing barriers [3]. The imbalance between the number of requests and the number of mangosteen provided caused by the level of productivity of the mangosteen is far below its potential.

Mangosteen plants generally propagated by seed. Mangosteen seeds in each fruit are 1-2 seeds and can only be obtained during the fruiting season only. Mangosteen seeds including apomixis seeds, namely fruits and seeds are formed without a marriage [4]. Apomixis also be recalcitrant seeds should be planted immediately after removing them from the fruit. The mangosteen generative propagation is
unfavourable because of its recalcitrant and do not have a dormancy period. While the vegetative propagation such as grafting is not recommended because it has a very small success rate and low yield [5]. Therefore it is necessary to do the mangosteen plant propagation to produce seeds in large numbers and superior through in vitro culture techniques.

**In vitro** culture is a method of plant propagation that can be used as an alternative way to obtain mangosteen seeds in large numbers within a short time [6]. The success **in vitro** culture methods influenced by the type of media, electoral explants and growth regulators. The basic media which is widely used is Murashige-Skoog medium (MS) because it contains high nitrate, potassium and ammonium [7].

Another factor that affects the success of **in vitro** culture is the explants. The explants were taken from parts of the plant tissues that are actively dividing meristematic or between other seeds. According [8] cleavage seed treatment can affect shoot regeneration. [9] states that the seed is cleaved to produce more number of shoots compared to seeds without a split. [10] also stated that seeds of mangosteen split into four pieces to produce nodules or buds small in a higher number because the mangosteen seeds split and placed facedown and the wound facing the media, which caused by a direct contact of media with wounded mangosteen seeds. The mangosteen seeds explants were quartered grown for 90 days after planting/DAP on MS medium + 5 mg / l BA was able to induce shoots up to 100% with the number of shoots that sprout 2.7.

The addition of growth regulators at appropriate concentrations is also necessary for the growth and differentiation of explants. Cytokinins such as BAP is a plant growth regulator that is widely used to stimulate the formation of buds with a strong activity that pushed the process of cell division [11]. [12] research results at 70 DAP reflect the addition of BAP concentrations of 1, 3, and 7 mg / l in the mangosteen seeds were quartered produce 100% the percentage of bud formation and the highest number of buds on the shoots 2.8531 with the treatment of 7 mg / l BAP.

Attempts to promote plant growth the **in vitro** culture techniques can be done by modifying the media, one of them with the addition of organic supplements such as honey. According to [13] honey is an additional compound in the media because it contains a variety of elements needed such as Ca, Na, K, Fe, P, S, Mn, vitamins, organic acids, carbohydrates and sugar which are able to stimulate cell growth. Research into the use of honey as an additional compound on MS medium in culture in vitro has not been done yet. One of them has been done on orchids which can be seen in [14]. A research has been conducted on the stems and shoots Grammatophyllum speciosum BL. MS medium for 84 DAP with the addition of Al Shifa honey at a concentration of 6 ml / l with an average number of shoots the highest (1.08 shoots), high shoots (26.58) and the number of leaves (4.83).

Based on this, we need to do research on the use of growth regulators BAP and honey as additional compounds on MS medium through the technique of **in vitro** culture, is expected in this study were able to produce mangosteen seedling in large numbers and quality by testing the use of plant growth regulators BAP and honey at different concentrations of the mangosteen seeds were quartered. The purpose of this study was to determine the effect of BAP and honey either singly or in combination to induce bud explants were quartered mangosteen seeds **in vitro** and determine the optimal concentration of BAP and honey on MS medium that induces mangosteen seed bud explants (Garcinia mangostana L.) that were quartered **in vitro**. The rest of this paper is organized as follow. Section 2 describes about methodology that used for research.

2. **Methodology**

2.1. **Materials of culture in vitro**

This investigation was carried out at Laboratory of Integrated Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau. Materials used in this research is the seeds of the mangosteen fruit explants derived from Riau, Indonesia. The mangosteen fruit is taken from the ripe to purplish-red skin colour fruit.

Implementation of research includes the preparation and sterilization of tools, preparation of
planting media, sterilization of explants, cultivation of explants and maintenance done in the incubation chamber to keep always clean and sterile conditions with a squirt of alcohol 70% within 2 days. The room temperature is set up to 23-25 °C for light irradiation. Observations were made at the end of the study (70 days after planting) by calculating the percentage of live explants (%), percentage of bud formation (%), the presence of shoots (days), shoot number, shoot length (cm) and number of leaves (leaf).

2.2. Methods of Culture In Vitro
This research used a Completely Randomized Design (CRD) with the concentration of BAP (3; 7 mg/l) and honey (3; 6; 9 ml/l), or in combination on MS medium with 5 replications. Accordingly obtained 12 level and each treatment was repeated 5 times, then the total amount is 60 experimental units. Each experimental unit consisted of one piece seeds mangosteen quartered. BAP concentration and honey used consisted of treatment are: M1: 0 ml / l (control), M2: 3 mg / l BAP; M3: 7 mg / l BAP; M4: 3 ml / l of honey; M5: 6 ml / l of honey; M6: 9 ml / l of honey; M7: 3 mg / l BAP + 3 ml / l of honey; M8: 3 mg / l BAP + 6 ml / l of honey; M9: 3 mg / l BAP + 9 ml / l of honey; M10: 7 mg / l BAP + 3 ml / l of honey; M11: 7 mg / l BAP + 6 ml / l of honey; M12: 7 mg / l BAP + 9 ml / l of honey. Data observations were then analyzed using Analysis of Variance (ANOVA). If there is a real effect, it is done by using Duncan's Multiple Range Test (DMRT) at the level of 5% to using SPSS. In the following section, we present the obtained results and discussion.

3. Results and Discussion
This section discuss the shoots inisiation and the growth of mangosteen shoots that have been produced in this experiment.

3.1. Shoots Inisiation
The results of the research that has been conducted against the mangosteen seeds explants quartered sections on MS medium (Murashige-Skoog) with the addition of of growth regulators BAP and honey concentration either singly or in combination in vitro on day 70 after plant showed the percentage of explants life and the percentage of bud formation were 100% in all treatments. Explants of living conditions are influenced by several factors such as age, size of the explants, the explants were cultured physiological conditions, sterilization method and media composition. In this study, the seed used comes from the selected mangosteen seeds which are ripe and large. Explant beans used only measured by similar relative size. The different maturity levels affect seed viability of explants culture for physiological conditions immature seeds affecting food reserves and the composition of the embryo in the seed [15]. The Unmatured seed physiology has insufficient food reserves during the seed germination process, which require energy for respiration. The high percentage of live explants in this study was also influenced by the content of nutrients in the growth medium which is available in sufficient quantities until 70 days after planting [16]. The medium MS containing a number of macronutrient and micronutrient used by plants to support optimal growth for plants in vitro [17].

Shoots that established directly from explants were marked by the appearance of buds on the surface of the seeds (organogenesis direct) and indirectly through the nodules and callus, wherein the callus initially looks like a mass of white greenish cells subsequently changed to yellow-green and form green buds (indirect organogenesis). The plant regeneration in vitro can be done through direct and indirect organogenesis. Direct organogenesis is the process of forming adventitious shoots direct from the explants, while indirect organogenesis is a process of forming adventitious buds through callus formation or nodule in advance [18].

The process of direct and indirect organogenesis on research begins with the planting of seeds (Figure 1A) then the color changes to pink at explant (Figure 1.B) became brownish red and then green appear small nodules and callus on indirect organogenesis (Figure 1.C). The nodules will form leaf primordia and will grow into the leaves intact. As for direct organogenesis nodules but not directly forming buds are green shoots (Figure 1.d.) and thereafter develop into mature shoots (Figure 1.E).
Nodules that appears on the mangosteen is a candidate bud explants. Shoots derived from callus and nodules in this study had a smaller size with several relatively more than in shoots that formed from seed. The formation of nodules in this study is consistent with research conducted which states that the mangosteen shoot induction produces bumps or nodules that are produced from the former shoots slices, and then swell. According [19] The colour that changed on explants, which were planted indicates that the explants have experienced the physical and chemical processes as an initial response to the media explant. The colour changes to red-brown thought to be caused by natural events of browning. Browning of explants in this study allegedly influenced by cutting and the production of phenolic compounds undergo oxidase enzyme activity. According [20], browning occurs due to the accumulation of polyphenol oxidase compounds that are released or synthesized in oxidized conditions when the tissue is injured. Explants undergo browning is still able to survive and grow to form a new plant organs such as buds. This is presumably because of the amount of phenolic compounds produced by explant not too much and browning is not occurs in all parts of the tissue so that some tissue explants can grow and unaffected the growth of shoots. The states phenolic compounds that appear on the explant would be toxic if the concentration is excessive and will inhibit growth. But the production of phenolic compounds is limited to explant or callus and can be tolerated by explant, so that culture can still grow.

3.2. The Growth of Shoots Mangosteen

The Analysis of Variance (ANOVA) results showed that addition of BAP growth regulators and honey as single treatment or in combination did not significantly affect the timing parameters of appearing shoots and leaves. BAP treatment and honey only have significant effect on the number of shoots and shoot length of explants mangosteen seeds. Mean and further test results are shown in Table 1.

Table 1. showed that the treatment combination of 7 mg / l BAP + 6 ml / l of honey (M11) tend to produce the fastest time appearing shoots which hst 6.00 compared to the control treatment. In almost all the parameters of the number of shoots is significantly different from the control treatment and some treatment, this shows an increasing number of shoot except in the treatment of 9 ml / l of honey and 7 mg / l BAP + 9 ml / l of honey. The treatment combination of 3 mg / l BAP + 3 ml / l of honey to produce long shoots better than in some other treatments. While the parameters of the number of leaves though not significantly, but there are differences in the average number of leaves.

The addition of plant growth regulator acts to increase the effectiveness of the amount of endogenous hormones on the seeds so with the addition of BAP it will be able to speed up the formation of buds. The addition of hormone at a certain concentration level is 6 ml/l to the culture medium can increase the activity of cytokines in the process of cell division and cell differentiation, because honey contains sugar as a carbon source because it is required in the metabolic processes during cell growth and it also contain a number of salts inorganic and vitamins. Meanwhile, in a single honey treatment with a concentration of 9 ml/l in this study, generally explants formed callus in advance, so it takes longer to form buds. This is presumably due to the addition of honey with a high concentration that increased the amount of sugar in the culture medium which is a source of carbon needed in the process of cell division [21]. The states that the higher the levels of sugars added, it will increase callus formation. In the parameter of the number of shoots, single BAP addition with a concentration of 7 mg/l is much useful to generate the highest number of shoots (8.20) compared to treatment with a single BAP concentration of 3 mg / l and control. While on the treatment of single honey from average figures show preferential treatment to 6 ml / l of honey to produce the highest number of shoot buds 4.40 compared to other single honey treatment (Table 2). Mangosteen seeds explant were planted on MS medium with a combination treatment of 7 mg / l BAP + 3 ml / l of honey to produce the highest number of shoot buds at 14.60, and highly significant with other treatments. The average number of shoots was the lowest for the treatment of 9 ml / l of honey amounted to 1.20 shoots.

The use of BAP with the concentration of 7 mg/l with 3 ml/l of honey to produce the highest number of shoots and combines the best treatment (Figure 2.A), thus allegedly honey with a low
concentration, namely 3 ml/l was able to optimize the work of BAP in increasing the number of shoots. BAP with high concentrations is required in the process of cell differentiation and supported by honey containing additional carbon source for cell metabolism processes, because most parts of the plants or explants cultured is non autotrof and has a low rate of photosynthesis due to the limited amount of CO2 in the culture bottles. Carbohydrates are added exogenously is a carbon source which replace the usual carbon that is obtained from the atmosphere in the form of CO2 for photosynthesis material. According to [22], addition of carbohydrates, sucrose and glucose can stimulate the formation of buds through energy and some carbon skeletons which are the basic essential ingredients in the formation of amino acids, nucleic acids, growth regulators and proteins that play a role in plant growth and development in vitro.

The results of analysis of variance (ANOVA) showed that the BAP and honey as single treatment or in combination provide a real impact on the length of shoots parameter produced from mangosteen seed explants quartered after 70 days of planting. The evident from the average length of shoots in all treatments (Table 1) shows the shoot length is lower than the control treatment, unless the treatment concentration of 3 mg/l BAP + 3 ml/l of honey to produce the highest shoot length of all treatments. Based on this, treatment concentration of 3 mg/l BAP combined with 3 ml/l of honey in the research is the optimum concentration to produce the longest shoots. These results suggest that the combination of growth regulators BAP and honey can increase the length of shoots, but the higher the concentration of BAP and honey given cause a decrease in length of shoots in the mangosteen seeds (Figure 3).

The length of mangosteen shoots is as the lowest of these results is suspected caused by the high concentration of the hormone cytokinin and honey given causing shoot growth becomes stunted. According to [23] the higher concentration of honey produced the longer shoots, but the addition of a concentration of 9 ml/l honey caused the length growth buds become less optimal treatment than other combinations. [24] The stated that the addition of high a concentration of cytokines that can inhibit the growth of shoots and low comparison between auxin and cytokinin causing imbalances in the explant.

The results of analysis of variance showed that the addition of BAP and honey on seed explants did not show any significant difference to the number of leaves formed. Although not significant, but there are differences in the average number of number of leaves as seen in Figure 4. The Figure 4 shows that almost all treatments produce a number of different leaves to the control except in the combination treatment of 3 mg/l BAP + 3 ml/l of honey and 3 mg/l BAP + 6 ml/l of honey as much as 2.80 leaves. The average number of leaves are most likely in the BAP treatment of 3 mg/l combined with honey each yield of 2.80 leaves (concentration 3 and 6 ml/l honey) and 3.20 strands at a concentration of 9 ml/l honey than with the addition of BAP 7 mg/l combined with honey. Treatment of 3 mg/l BAP + 9 ml/l of honey to produce the highest number of leaves compared with other treatments. Meanwhile, the lowest number of leaves found in 9 ml/l of honey and 7 mg/l BAP + 9 ml/l of honey as much as 0.40 leaves. This shows that the use of BAP concentration of 3 mg/l is the best concentration to produce the highest number of leaves. According to [25], the number of leaves in in vitro culture is influenced by plant growth regulators BAP in the media especially from the class of cytokines. This is allegedly due to the treatment at 3 mg/l BAP, the addition of organic honey supplement with a high concentration which is 9 ml/l of honey that is believed to accelerate the formation of leaf organs.

The honey contains a variety of nutrients, vitamins and elements of magnesium and calcium. In addition, protein and amino acids in the honey can be used for the development of plant tissues [26]. The content of nitrogen in the media is very important for plants since it is one of the amino acids constituent, chlorophyll and metabolic processes. However, the lowest number of leaves of in this study contained at treatment concentrations of 9 ml/l of honey without BAP and concentration of 7 mg/l BAP + 9 ml/l of honey, since the average seeds explants in the treatment of 7 mg/l BAP + 9 ml/l of honey to form a callus and have the number and size of the smaller buds that have not been able to form leaves.

The results showed that the combination of BAP and honey concentration of 7 mg/l BAP + 3 ml/l of honey is the best combination because it produced the highest number of shoots (14.60 shoots) than
other treatments. However, the average length of shoots produced in this treatment, the small size of 1.62 cm. This is presumably because the number of buds which led to form competition for nutrients in the media, it is necessary to do further research by explant subcultures on the same medium to address nutrient deficiencies that becomes more optimal explant growth. Shoots in vitro of the results of the shoot multiplication as the best treatment for the purpose of propagation get large numbers of seedlings from seeds of mangosteen on MS medium with the addition of BAP at various concentrations. [10] conducted a multiplication of axillary buds mangosteen on media MS + 3 mg/l BAP is best to additional multiplication medium height, number of leaves and number of branches. [27]) conducted a subculture of mangosteen on MS medium with the addition of 2 mg / l BAP provides the most number of buds on each subculture of 39.56 shoots per explant.

**Table 1.** The average growth of shoots explant mangosteen seeds to the addition of BAP and honey at 70 days after planting

| Code | BAP (mg/l) | honey (ml/l) | Shoot initiation period ± sd (days) | number of shoots ±sd (shoots) | heighest of shoots ±sd (cm) | Number of leaves ±sd (leaves) |
|------|------------|-------------|----------------------------------|------------------------------|-----------------------------|------------------------------|
| M1   | -          | -           | 9.20 ± 2.58                      | 1.80 ± 0.83                 | 4.20 ± 1.92                 | 2.80 ± 1.09                  |
| M2   | 3          | -           | 11.20 ± 7.50                     | 3.20 ± 3.19                 | 1.64 ± 0.86                 | 1.60 ± 2.19                  |
| M3   | 7          | 3           | 7.40 ± 0.54                      | 8.20 ± 6.18                 | 2.50 ± 1.14                 | 1.80 ± 2.04                  |
| M4   | -          | 3           | 7.40 ± 0.54                      | 2.20 ± 1.09                 | 4.94 ± 2.40                 | 1.60 ± 0.89                  |
| M5   | -          | 6           | 7.80 ± 1.64                      | 4.40 ± 7.63                 | 2.18 ± 2.78                 | 0.80 ± 1.09                  |
| M6   | -          | 9           | 16.40 ± 11.71                    | 1.20 ± 0.83                 | 1.48 ± 2.00                 | 0.40 ± 0.89                  |
| M7   | 3          | 3           | 11.80 ± 8.76                     | 9.20 ± 7.94                 | 5.10 ± 3.27                 | 2.80 ± 2.68                  |
| M8   | 3          | 6           | 10.80 ± 6.22                     | 6.60 ± 3.64                 | 3.64 ± 2.71                 | 2.80 ± 1.78                  |
| M9   | 3          | 9           | 8.80 ± 4.60                      | 6.00 ± 6.24                 | 4.46 ± 2.32                 | 3.20 ± 1.09                  |
| M10  | 7          | 3           | 7.80 ± 2.38                      | 14.60 ± 11.19               | 1.62 ± 0.72                 | 1.20 ± 1.78                  |
| M11  | 7          | 6           | 6.00 ± 0.70                      | 10.40 ± 1.51                | 3.20 ± 1.94                 | 2.20 ± 1.41                  |
| M12  | 7          | 9           | 7.60 ± 1.67                      | 1.60 ± 0.54                 | 1.22 ± 0.48                 | 0.40 ± 0.89                  |

The different letter within the column showed significant difference at 5% analyzed by Duncan’s Multiple Range Test (DMRT).

**Figure 1.** Developmental stages explants seeds mangosteen form buds (A) seeds at the beginning of planting, (B) the seeds change color 5 days after planting, (C) nodules (prospective shoots) 14 days after planting, (D) the buds sprout green 14 days after planting, (E) shoots adult 70 days after planting.
Figure 2. Response in vitro growth of mangosteen seed shoots explant which shows the number of shoots at the end of treatment (70 days after planting), (A) the highest number of shoots highest in the treatment 7 mg/l BAP + 3 ml/l of honey (M10), (B) the lowest number of shoots in treatment of 9 ml/l of honey (M6) and (C) controls.

Figure 3. The response in vitro growth of shoots mangosteen seed explant showed high buds at the end of treatment (70 days after planting), (A) long-best buds in treatment 3 mg/l BAP + 3 ml/l of honey (M7), (B) long shoots lowest in treatment of 7 mg/l BAP + 9 ml/l of honey (M12) and (C) controls.

Figure 4. The response in vitro growth of shoots mangosteen seed explant showed the number of leaves at the end of the observation, (A) the highest number of leaves of in the treatment 3 mg/l BAP + 9 ml/l of honey (M9), (B) the lowest number of leaves in the treatment of 7 mg/l BAP + 9 ml/l of honey (M12) and (C) Controls.

4. Conclusion
In this study, we have successfully presented the effect of 6-benzylaminopurine and honey for in vitro shoots initiation from mangosteen seed explant (Garcinia mangostana L.) which taken from Riau Indonesia. The conclusion of this study are (1) the percentage of live mangosteen seeds explants and a percentage of explants that formed buds reached 100% in both the control and all treatments with the addition of BAP 3 and 7 mg/l and honey with a concentration of 3, 6 and 9 ml/l. (2) Addition of
BAP and honey significantly affected number of shoots parameter and long shoots on seeds mangosteen explants that were quartered and give the best results of shoots growth on the treatment of 7 mg / l BAP + 3 ml / l of honey with a number of shoots of 14.60 shoots, shoot length which are best resulted from treatment 3 mg / l BAP + 3 ml / l of honey and the highest number of leaves on a combination treatment of 3 mg / l BAP + 9 ml / l of honey each as much as 5.10 cm and 3.20 strands leaf.

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