The Effects Of Plant Growth Regulators (Naa+Bap) And Explant Types On Propagation Buds Of Asam Gelugur (Garcinia Atroviridis Griff)

Nurcholis Alfarisi, Luthfi Aziz Mahmud Siregar, Tengku Chairun Nisa

Abstract—The research was aimed to determine the effect ratio combination of Naphthaleneacetic acid (NAA) and 6-Benzylaminopurine (BAP) plant growth regulators and explant types of Asam Gelugur plant (Garcinia atroviridis Griff) and their interaction in the Murashige Skoog (MS) media. This research was conducted in the Tissue Culture Laboratory, Faculty of Agriculture, Universitas Sumatra Utara, Indonesia in February until June 2018. The research was used the completely randomized design with 2 treatment factors with three replications. The first factors is combination of NAA and BAP dosage with nine rates, including G1 (0.2 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G2 (0.2 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G3 (0.2 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP); G4 (0.4 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G5 (0.4 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G6 (0.4 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP); G7 (0.6 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G8 (0.6 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); and G9 (0.6 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP). The second factors is the explant types such as apical shoot (E1) and axillary bud (E2). The results were showed that the addition of 0.2 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP (G2) in the MS media had significant effect on the percentage of bud formed, number of buds, length of buds, and number of leaves. The axillary bud explant (E2) had significant on the number of buds. The interaction of E2G2 had significant on the number of buds in G. Atroviridis plant. The combination of E2G2 in vitro can propagation of buds then recommended to prevent the scarcity of G. atroviridis plant.

Index Terms— apical shoot, axillary bud, BAP, Garcinia atroviridis, NAA.

1. INTRODUCTION

The State of Indonesia is one of the tropical countries referred to as “Mega Biodiversity”. Indonesia has a biodiversity of 325,350 species of flora and fauna. Asam Gelugur (Garcinia atroviridis Griff) is a type of flora in Indonesia. Plants of the Garcinia species have 77 species recorded in Indonesia and 22 of them are found in North Sumatra [1]. G. atroviridis plant is widely used by the public as cutting acid which is used as a spice in cooking, sweets, syrup, and medicine. Besides, G. atroviridis is an antioxidant that is beneficial for weight loss and cholesterol [2-4]. A large amount of demand for Asam Gelugur has increased causing farmers to need the availability of superior plant material. One way to obtain superior seeds is by the propagation of plant material in vitro (tissue culture). Tissue culture techniques can produce large numbers of seeds in a relatively short time [5]. Explants used were microshoot propagation. Factors that influence the multiplication of micro apical shoots are the type of plant material, including the type of organ or tissue used as a source of explants [6]. In addition to the source of explants, the composition of the growth media also influences the success in the propagation of micro apical shoot, because in it there are components of growth regulators consisting of auxins, cytokinins, gibberellin, abscisic acid, and ethylene [7]. Plant growth and development are controlled by chemical substances with very low concentrations, called plant growth substances, growth hormones, phytohormones or plant growth regulators (plant growth regulators = PGRs) [8]. The development of hormones develops when advances in biochemical knowledge and chemical industry engineering make it possible to produce synthetic compounds that have physiological effects similar to plant hormones. Because hormones contain an understanding of organic compounds instead of nutrients that are synthesized in one part of the body of the plant and transferred to other parts in low concentrations capable of causing biochemical, physiological and morphological responses. Thus, synthetic substances such as hormones, because they are not synthesized in plants do not include plant hormones. From this comes the name of growth regulators (PGR) or plant growth regulators for these synthetic compounds [9].

In tissue culture, there are two very important groups of PGR, namely cytokinins and auxins. Interaction and balance between PGR given in the media and produced by cells endogenously determine the direction of development of a culture, thus affecting the processes of growth and morphogenesis [10]. Where the higher auxin ratio than cytokinin will stimulate root formation, while the higher cytokinin ratio than auxin will induce the formation of apical shoot. If auxin and cytokinin are at the same concentration (ratio 1), a callus will form [11]. Wherein it is known that auxin influences stem length increase through shoot cell lengthening, growth, differentiation, adventitious root formation, fruit development, apical dominance, phototropism, and geotropism, while cytokinins function as hormones that act as cell division and regulate the development and increase metabolism in the body plants [12]. Due to the lack of information about the multiplication of Asam Gelugur through the propagation of micro apical shoot, as well as the composition of the most appropriate media as a growth medium. So it is necessary to study the effect of explant types and growth regulators on the induction of micro apical shoot. The research was aimed to determine the best types of explants as well as different compositions and administration of multiplication growth regulators most appropriate for induction, the multiplication of shoot lengthening for Asam Gelugur.

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2 MATERIALS AND METHODS

2.1 Sterilizer Equipment of The Laboratory
The equipments (culture bottles, petridish, scalpel, forceps) were washed by the soap and dried, then wrapped by aluminum foil. The equipments were sterilized in the autoclave at 121°C with the pressure of 17.5 psi for 60 minutes. This research was conducted in the Laboratory of Tissue Culture, Faculty of Agriculture, Universitas Sumatera Utara. This research was conducted in February until July 2018.

2.2 Source Explant
The explants of Asam Gelugur (Garcinia atroviridis Griff) plant were collected from the Timbang Lawan Village, Bahorok Subdistrict, Langkat District, North Sumatra Province, Indonesia. The explants were taken by the middle branch 5 cm of Asam Gelugur plant, then were cleaned with detergent and rinsed with running water

2.3 Sterilized Explant
The explant was soaked in the Dithane solution of 3 g l⁻¹ aquadest for 30 minutes then rinsed with aquadest 3-fold. The explant was soaked in the Benlate solution with 3 g l⁻¹ aquadest for 30 minutes then rinsed with aquadest 3-fold. The explant was soaked in the Clorox 15%, l⁻¹ aquadest for 5 minutes then rinsed with aquadest 3 fold. The explant was soaked in the Alcohol 75% for 5 minutes and continued with betadine 5%, l⁻¹ aquadest for 5 minutes, then rinsed with aquadest 3-fold.

2.4 Making Media of Tissue Culture
The media was used the Murashige and Skoog (MS) solid media [13]. The stock solution of PGR (NAA and BAP) were conducted to use 1 mg l⁻¹ aquadest. The stock solution of MS macronutrient with concentration 20-fold of 50 ml, the MS micronutrient with concentration 200-fold of 5 ml, vitamin solution with concentration 200-fold of 5 ml, myo-inositol 0.1 g, agar 7 g as a gelling agent in tissue culture, and sucrose 30 g. The media (macro, micro, vitamin, myo-inositol, agar, sucrose) were mixed until homogeneous with aquadest up to 1 l. The acidity of the solution was measured by the pH meter (required pH 5.8). An increase in the pH was used 0.1N HCl and a decrease in the pH was used 0.1N KOH. After that, all ingredients were mixed using a magnetic stirrer. Then the solution is transferred to the erlemeyer tube and covered with aluminum foil. Then the MS media was sterilized at 17.5 psi at 121°C for 20 minutes in the autoclave. After the sterilization process was completed, the media into the culture bottles amounted to 15 ml tube⁻¹ then included by NAA and BAP treatment, respectively. The culture bottle covered with aluminum foil then stored in the culture room.

2.5 Experimental Design
This study uses a completely randomized design (CRD) method with two treatment factors. The first factor is BAP and NAA growth regulators consisting of 9 levels of concentration: G1 (0.2 mg/l NAA + 0.5 mg/l BAP); G2 (0.2 mg/l NAA + 1 mg/l BAP); G3 (0.2 mg/l NAA + 1.5 mg/l BAP); G4 (0.4 mg/l NAA + 0.5 mg/l BAP); G5 (0.4 mg/l NAA + 1 mg/l BAP); G6 (0.4 mg/l NAA + 1.5 mg/l BAP); G7 (0.6 mg/l NAA + 0.5 mg/l BAP); G8 (0.6 mg/l NAA + 1 mg/l BAP); and G9 (0.6 mg/l NAA + 1.5 mg/l BAP). The second factor was explant apical shoot consisting of E1 (apical shoot) and E2 (axillary bud) with 18 treatment combinations. This research was used 3 replications.

2.6 Planted Explant
The explant of this research was used apical shoots and the axillary buds of Asam Gelugur were sterilized can be planted in the culture bottles that contain MS media. The size explant was used of 1 cm, respectively. Then the explants were planted into culture bottles according to the treatment of 1 explant bottle⁻¹ and covered with aluminum foil. After planted, the culture bottle was placed in a culture room with a temperature of 20 to 25°C and light intensity of 2000 Lux.

2.7 Variable and Analysis
Variables were observed include percentage of buds formed, number of buds, length of buds, and number of leaves. The percentage of buds formed was calculated at 3 Weeks After Planted (WAP) using the formula:

\[
\% \text{ of buds formed} = \frac{\text{number of buds formed}}{\text{number of explants planted}} \times 100\% \quad (1)
\]

The number of buds was observed at 6 WAP by counting the number of buds that appearance from the explant surface. The length of buds was measured at the highest shoot using millimeter paper measured from the appearance buds (base) until the highest buds. Measurement was observed at 6 WAP. The number of leaves was observed by count the leaves formed on the plantlet with the structure midrib of the leaf. Measurement was observed at 6 WAP. The variables were analyzed using the one-way ANOVA and were continued by the DMRT (Duncan Multiple Range Test) at the level of 5%.

3 RESULTS AND DISCUSSION

3.1 Percentage of Buds Formed (%)
Percentage of buds formed in the giving of growth regulators (PGR) with 9 combinations of NAA and BAP concentrations, explant types of Asam Gelugur and their interaction can be seen in Table 1. The giving of 9 combinations of NAA and BAP concentrations, explant types of Asam Gelugur and their interaction was not significant on the percentage of buds formed at 3 WAP. In this study the highest percentage of shoot formation was seen in the explant treatment E1 (apical shoots) while for the combination of PGR treatment the highest percentage was in the treatments G2, G5, G6, G7, and G8 while the lowest percentage was found in the combination treatment of the PGR G9 (Table 1).
The different growth buds formed of explant types can be seen in Figure 1.

**Fig 1. The different growth buds formed of Asam Gelugur at 3 WAP. A=apical shoots; B = axillary bud.**

The low percentage of shoot formation is due to the reaction of some explants that have browning. Browning explants in plant apical shoot results in not being able to produce new apical shoot to support the life of these plants because cells in plants can no longer stimulate plant growth. Thus, different growth of buds formed from apical shoots more highest compared to axillary buds (Figure 1). [14] suggested that browning in tissue culture was caused by increased production of phenolic compounds followed by oxidation by oxidase enzymes and their polymerization activities where browning in explants mostly occurred in hardwood species. One of the main causes of browning in vitro culture is injury due to tissue cutting. These injuries stimulate stress and cause an increase in phenylpropanoid production activity and cause browning.

### Table 1

<table>
<thead>
<tr>
<th>Explant Types</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>G8</th>
<th>G9</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1 (apical shoot)</td>
<td>100.00</td>
<td>100.00</td>
<td>66.67</td>
<td>66.67</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>66.67</td>
<td>88.89</td>
</tr>
<tr>
<td>E2 (axillary bud)</td>
<td>66.67</td>
<td>100.00</td>
<td>66.67</td>
<td>66.67</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>33.33</td>
<td>81.48</td>
</tr>
<tr>
<td>Average</td>
<td>83.33</td>
<td>100.00</td>
<td>66.67</td>
<td>66.67</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>50.00</td>
<td>85.19</td>
</tr>
</tbody>
</table>

Note: The numbers followed by the same letters on the same line show no significant difference in the distance test at the level of 5%. G1 (0.2 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G2 (0.2 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G3 (0.2 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP); G4 (0.4 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G5 (0.4 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G6 (0.4 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP); G7 (0.6 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G8 (0.6 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G9 (0.6 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP).

Table 2 showed that the interaction of apical shoot and axillary bud explants of Asam Gelugur with PGR 9 combination of NAA and BAP concentrations resulted in the highest number of buds found in the E2G2 treatment (axillary bud explants with 0.2 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP) amounted to 2.00, which differs from all other treatment combinations (Figure 2). While the number of buds was lower found in the E1G1, E1G2, E1G3, E1G4, E1G5, E1G6, E1G7, E1G8, E1G9, E2G1, E2G3, E4G4, E1G7, E1G8, dan E1G9 with an average of 1.00, respectively. The addition of cytokines (ex: BAP) in the match concentration gives a good influence on the formation of the apical shoot and produces the highest number of buds. Parameters the number of buds of Asam Gelugur can see that for the highest number of buds found in the axillary bud explants type and the lower found in the apical shoot. This is because the axillary bud explant can be formed in two buds while the apical shoots only formed one bud of Asam Gelugur. In this case, the treatment of giving PGR to the explant can affect the formation of the number of buds wherein giving the right PGR concentration to the explant type will produce a large number of micro buds at the tissue subculture stage. The stage of subculture to new media with the appropriate PGR will result in the multiplication of micro apical shoot in mass quantities. According to [15] stated that the giving BAP and NAA in MS media were showed significantly different results on the appearance of buds, the number of buds, length of buds, with the better treatment resulted in the BAP 0.5 mg.l\(^{-1}\) + NAA 0 mg.l\(^{-1}\). According to [16] stated that the giving combination of NAA and BAP on Woody Plant Medium (WPM) media, can effect on explants forming buds found in the BAP 0.5 mg.l\(^{-1}\) + NAA 0.25 mg.l\(^{-1}\). [17] stated that the cytokinins have two important roles for propagation in vitro, stimulators of cell division in the tissue made of explants and stimulate the growth of leaf buds. Buds can arise because the explants had buds eye so that the explants are planted in the culture media the buds eye are elongated. [18] stated that the auxin

### Table 2

<table>
<thead>
<tr>
<th>Explant Types</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>G8</th>
<th>G9</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1 (apical shoot)</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,00</td>
</tr>
<tr>
<td>E2 (axillary bud)</td>
<td>1,00 c</td>
<td>2,00 a</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,33 b</td>
<td>1,33 b</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,00 c</td>
<td>1,19</td>
</tr>
<tr>
<td>Average</td>
<td>1,00</td>
<td>1,50</td>
<td>1,00</td>
<td>1,00</td>
<td>1,17</td>
<td>1,17</td>
<td>1,00</td>
<td>1,00</td>
<td>1,00</td>
<td>1,09</td>
</tr>
</tbody>
</table>

Note: The numbers followed by the same letters on the same line show no significant difference in the distance test at the level of 5%. G1 (0.2 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G2 (0.2 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G3 (0.2 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP); G4 (0.4 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G5 (0.4 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G6 (0.4 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP); G7 (0.6 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G8 (0.6 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G9 (0.6 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP).
generally inhibits the growth of buds if higher concentrations, whereas the combination of higher cytokinin concentrations with lower auxin is important in the formation of buds and leaves. In tissue culture, both classes of PGR have been shown in the role in supporting tissue growth when used at the match concentration. [19] stated that the in vitro culture, plants need cytokinins for the formation of buds and leaves, while the auxin was inhibited the buds. The effect of NAA and BAP concentrations combination from the apical shoot and axillary bud on growth Asam Gelugur on at 5 WAP can be seen in Figure 2.

Fig 2. The effect of NAA and BAP concentrations combination from apical shoot and axillary bud on growth Asam Gelugur at 5 WAP. Explant types (E1 = apical shoot; E2 = axillary bud). Combination of NAA and BAP concentrations (G1 = 0.2 mg.l⁻¹ NAA + 0.5 mg.l⁻¹ BAP; G2 = 0.2 mg.l⁻¹ NAA + 1 mg.l⁻¹ BAP; G3 = 0.2 mg.l⁻¹ NAA + 1.5 mg.l⁻¹ BAP; G4 = 0.4 mg.l⁻¹ NAA + 0.5 mg.l⁻¹ BAP; G5 = 0.4 mg.l⁻¹ NAA + 1 mg.l⁻¹ BAP; G6 = 0.4 mg.l⁻¹ NAA + 1.5 mg.l⁻¹ BAP; G7 = 0.6 mg.l⁻¹ NAA + 0.5 mg.l⁻¹ BAP; G8 = 0.6 mg.l⁻¹ NAA + 1 mg.l⁻¹ BAP; G9 = 0.6 mg.l⁻¹ NAA + 1.5 mg.l⁻¹ BAP).

3.3 Length of Buds
Observation data for the length of buds in the giving PGR with 9 combinations of NAA and BAP concentrations using apical shoot and axillary buds of Asam Gelugur (Table 3). The giving NAA + BAP concentrations were showed that a significant effect on the length of Asam Gelugur buds. Table 3 was showed that the 9 combinations of NAA and BAP concentrations applied to the apical shoots and axillary buds explants of Asam Gelugur for the highest length of buds was found in the G6 (0.4 mg.l⁻¹ NAA + 1.5 mg.l⁻¹ BAP) of 1.13 cm that is significantly different from all other treatments. Whereas the lowest buds length was found in G1 (0.2 mg.l⁻¹ NAA + 0.5 mg.l⁻¹ BAP) of 0.14 cm. Similarly, according to [20] stated that the highest buds length was found in media treatment with NAA 0 mg.l⁻¹ and BAP 2.5 mg.l⁻¹ on the garlic plants. In this case, the determination of the appropriate concentration of auxin and cytokinin in the plant types is very influential, where plant types have a different response to the reaction giving PGR in the tissue culture. Therefore, need necessary to determine the match composition of PGR for plant types. The function of auxin as cell elongation while cytokinin acts as cell division, therefore with the match combination of auxin and cytokinin will produce great elongation of buds. In tissue culture, very important to observe changes in the length of buds because increasing the length of buds will produce a lot of micro buds than the results of the subculture process. According to [11] stated that the concentration of cytokinin is higher than the auxin concentration in the culture media will inhibit root growth and will stimulate the formation of buds.
### Table 3
Length of buds in the 9 media combination of NAA and BAP concentrations of apical shoots and axillary bud explants of Asam Gelugur at 6 WAP.

<table>
<thead>
<tr>
<th>Explant Types</th>
<th>Combination of NAA + BAP</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>G2</td>
<td>G3</td>
</tr>
<tr>
<td>E1 (apical shoot)</td>
<td>0.13</td>
<td>0.43</td>
</tr>
<tr>
<td>E2 (axillary bud)</td>
<td>0.15</td>
<td>0.80</td>
</tr>
<tr>
<td>Average</td>
<td>0.14 d</td>
<td>0.62 c</td>
</tr>
</tbody>
</table>

Note: The numbers followed by the same letters on the same line show no significant difference in the distance test at the level of 5%. G1 (0.2 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G2 (0.2 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G3 (0.2 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP); G4 (0.4 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G5 (0.4 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G6 (0.4 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP); G7 (0.6 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G8 (0.6 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G9 (0.6 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP).

### Table 4
The number of leaves in 9 combinations of NAA and BAP concentrations from the apical shoot and axillary bud explants of Asam Gelugur at 6 WAP.

<table>
<thead>
<tr>
<th>Explant Types</th>
<th>Combination of NAA + BAP</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>G2</td>
<td>G3</td>
</tr>
<tr>
<td>E1 (apical shoot)</td>
<td>2.00</td>
<td>4.67</td>
</tr>
<tr>
<td>E2 (axillary bud)</td>
<td>2.00</td>
<td>6.67</td>
</tr>
<tr>
<td>Average</td>
<td>2.00g</td>
<td>5.67c</td>
</tr>
</tbody>
</table>

Note: The numbers followed by the same letters on the same line show no significant difference in the distance test at the level of 5%. G1 (0.2 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G2 (0.2 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G3 (0.2 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP); G4 (0.4 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G5 (0.4 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G6 (0.4 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP); G7 (0.6 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP); G8 (0.6 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP); G9 (0.6 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP).

### 3.4 Number of Leaves
The results of observational data and ANOVA on the number of the parameters of leaves on giving PGR with 9 combinations of NAA and BAP concentrations using apical shoots and axillary bud explants of Asam Gelugur (Table 4). The giving 9 combinations of PGR were showed that had a significant effect on the number of Asam Gelugur leaves. Table 4 was showed that the giving 9 combinations of NAA and BAP concentrations were applied on the apical shoots and axillary buds explants of Asam Gelugur plant for the highest number of leaves was found in the G8 (0.6 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP) of 7.00 sheets, while in the lower number of leaves was found in the G1 (0.2 mg.l\(^{-1}\) NAA + 0.5 mg.l\(^{-1}\) BAP) of 2.00 sheets. This is suspected by the addition of cytokinins (BAP) to the media that can encourage meristem cells in explants to divide and influence other cells to develop into buds and formed leaves.

### 4 CONCLUSIONS
The giving PGR combination of 0.4 mg.l\(^{-1}\) NAA + 1.5 mg.l\(^{-1}\) BAP (G6) can produce a greater length of Asam Gelugur buds. The combination of 0.6 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP (G8) can produce the greater number of Asam Gelugur leaves. The interaction of axillary bud explant and combination of 0.2 mg.l\(^{-1}\) NAA + 1 mg.l\(^{-1}\) BAP (E2G2) on MS media can produce a greater number of Asam Gelugur buds.

### REFERENCES


