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Article

Growth Response of Banana Kepok Tanjung (*Musa acuminata balbasiana*) with the Application of BAP (*Benzyl Amino Purine*) and Cow's Milk *In Vitro*

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Abstract. Tissue culture is a plant propagation technique using artificial media by adding particular growth regulators (ZPT) to produce plants as expected. Although, the availability of other issues such as browning and contamination can inhibit the process of explant propagation. The addition of cow's milk to the culture media and BAP is a type of cytokinin is expected to regenerate the growth of banan Kepok Tanjung variety optimally. This study aims to have best concentration of BAP and cow's milk for the growt Kepok Tanjung explants *in vitro*. The experimental design used was a randomized with 16 treatment combinations. The result showed a significant of the combination of media treatment of banana Kepok Tanjung variety on the number of leaves (24.2 sheets) and explant height (25.62 cm) starting from 3-12 WAP.

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1. Introduction

Based on the economic aspect, the need for bananas continues to increase, one of which is the type of banana Kepok Tanjung. The selection of this variety is based on the advantages of the variety, which is able to control diseases caused by the bacterium *Ralstonia solanacearum*, because this variety does

not have stamen or banana blossom [1-2]. However, the availability of Kepok Tanjung seeds is still small. So it is necessary to do the right propagation technique, one of which is by using tissue culture techniques [1].

With tissue culture techniques, it is expected to produce seeds en masse without requiring many broodstock in a relatively short time [3]. Tissue culture is a method to isolate parts of plants such as protoplasm, cells, tissues, organs then grow them under aseptic conditions [4]. Tissue culture is a technique of plant propagation *in vitro* for a long time to improve plant genetics [5].

Tissue culture techniques help in eliminating pathogens by selecting parts or cells that do not contain pathogens, especially viruses, and then regenerating them into healthy plants [5]. The success of tissue culture techniques is influenced by the provision of adequate nutrients in the growing medium [4][5]. The composition of the media used usually consists of mineral salts, vitamins and growth regulators [6].

Growth Regulators (ZPT) used in tissue culture media are auxins and cytokinins [7]. Cytokinins are hormones that can affect the emergence of buds [8]. The addition of cytokinins to tissue culture media can increase the concentration of growth regulators in cells and become one of the important factors in the process of tissue growth and development [9]. *Benzyl Amino Purine* is a cytokinin that is always used because it has a high effectiveness against stimulating bud formation in plants [10].

Until now, the growth in the number of shoots using BAP has had a major influence on the formation of explants [1][5]. Based on research [11], BAP concentration of 2 ppm is the optimum level of BAP administration to increase the morphology of growth of pineapple plants (*Ananas comosus* L. Merr) and orchids (*Phalaenopsis* sp.). Other studies also explained, giving 0-1 mg/l BAP has a very strong influence on root formation, but when the concentration of BAP given is higher, it will inhibit the process of root emergence [10]. Thus, the use of ZPT will provide convenience in multiplying plant seeds *in vitro*.

Tissue culture media not only provides macro and micro nutrients, but also a source of carbohydrates in the form of sucrose [4]. Sucrose serves to replace carbon that is usually obtained to carry out the process of photosynthesis [4][12]. However, the use of other carbohydrates such as lactose, galactose, raffinose, maltose, and starch is considered to respond poorly to plant propagation and growth *in vitro* [12].

The use of organic compounds in culture media is a source of natural growth regulators in which there are several hormones such as cytokinin and auxin groups [13]. To get better results, vitamins can also be added vitamins, amino acids, and other growth regulators [4]. In general, milk is a source of animal protein needed for health because it contains high nutrition [14]. Milk contains proteins, fats, carbohydrates, minerals and vitamins [14-15]. Protein is the main nutrient in milk because it contains essential amino acids [15]. Milk proteins are formed from three main sources namely peptides, plasma, and amino acids that promote plant growth through facilitating the availability of nitrogen to plants [15-17].

In plants, proteins are the basic ingredients of protoplasm and mostly form enzymes, then enzymes enter the meristem area which can promote leaf and root growth [13][16]. Some research results [17], the use of casein hydrolysate (200-500 mg/l) containing amino acids is effective in the growth of the number of leaves and the number of shoots on *Rosa canina* plants.

This research was conducted because various kinds of media affect the growth and development of plants. By using a combination of culture media treatments, it is expected to increase the number of shoots, number of leaves, and explant height in the growth of banana explants of cape kepok varieties. The purpose of this study was to obtain the best concentration of BAP and cow's milk for the growth of Kepok Tanjung banana explants *in vitro*.

2. Experimental Section

2.1. Materials

The tools used in this experiment are culture bottles, spatula, beaker glass 100 ml up to 1 L, petridish, dropping pipet 3 ml, scalpel blade, forcep, *Laminar Air Flow* (LAF), autoclave, bunsen, hand sprayer, analytical balance, ruler, stirrer magnetic, syringe 1 cc up to 50 cc, stove, note book, pen, marker, and camera. The materials for experiment are explant bananas kepok tanjung, pH meter, murashige & skooge, BAP (6-*Benzyl amino purine*), cow's milk, 5 L aquades, sucrose 30 g/L, NaOH 1 M, alkohol 70% and 96%, tissue, alumunium foil, jelly, wrapping plastic, PPM (*Plant Preservative Mixture*).

2.2. Methods

This experiment was conducted at Tissue Culture Laboratory, Seed Hall and Forest Protection, Serang, Banten from October 2021 to March 2022. The research flowchart is shown in Figure 1-2. The tools and materials to be used are first sterilized using an autoclave with a temperature of 121^0C at a pressure of 1 atm for 30 minutes. While materials such as aquades and culture media are sterilized using an autoclave for 15 minutes at a temperature of 121^0C at a pressure of 1 atm. Tools such as syringes also need to be sterilized using 70% alcohol and then put into *Laminar Air Flow* (LAF) to be given UV light for 30 minutes.

The second, in the manufacture of culture media, initially some components of MS media are dissolved with aquades as much as 500 ml. Then add 30 g/1 of sucrose and homogenize. After the solution is homogeneous, enter the stock of BAP hormone according to the treatment then measure the pH of the solution in the range of 5.6-5.8. After that, add the media solution with sterile aquades to 1 L, then mix the gelatin into the culture medium. Heat the solution and then transfer the media solution into 30 ml culture bottles in each bottle. Close tightly using plastic wrapping and aluminum foil. Then, sterilize the media into an autoclave with a temperature of 121^0C 1 atm for 15 minutes. After the media is sterile, transfer the culture media into *Laminar Air Flow* (LAF) to be given additional milk treatment according to the treatment, then close again. Place the culture medium inside the incubation chamber. After the media has been incubated for one week, if the media is sterile (not contaminated) the media can be used to subculture. Prepare the planting room, make sure that everything is in a sterile state. Then, the Kepok Tanjung banana explant will be subcultured using a combination of BAP and cow's milk.

Parameters observed were buds time appear, roots time appear, the number of buds, the number of leaves, and plant height. There were 2 treatments combination, the first treatment was BAP (0 ppm; 2 ppm; 4 ppm; and 6 ppm). And the second treatment was cow's milk (0 mg/l; 50 mg/l; 100 mg/l; and 150 mg/l). Each treatment was repeated 5 times so that there were 80 experimental units. Each experimental unit consisted of 1 plant, so there were 80 Kepok Tanjung explant. Data analysis was performed using the Microsoft Excel application and IBM SPSS 25 T test 5% significance level.

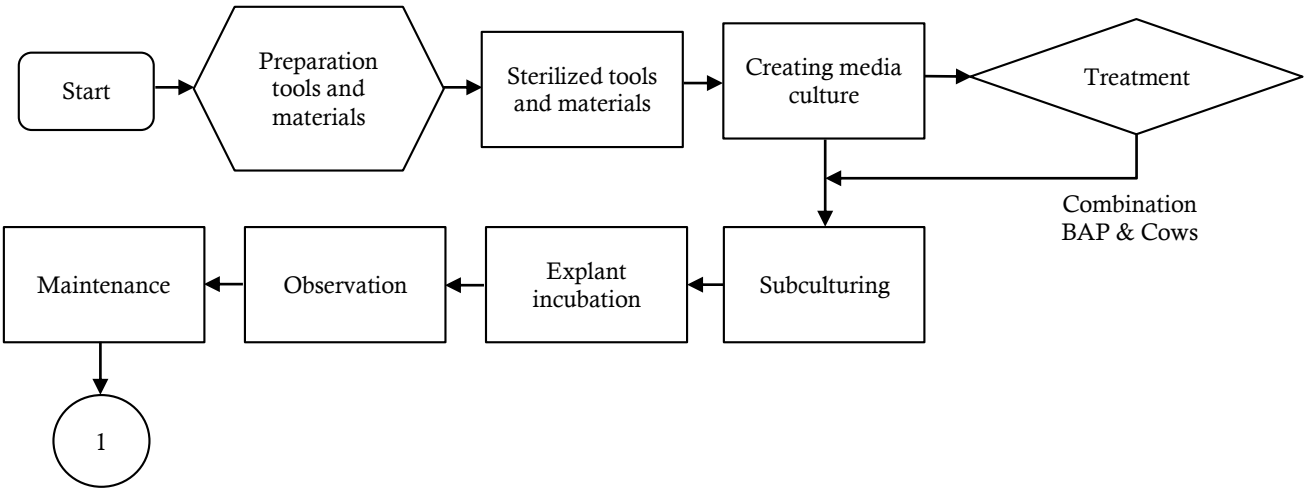


Figure 1. Schematic/flowchart of research

Number one (1) is the next step in treatment. Number two (2) is the next step in data processing (Ms. Excel and IBM SPSS 25). Number three (3) is a non-significant result in the analysis of variance. Y is a sign of a normal or significant result and N is a sign of an abnormal or significant result from the test.

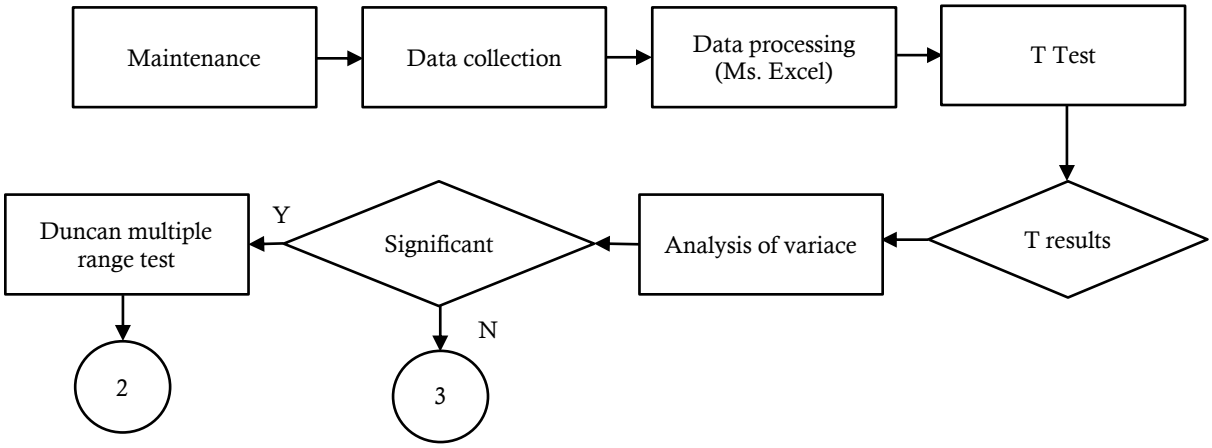
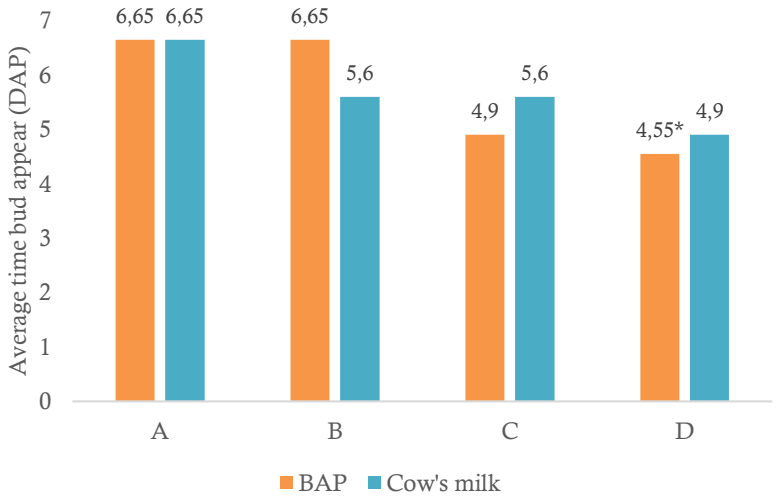


Figure 2. Schematic/flowchart of research

3. Results and Discussion

3.1. Bud Time Appear (DAP)

Based on the results of the analysis in Figure 3, showed that the timing of budding significantly affected each treatment up to 1 WAP. The average time of emergence of shoots ranges from 4.5-6.7 DAP. In the results, it can be seen that the fastest bud emergence time is in the 6 ppm BAP treatment with a bud emergence time of 4.6 DAP, while in the cow's milk concentration treatment of 150 mg/l the average bud emergence time is 4.9 DAP.



Annotation. BAP (A = 0 ppm; B = 2 ppm; C = 4 ppm; D = 6 ppm); Cow's milk (A = 0 mg/l; B = 50 mg/l; C = 100 mg/l; D = 150 mg/l).

Figure 3. Effect of BAP and cow's milk concentration on bud emergence time parameters

The speed of budding time occurs due to the interaction between endogenous and exogenous hormones of the explant. Different endogenous hormone levels in each explant will affect the response of an explant to ZPT [18].

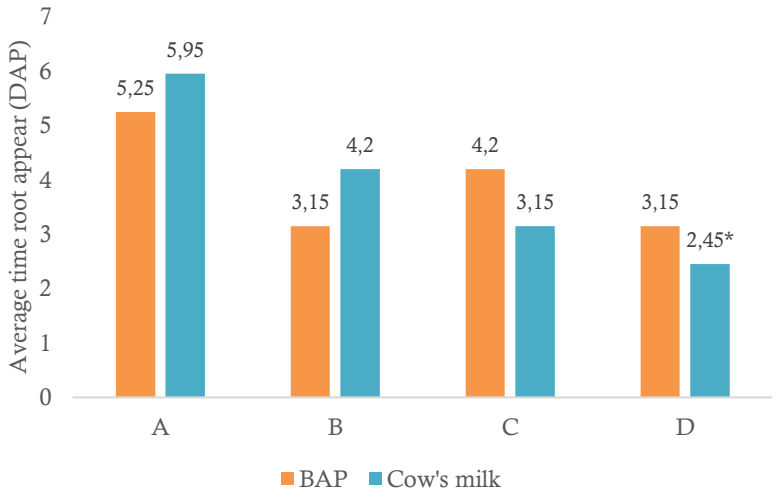
Based on the Figure 3, the average control time of bud emergence was 6.65 DAP. The time of emergence of shoots in the control treatment results are not much different from BAP 2 ppm. This is because in the control there is no addition of growth regulators so that the time to appear shoots produced is longer, while at a BAP concentration of 6 ppm the use of cytokinin growth regulators is able to stimulate physiology in banana explants. In research [20], the influence of BAP on early growth is very dominant, so it is often used to stimulate growth and development.

In addition, this study is supported by statement [19], which explains that the addition of BAP 2 ppm slows down the time of bud emergence in *Siamese citrus* tissue culture. However, this statement is in contrast to research [19], which explains that the use of BAP 2 ppm accelerates the growth of shoots against the explant of kasturi orange [20]. This is because the increase in cytokinins in bud regeneration increases the content of hydrogen peroxide which can damage structural cell elements and degradation which can cause cell death so as to slow down bud regeneration.

While at the time of bud growth using the concentration of cow's milk at 50 mg/l treatment is not much different from the treatment of cow's milk concentration of 100 mg/l. This is thought to be because cow's milk contains protein composed of amino acids that play a role in stimulating the growth and development of buds on the explant. These results are similar to research [17], that giving casein hydrolysate 25-50 mg/l in banana plant explants combined with 4 ppm BAP significantly increases the regeneration of banana buds.

3.2. Root Time Appear (DAP)

Based on the analysis results in Figure 4. shows that the time of root appearance does not affect the combination of treatments (BAP and cow's milk), but affects each concentration of BAP and cow's milk for less than 1 WAP. The average time of root appearance starts from 2.5 DAP - 6 DAP. Root growth increased until the end of observation (12 WAP).



Annotation. BAP (A = 0 ppm; B = 2 ppm; C = 4 ppm; D = 6 ppm); Cow's milk (A = 0 mg/l; B = 50 mg/l; C = 100 mg/l; D = 150 mg/l).

Figure 4. Effect of BAP and cow's milk concentration on root emergence time parameters

In Figure 4. it can be seen that the fastest root appearance time is in the treatment of 150 mg/l concentration of cow's milk with an average root appearance time of 2.45 HST. It is thought that the effect of cow's milk concentration can stimulate root growth because cow's milk contains amino acids, calcium, vitamins and other macro elements that are able to stimulate root growth. Root formation occurs due to the movement of auxin towards the bottom of the plant, carbohydrates contained in plants will gather with auxin in the explant treatment area, then encourage cells to form roots through the process of cell differentiation [21].

While in the treatment of BAP concentration of 2 ppm gives the fastest root growth time with an average root growth time of 3.15 DAP. In this study, the average root appearance time control started from 5-6 DAP. The root appearance time in the control treatment was longer than the addition of growth regulators. This is thought to be because the auxin content contained in the explants is not sufficient to fulfil root growth so that if added with exogenous auxin, root emergence will be faster.

In addition, it is suspected that the content of BAP which is one of the cytoknin hormones that functions in inhibiting root growth. This is in accordance with the statement [19] that too high a concentration of BAP can cause inhibition of root growth. Without the provision of sucrose at all concentrations, root growth was still found, although it was slow in growth. This is thought to be due to the presence of endogenous auxin which is able to stimulate root growth in explants so that such results are obtained even though they are not given exogenous auxin. Statement [22], that the formation of roots is due to the influence of exogenous auxin (NAA) added to tomato media. In research [23], that at a concentration of 59 mg/l sucrose can increase root growth in sugarcane explants.

3.3. Number of Buds (bud)

One indication of success in tissue culture techniques is the multiplication. In (Table 1) it can significantly be seen that the number of buds produced at 12 WAP is in the treatment of 0 mg/l cow's milk concentration or control with an average number of buds that appear as many as 8.85 buds.

The maximum number of buds produced in the combination of BAP and cow's milk is in the combination of BAP 2 ppm treatment and without the provision of cow's milk concentration with the number of buds that appear as many as 18.6 buds. According to the opinion [24] that differences in media composition, composition of growth regulators and the type of media used will greatly affect the growth and regeneration of cultured planlets.

Table 1. The Effect of Giving BAP and Cow's Milk Concentrations on the Number of Buds Parameter (Buds)

Treatment	Cow's milk				Average
BAP	0 mg/L	50 mg/L	100 mg/L	150 mg/L	
0 mg/L	1.8	2.4	1.8	3	2.25
2 mg/L	18.6	5.4	3	2.4	7.35
4 mg/L	6.6	1.2	1.2	1.2	2.55
6 mg/L	8.4	2.4	0.6	1.2	3.15
Average	8.85^{A}	2.85^{B}	1.65^{B}	1.95^{B}	

Based on (Tabel 1 and Figure 5) at 12 WAP it can be seen that the higher the concentration of growth regulators given, the lower the number of buds produced. It is suspected that the concentration of BAP, which is included in the cytokinin hormone, the higher the concentration will inhibit the explants to form buds, so that the resulting buds are reduced. However, this is not in line with research [25] that in the 4 ppm treatment the number of cavendish banana buds produced was an average of 3.06 buds. In addition, research [26] also explains that the growth of the highest number of shoots is in the combination of BAP treatment 6 mg/l and control which has a total of 6 shoots.

The endogenous hormone auxin acts in cell proliferation, inhibits bud proliferation, stimulates root proliferation, and contributes to the development of xylem and phloem structures, as well as affecting root growth. This results in the higher growth of dividing cells without cow's milk treatment than without cow's milk treatment.

(a) (b)

Figure 5. (a) Explant with cow's milk 50 mg/L; (b) Explants without cow's milk

Based on the data contained in Figure 5, the higher the concentration of cow's milk given can prevent the development of explants to grow buds, this cow's milk is thought to be similar to the growth regulator auxin which only stimulates the growth of height and size in explants. The imbalance of endogenous auxin and exogenous auxin content causes inhibition of the formation of new shoots derived from cell division [18][20][27]. However, this is different from the opinion of [17], that the use of casein hydrolysate (200-500 mg/l) containing amino acids is effective in the growth of the number of leaves and the number of buds on *Rosa canina* plants. Based on research [28], the provision of casein hydrolysate containing amino acids as much as 10-30 mg/l with the addition of BAP 2 ppm was able to regenerate *Chlorophytum borivilianum* buds after 30 and 45 HST.

3.4. Number of Leaves (sheets)

Based on the results of the analysis in Table 2, it shows that the combination treatment of BAP and cow's milk gives a significant effect on the number of leaves on banana explants of Kepok Tanjung variety.

Table 2. The Effect of the Interaction of BAP and Cow's Milk Concentration on the Parameter of the Number of Leaves (Sheets)

Treatment	Cow's milk			
BAP	0 mg/L	50 mg/L	100 mg/L	150 mg/L
0 mg/L	5.4 b	10 b	1.4 b	10.8 b
2 mg/L	24.2 a	5.6 b	5.2 b	4.4 b
4 mg/L	7.8 db	1.8 b	3 b	2.8 b
6 mg/L	8.6 b	8 b	4 b	1.2 b

The average number of leaves increased until 12 weeks after planting, while the combination of BAP and cow's milk treatment in (Table 2) showed the highest number of leaves was in the treatment of BAP concentration of 2 ppm and 0 mg/l cow's milk with an average number of leaves that appeared as many as 24.2 strands. It is suspected that the ratio of cytokinin which is greater than auxin in in vitro culture will stimulate the growth of shoots and leaves, but for BAP concentrations that are too high will inhibit leaf growth. Based on research [29], the formation of plant organs is by adding growth regulators auxin and cytokinin with the right concentration in tissue culture media. Too high a concentration of cytokinin can inhibit shoot growth components, one of which is the number of leaves [19].

In addition, the provision of cytokinin that exceeds the optimal limit of plant needs will inhibit plant growth. The same thing happened in research [19] that, the number of leaves from 2-3 ppm treatment can significantly reduce the number of leaves.

However, it is inversely proportional to the treatment of cow's milk concentration, based on (Table 2), the lower the concentration of cow's milk given will increase the number of leaves. This is thought to be due to the nitrogen content contained in cow's milk if the concentration increases, it will inhibit the growth of shoots, so that the number of leaves that will be produced is also low. Based on research [30], that nitrogen contained in casein hydrolysate affects leaf growth.

Based on (Table 2) the provision of cow's milk concentration of 50-100 mg/l showed results that were not much different in the parameter of the number of leaves. Based on research [30] also explained that the treatment of casein hydrolysate 100 ppm containing amino acids can stimulate plant growth where the organic nitrogen axis contained in casein hydrolysate is more quickly absorbed by plants.

3.5. Plant Height (cm)

Based on the analysis in (Table 3) combination of BAP concentration and cow's milk significantly influenced the height of explants until 12 weeks after planting. Compared to the control treatment at 12 weeks post-planting, the maximum height of plantlets was found in the concentration of BAP 2 ppm and 0 mg/l cow's milk, followed by the concentration of BAP 4 ppm and 6 ppm with an average explant height of 25.62 cm - 21.66 cm.

Table 3. Effect of Interaction of BAP and Cow's Milk Concentration on Explant Height Parameters (cm)

Treatment	Cow's milk			
BAP	0 mg/L	50 mg/L	100 mg/L	150 mg/L
0 mg/L	17 abc	16.84 abc	9.94 bcd	24.6 a
2 mg/L	25.62 a	10.8 bcd	5.18 cd	6.64 cd
4 mg/L	24.7 a	5.06 cd	12.1 abcd	1.98 d
6 mg/L	21.66 ab	15.14 abcd	5.34 cd	4.32 cd

This is thought to be because BAP is able to stimulate cell division which is then followed by enlargement and elongation. According to the statement [8], the treatment of low concentrations of BAP has the potential to increase the height of the planlets. The other factors that cause the height of the explants are thought to be due to the influence of cytokinin hormones contained in BAP when combined with other concentrations such as auxin hormones in the explants can also cause cell elongation so as to increase the height of the shoots [19].

In the treatment of cow's milk concentration compared to the control, the concentration of cow's milk 50 mg/l gives an average value of explant height of 16.48 cm. It is suspected that the higher the concentration of cow's milk given can inhibit the growth of explants height can be seen in (Table 3). This concentration of cow's milk contains casein which consists of nitrogenous composition. The higher the content of milk given to the explants, the lower the height growth of the explants. This is in accordance with research [31] that amino acids act as a source of organic nitrogen that can stimulate growth and development, the higher the concentration of amino acids if given to explants can make the growth of plant height inhibited due to high nitrogen content.

4. Conclusion

There was an effect on the combination of BAP and cow's milk treatment on the parameters of the number of leaves as many as 24.2 strands and explant height of 25.62 cm at the age of 12 weeks after planting. BAP concentration of 2 mg/l gave the best effect on the number of buds (4.3 buds) at the observation of 12 weeks after planting. Meanwhile, the concentration of BAP 6 mg/l gave the best effect on the fastest shoot emergence time at 4.55 DAP. Cow's milk concentration of 150 mg/l influenced the time of root emergence at 2.45 DAP.

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