Vol. 13, No. 4 (2024): 1013 - 1022 http://dx.doi.org/10.23960/jtep-l.v13i4.1013-1022

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Effect of Benzylaminopurine (BAP) and Coconut Water on the Growth of *Bucephalandra* sp. in Vitro

Alfira Damayanti Septianingsih[1], Makhziah[1,✉], Sukendah[1]

[1] Department of Agro-Technology, Faculty of Agriculture, UPN "Veteran" Jawa Timur, Surabaya, INDONESIA.

Article History:

Received : 03 April 2024
Revised : 26 May 2024
Accepted : 06 June 2024

Keywords:

Aquatic plant,
Endemic plant,
Plant growth regulators,
Tissue culture.

Corresponding Author:
✉ makhziah.agro@upnjatim.ac.id
(Makhziah)

ABSTRACT

Tissue culture is useful as a way to obtain seeds in large quantities of seedlings in a short time as an effort to preserve Bucephalandra *sp. in nature. The use of PGR has problems such as expensive prices and difficult to find, therefore its use can be replaced by natural PGR that are easy to obtain and have relatively cheap prices such as coconut water. The aim of this research was to determine the effect of Benzylaminopurine (BAP) and coconut water on the growth of* Bucephalandra *sp. in vitro. This research is a 2-factor factorial experiment using a completely randomized design. The factors are concentration of BAP (0; 1; 3; 5 mg/l) and concentration of coconut water (0; 50; 100 ml/l). The results showed Murashige and Skoog (MS) media with the addition of BAP 1 mg/l + coconut water 50 ml/l (4.11 shoots), BAP 5 mg/l + coconut water 50 ml/l (4.33 shoots), and BAP 3 mg/l (6.22 shoots) were able to provide the number of shoots with high results. The addition of coconut water did not affect the growth of* Bucephalandra *sp. MS media can grow shoots with the best results in measurements of shoot emergence time, number of roots, shoot and root length.*

1. INTRODUCTION

Bucephalandra sp. is an endemic ornamental plant of Borneo Island which generally has a living habitat in river areas. *Bucephalandra* sp. belongs to the *Araceae* family which has a unique morphology with each species having differences in color, leaf shape and leaf edges. The beauty of this plant is used as a design object in aquaspace to paludarium. As an aquatic plant, *Bucephalandra* sp. is highly favored by aquascape enthusiasts both domestically and internationally. This causes the selling price of this plant to be relatively high (Nugraha *et al*., 2020).

Conventional propagation can take quite a long time with limited quantities of seedlings produced, because *Bucephalandra* sp. plants grow slowly (Tustin, 2013; Sholichah *et al*., 2020). Continuous exploitation of nature leads to the threat of extinction for *Bucephalandra* (Yunita *et al*., 2020). Conventional propagation faces various obstacles such as field techniques, quality, and time (Basri, 2016). Tissue culture is useful as a way to generate a high quantity of seedlings in a short time as an effort to preserve *Bucephalandra* sp. plants in nature. Tissue culture techniques can help overcome the problem of slow growth of *Bucephalandra* sp. plants (Laohavisuti *et al*., 2017). The advantages of *in vitro* cultivation are that the number of plants produced does not require a large area, fast and free from attacks by other pest organisms (Yunita *et al*. 2020). Tissue culture requires a suitable media composition for optimal plant growth, which is achieved by using growth regulators. Growth regulators are divided into two categories: synthetic and natural. Examples of synthetic growth regulators include BAP (Benzylaminopurine), TDZ (Thidiazuron), IAA (Indole Acetic Acid), etc (Abror & Noviyanti, 2019). Examples of natural growth regulators include banana extract, tomato extract, and coconut water.

Yunita *et al*. (2020) reported the combination of BAP and TDZ, both synthetic plant growth regulators (PGR),

resulted in the highest number of shoots with BAP 2 mg/l and TDZ 0.1 mg/l. Changing the variety and concentration level of PGR can be done to growth optimally in tissue culture media. Synthetic PGR price is relatively expensive, therefore this can be replaced by using natural growth regulators that it's more easier and low prices such as coconut water (Handayani & Isnawan, 2014). Coconut water is a sterile natural water that has a high mineral content of K and Cl (Kristina & Syahid, 2012). Coconut water is included in cytokinin and auxin hormones (Trubus, 2021). Coconut water contains growth regulators including zeatin, 9-β-D ribofuranosyl zeatin, 2-(3-methylbut-2-enylamino)-purin 6-one and N-N-Diphenyl urea (Habibah *et al*., 2021). The content of nucleic acids and protein in young coconut water can stimulate plant metabolism so that it can spur the growth of sugarcane cuttings to the maximum compared to other growth regulators treatments (Maruapey & Sangadji, 2022).

The effect of coconut water on culture media was significantly on plant growth and development of C*hrysanthemum* (Solihah *et al*., 2021). Coconut water 50 ml/l resulted in a shoot emergence time of 76.67 days, with shoot height reaching 8.37 cm on *Cocos nucifera* explants (Maulida & Erfa, 2020). Coconut water 100 ml/l has an impact on the quantity of planlet roots. The combination of coconut water 100 ml/l + BAP 1 mg/l resulted the highest percentage value of shoot production rate of *Ficus carica* explants (Sophia *et al*., 2021). The aim of this research was to determine the effect of BAP and coconut water on the *in vitro* growth of *Bucephalandra* sp.

2. MATERIALS AND METHODS

This study was carried out from August - December 2023 at the Tissue Culture Laboratory, DKPP Surabaya. The research used *Bucephalandra* sp. explants from the SEAMEO BIOTROP Bogor collection. The materials used in the study were BAP, green young coconut water aged 6 - 8 months, culture media materials such as stock solution, agar media, 10% KOH, 1N HCl, and sugar. The tools used in the study were culture bottles, scalpel, tweezer, knife, Bunsen, Petri dishes, magnetic stirrer, hot plate, millimeter paper, camera, and stationery.

2.1. Research Procedure

Research is carried out by preparing the tools and materials to be used. Make sure the equipment and materials to be used are in sterile condition. Coconuts used are green coconuts aged 6-8 months. Coconut water is filtered using filter paper to separate water from dirt and pulp. Coconut water is put together in one container and then stirred until smooth. Coconut water was measured according to each treatment requirement. Preparation of BAP stock solution was done by dissolving 0.1 gram of BAP in a few drops of KOH 10% and then adding 1000 ml sterile distilled water. The solution was stirred until homogeneous using a magnetic stirrer.

Tissue culture generally uses Murashige and Skoog (MS) media which contains macro and micro nutrients essential for plant growth. The preparation of MS media involves adding stock solutions A, B, C, D (macro nutrients), E (iron solution), and F (micro nutrients) according to their concentrations. Add sterile distilled water to the MS media solution and mix with a magnetic stirrer. Additionally 30 gr/l of sugar, BAP, and coconut water are included for each treatment, along with extra distilled water that has been sterilized. The treatment media pH is raised to 5.8. If the pH is lower, 10% KOH is added, while if it is higher, 1N HCl is added until the pH reaches the desired level. Agar (6.8 grams/l) is then added to the media, which is heated using a hotplate until boiling. Once the media is prepared, it is dispensed into sterile culture bottles (30 ml each). The bottles are covered with aluminum foil and sterilized using an autoclave for 30 minutes at 0.1 MPa pressure and temperature 121°C.

The explants were cut inside the LAF. This involved cutting plant organs such as leaves and roots, separating the shoots, and detaching the explants from the agar media. Explants were cut to a length of ± 1 cm. Explants were located in the treatment media, with each culture bottle containing one explant. These bottles were then stored on a culture rack. Maintenance involved placing the culture bottles containing explants on a rack under 12 hours of irradiation. The room temperature was maintained at 25°C (Laohavisuti *et al*., 2017; Yunita *et al*., 2020). Observations of the explants included the percentage of explant growth (%), shoot emergence time (days), number of shoots, number of leaves, shoot length (cm), number of roots, root length (cm), and visual observations. These observations were conducted weekly and at the end of the observation period, namely 10 WAP (weeks after planting).

2.2. Experimental Design and Data Analysis

This study used a 2-factor factorial experiment in a completely randomized design (CRD). The first factor was the concentration of BAP (0, 1, 3, and 5 mg/L), and the second factor was the concentration of coconut water (0, 50, and 100 mL/L). The observation data will be analyzed using ANOVA for the linear model of the factorial CRD to determine whether there are any significant differences in the observed effects. Test results that show significant differences will be subjected to further analysis Duncan Multiple Range Test (DMRT) at a 5% significance level to determine the effects of each treatment.

3. RESULTS AND DISCUSSION

The test results using analysis of variance showed that there were several parameters that produced interactions in the application of BAP and coconut water in the culture medium. The interaction occurred in the parameters of percentage of explant growth, shoot emergence time, number of shoots, shoot length, number of roots, and root length. The addition of BAP produced an effect on all treatments except percentage of explant growth and number of leaves. The addition of coconut water alone affects only the parameters of shoot emergence time, number of roots, and root length.

Table 1. Analysis of variance (ANOVA) of each treatment for all response variables

ANOVA →	E	B		A		B×A	
	-	F5% = 3.01		F5% = 3.40		F5% = 2.51	
Treatment	MS	MS	F	MS	F	MS	F-value
Percentage of Explant Growth (%)	0.02	0.04	1.46^{tn}	0.04	1.50^{tn}	0.07	2.83*
Shoot Emergence Time (days)	3.13	53.10	16.98*	20.52	6.56*	19.30	6.17*
Number of Shoots	1.35	7.55	5.59*	1.77	1.31^{tn}	6.54	4.84*
Number of Leaves	0.36	1.08	3.00^{tn}	0.79	2.19^{tn}	0.57	1.60^{tn}
Shoot Length (cm)	0.08	0.20	2.44^{tn}	0.14	1.77^{tn}	0.51	6.32*
Number of Roots	0.04	3.79	87.69*	3.12	72.21*	3.09	71.55*
Root Length (cm)	0.01	0.39	69.58*	0.35	62.25*	0.32	57.27*

Note : E: error, B: BAP, A: coconut water, B×A: combination of BAP and coconut water, MS: mean of squares

3.1. Percentage of Explant Growth (%)

The results revealed that there was an effect of BAP and coconut water added to MS media on the percentage of explant growth. Treatments with BAP and coconut water individually did not result in any apparent effects. Table 2 shows the impact of BAP and coconut water on the percentage of explant growth. Table 2 shows the percentage of explants that grew, ranging from 66.67% to 100%. The variation in the growth percentage is attributed to obstacles during explant growth, such as contamination and browning. Explants with bacterial contamination will show symptoms of white to yellowish brown mucus at the bottom of the explant and expand to cover the media. According to Wati *et al*. (2020), bacteria can enter tissue culture media through wounds caused by cutting explants, leading to contamination and explant death. Explants contaminated by fungi exhibit the growth of white to gray-black hyphae on the media. The cause of contamination in tissue culture according to Hapsoro & Yusnita (2018) is that culture media stored for a long time in a humid and dirty location can cause a trigger for contamination. This might due to the high population of microorganism inoculum in the air when the air was humid or the lack of sterilization of the culture bottles used. Explants browning show the characteristics of the explant body that is blackish brown to the inside of the explant. Browning occurs because there is a reaction of phenolic.

Table 2. Effect of BAP and coconut water on the percentage of explant growth (%)

Coconut Water (ml/l)	BAP (mg/l)			
	0	1	3	5
0	100 b	77.78 ab	100 b	66.67 a
50	100 b	100 b	66.67 a	100 b
100	100 b	100 b	100 b	88.89 ab

Note : A significant difference is not seen in the 5% DMRT test for numbers that are followed by the same letter.

phenolic compounds with the enzyme Polyphenol Oxidase (PPO) which will result in the appearance of a brown color at the opening of the explant, if not overcome it will cause the death of the explant (Helena *et al*., 2022).

The addition of BAP 5 mg/l which has a growing explant percentage value of 66.67% shows that the explants experience death due to browning. (Prameswari *et al*., 2019) stated that the use of BAP concentrations ≥ 5 mg/l in tissue culture media can cause explant death. In the opinion of Mastuti *et al*. (2018), the addition of PGR in culture media with high concentrations can cause a higher chance of browning.

Media with BAP 1 mg/l, BAP 3 mg/l + coconut water 50 ml/l, and BAP 5 mg/l + coconut water 100 ml/l showed the percentage value of growing explants of 77.78%; 66.67%; and 88.89%. These treatments were decreased in the percentage of growing explants caused by contamination of the explants. The aplication of coconut water in the media could cause contamination due to lack of sterilization of the media. Coconut water is an organic material that comes from nature, so the lack of sterilization stage can be a source of contaminants. The lack of sterilization stages on the equipment and materials used can also be a source of contaminants. This was supported by Apriliyani & Wahidah (2021), regarding explants and culture media materials that are less sterile can trigger contamination. Contamination can be caused by the use of culture bottles or from media materials that are less sterile which results in the growth of microorganisms in the culture media. The use of sterile tools and media is necessary to prevent the death of explants by contamination.

3.2. Shoot Emergence Time (days)

The results revealed that there was an effect of BAP and coconut water added to MS media on the shoot emergence time. Every application of BAP or coconut water by itself has a noticeable impact. Table 3 shows the impact of BAP and coconut water on the shoot emergence time.

Table 3. Effect of BAP and coconut water on the shoot emergence time (days)

Coconut Water (ml/l)	BAP (mg/l)			
	0	1	3	5
0	6.67 a	11.33 c	11.67 c	12.78 c
50	10.67 bc	11.22 c	17.44 e	12.33 c
100	13.56 cd	7.78 ab	16.67 de	13.33 cd

Note : A significant difference is not seen in the 5% DMRT test for numbers that are followed by the same letter.

Table 3 revealed that the media treatment of BAP 3 mg/l + coconut water 50 ml/l has the longest shoot emergence time of 17.44 days after planting. This treatment was not significantly different from BAP 3 mg/l + coconut water 100 ml/l which has a shoot emergence time value of 16.67 days. MS media (control) gave the fastest shoot emergence time of 6.67 days after planting compared to other combinations. The application of BAP and coconut water did not show an effect on the time of shoot emergence of *Bucephalandra* sp. explants. This is because the addition of PGR provides a growth response from the explants. Changes in the content of culture media cause differences in explant growth response. The application of PGR to culture media results in differences in explant growth response (Putriana *et al*., 2019). The use of explant media types before research and when used has the same chemical composition, namely MS media, so that the explants do not experience an adaptation phase. Explants will adapt to adjust metabolism to a new media environment that is different from the previous media. Prihantini *et al*. (2007) stated that cells that are inoculated will undergo chemical and physiological changes to adjust metabolic activity so that cells can grow in new media.

3.3. Number of Shoots and Shoot Length (cm)

There was an interaction between BAP and coconut water on the number of shoots and shoot length. Media with BAP gives a significant effect on the number of shoots but not significant impact on shoot length. Coconut water treatment alone gives no significant effect. Table 4 shows the impact of BAP and coconut water on the number of shoots and shoot length. BAP 3 mg/l media without coconut water showed the highest number of shoots (Table 4). This shows the use of BAP 3 mg/l can grow shoots optimally. Klaocheed *et al*. (2020) stated that *Cryptocoryne wendtii* plants in vitro

on media containing BAP 3 mg/l were able to produce the highest average number of shoots compared to other treatments. The results of research by Kanchanapoom *et al*. (2012) on *Anubias barteri* var. nana plants with the application of BA 3 mg/l resulted in the growth of the number of shoots among other treatments.

Table 4. Effect of BAP and Coconut Water on the Number of Shoots and Shoot Length (cm)

Coconut Water (ml/l)	BAP (mg/l)			
	0	1	3	5
Number of Shoots				
0	1.44 a	2.33 abc	6.22 d	1.78 ab
50	2.33 abc	4.11 cd	2.78 abc	4.33 cd
100	1.44 a	3.89 bc	2.56 abc	2.61 abc
Shoot Length (cm)				
0	2.30 c	1.06 a	1.41 ab	1.13 a
50	1.22 ab	1.70 b	1.10 a	1.43 ab
100	1.22 ab	1.22 ab	1.42 ab	1.17 a

Note : A significant difference is not seen in the 5% DMRT test for numbers that are followed by the same letter.

The media treatment of BAP 3 mg/l without coconut water was not significantly different from the media of BAP 5 mg/l + coconut water 50 ml/l and BAP 1 mg/l + coconut water 50 ml/l which had a shoot number value of 4.33 and 4.11. MS media with BAP 1 mg/l + coconut water 50 ml/l shows that it has been able to grow the number of shoots in high yields. This can occur due to the balance of concentration between BAP and coconut water in the media. The use of PGR concentration needs to be considered with the right suitability so that it can produce maximum growth and development of explants (Maryam *et al*., 2024). The selection of growth regulators to be used for media in addition to considering the effect on explant growth also considers economic use. The use of PGR with low concentrations can be profitable by spending much less cost with the same effect on explant growth.

MS media without the addition of PGR has the highest mean value for shoot length, which is 2.30 cm. This might due to the explants do not need to make adjustments to the new media which has the same composition as the previous media so that the explants grow like the original nature of the parent. The incorporation of growth regulators modifies the molecular chemical structure of the culture media, necessitating adjustments for the explants to ensure optimal growth. The Laohavisuti *et al*. (2017) research also revealed the best shoot length results of *Bucephalandra* sp. plants in MS media treatment compared to other treatments. Endogenous hormones can influence the growth of explants *Bucephalandra* sp.

BAP and coconut water on MS media showed data not significantly different on the shoot length. This might due to the influence of changes in the content of nutrients contained in the subtrate due to the addition of growth regulators that make explants give different growth responses. Explants without the addition of PGR will grow like the parent plant because there is no change in the content of nutrients contained in the media. According to Nugraha *et al*. (2020), *Bucephalandra* sp. generally has a growth size of about 2-60 cm. The addition of PGR can cause changes in the shape of the explants with the length of the explants being low compared to the growth of bucephalandra in general. This might due to the addition of PGR changes the shoot growth response of *Bucephalandra* sp. explants. Figure 1 showed the MS media treatment (control) provide greater growth than the other treatments. However, the MS media treatment (control) only had a shoot number of 1.44 shoots. This might due to the addition of growth regulators changing the shoot growth response of *Bucephalandra* sp. explants.

Visually, *Bucephalandra* sp. growth on MS media without growth regulators (control) showed the same results as their parents. The visual characteristics displayed by *Bucephalandra* sp. plants on MS media without growth regulators are wide leaves, wavy leaf edges, long petioles, and shoots that tend to be red in color. Getting many seedlings with the same desired traits as the parent plant in a short time is an advantage of tissue culture (Ziraluo, 2021).

Differences in each treatment could be caused by the addition of different concentrations of growth regulators so that the nutrient content of the media is different from one another. This affects the visual form of each explant. Media

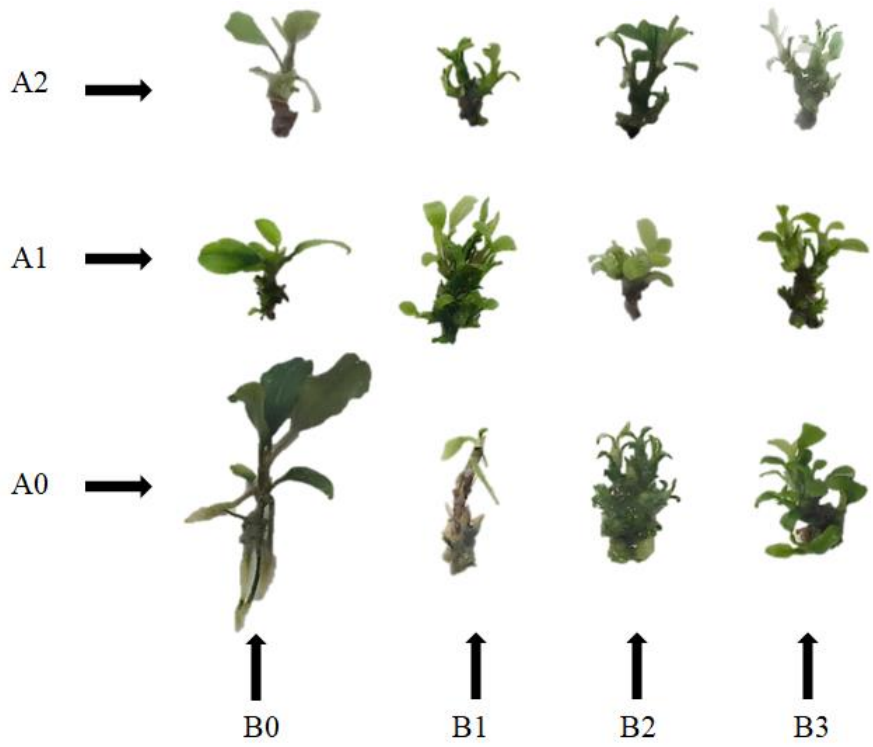

Figure 1. Visual of *Bucephalandra* sp. explants in various treatments (B0: BAP 0 mg/l; B1: BAP 1 mg/l; B2: BAP 3 mg/l; B3: BAP 5 mg/l; A0: coconut water 0 ml/l; A1: coconut water 50 ml/l; and A2: coconut water 100 ml/l)

with BAP addition showed higher shoot growth compared to media without BAP. Figure 1. shows a significant difference in the shape of the explants. The application of BAP which is a cytokinin can stimulate shoot growth in explants. In plant growth and development, cytokinins play an important role. Cytokinins can increase cell division, shoot and callus proliferation, and shoot morphogenesis (Immalasari, 2018).

Explants growth on media with the addition of BAP and coconut water were visually different from the parent plant. This was due to changes in the content of different chemical nutrients so that the metabolism of explants changes which have a visual impact. Treatments BAP 0 mg/l + coconut water 50 ml/l and 100 ml/l had slightly wider leaf growth than treatments BAP 1 mg/l, 3 mg/l, and 5 mg/l without coconut water, BAP 1 mg/l + coconut water 50 ml/l, BAP 1 mg/l + coconut water 100 ml/l, BAP 3 mg/l + coconut water 50 ml/l, BAP 3 mg/l + coconut water 100 ml/l, BAP 5 mg/l + coconut water 50 ml/l, and BAP 5 mg/l + coconut water 100 ml/l which have small leaf shape. All treatments have the same stem color which is greenish brown. All treatments on average have green shoots except for treatment MS media without growth regulators (control) which has shoots that tend to be red.

3.4. Number of Leaves

The results revealed no interaction between the effect of adding BAP and coconut water on the number of leaves. The treatment of BAP singly gave a no significant impact. Coconut water treatment alone gives no significant impact. Table 5 shows the impact of BAP and coconut water on the number of leaves. Table 5 reveals that the addition of BAP alone shows no effect on the growth of the number of leaves. This is due to observations made when the explants were 70 days old, indicating that the explants had entered the subculture stage to be transferred to new media. Budiarto (2015), stated that subculture activities are carried out every month by replacing new media. This aim to increase the nutrient contained in the media so that the explants can grow and develop optimally. Media without the addition of growth regulators (control) was able to grow the number of leaves with an average of 4.89 leaves per explant at the age of 70 days after planting. This is due to the ability of endogenous hormones to support explant growth. Eriansyah *et al*. (2018) stated that each plant has endogenous hormones that can affect the growth of the plant itself.

Table 5. Effect of BAP and Coconut Water on the Number of Leaves

Treatment	Number of Leaves
BAP (mg/l)	
0	4.89
1	4.74
3	2.85
5	2.37
Coconut Water (ml/l)	
0	2.89
50	5.06
100	3.19

Note : A significant difference is not seen in the 5% DMRT test for numbers that are followed by the same letter.

Media MS with BAP 3 and 5 mg/l were able to reduce the number of leaves produced by *Bucephalandra* sp.. Nuraini *et al*. (2022) found in their research that the application of BAP had a significant effect on the number of leaves of *Boehmeria nivea*, with an increase in BAP concentration of 2 and 3 mg/l resulting in a reduction of the number of leaves compared to the control treatment. According to the opinion of Tilaar *et al*. (2015) state that the application of BAP with a low concentration gives the result of a higher number of leaves and as the concentration of BAP increases, the number of leaves produced decreases. This is in agreement with a study by (Jannah *et al*., 2023), which shows that increasing BAP concentration can slow the growth of *Clitoria ternatea* leaves, whereas applying BAP 1 mg/l alone yields the maximum number of leaves.

Coconut water on MS media did not affect the growth of *Bucephalandra* sp. *in vitro*. This can be caused by the use of coconut water concentration on MS media as PGR given is still not in accordance with the needs of optimal growth in *Bucephalandra* sp. This is supported by Sholikhah & Azizah (2011), which states that the application of inappropriate organic hormones doesn't have a direct effect and can even inhibit the process of growth and cell differentiation. Silvina & Murniati (2007) stated that the application of young coconut water to MS media for *in vitro* propagation is a concentration ranging from 5 – 50% depending on the type of plant. Alfiana (2020) states that the application of coconut water alone is not enough to induce the number of shoots of *Pennisetum purpureum* so it requires additional growth regulators.

3.5. Number of Roots and Root Length (cm)

There was an effect of BAP and coconut water added to MS media on the number and length of roots. Every application of BAP and coconut water by itself has a noticeable impact. Table 6 shows the impact of BAP and coconut water on the number and length of roots. Table 6 displays the same letter notation on the quantity and length of roots suggested that the application of BAP and coconut water didn't significantly differ from one another in the results of the DMRT 5% additional test. This is significantly different from the MS media without the addition of PGR (control). In comparison to other treatments, the results indicated that the MS media without PGR produced the greatest amount and length of roots, measuring 3.67 roots and 1.19 cm. Media MS with BAP and coconut water has a negative effect on root growth. This is due to the effect of changes in nutrient content contained in the media due to the addition of growth regulators that make the explants give different growth responses. This is due to the effect of changes in nutrient content contained in the media due to the addition of growth regulators that make the explant growth response different. Root growth in *Bucephalandra* sp. explants is by the influence of endogenous hormones contained in the explants. Media with addition of growth regulators can inhibit the root growth of *Bucephalandra* sp. The addition of exogenous hormones can increase the endogenous hormone content in the plant tissue. According to the opinion of Khair & Hamdani (2013), stating that the use of hormones that exceed the concentration needed by plants can cause these hormones to be ineffective to affect plant growth. Laohavisuti *et al*. (2017) expressed the opinion that root growth in *Bucephalandra* sp. explants growth on MS media (control) shows that there is an endogenous auxin hormone that can spur root growth and *Bucephalandra* sp. plants on MS media have sufficient nutrients to be able to induce root formation due to the hormones contained in the explants themselves. Tissue culture MS media has a complex nutrient content that has been formulated according to the needs for plant growth (Yulis, 2023).

Table 6. Effect of BAP and coconut water on the number of roots and root length (cm)

Coconut Water (ml/l)	BAP (mg/l)			
	0	1	3	5
Number of Roots				
0	3.67 b	0.11 a	0.00 a	0.00 a
50	0.00 a	0.00 a	0.00 a	0.00 a
100	0.33 a	0.00 a	0.22 a	0.00 a
Root Length (cm)				
0	1.19 b	0.08 a	0.00 a	0.00 a
50	0.00 a	0.00 a	0.00 a	0.00 a
100	0.11 a	0.00 a	0.09 a	0.00 a

Note : A significant difference is not seen in the 5% DMRT test for numbers that are followed by the same letter.

4. CONCLUSION

The addition of BAP 1 mg/l + coconut water 50 ml/l (4.11 shoots), BAP 5 mg/l + coconut water 50 ml/l (4.33 shoots), and BAP 3 mg/l (6.22 shoots) were able to provide the number of shoots with high results. The addition of coconut water did not significantly affect the in vitro growth of *Bucephalandra* sp. The concentration of coconut water used did not have an impact on the growth of *Bucephalandra* sp. The MS media without plant growth regulators was able to grow shoots for measurement of shoot emergence time, number of roots, shoot and root length.

ACKNOWLEDGMENTS

The authors would like to express thanks to the Food Security and Agriculture Department Surabaya for facilitating the laboratory unit to carry out this research activity.

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