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Research paper

The effect of coconut water and *benzyl amino purine* (BAP) addition to the growth of pineapple from Sipahutar North Sumatera *in vitro* condition

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Abstract

Background: Pineapple fruit used in this study was obtained from Sipahutar sub-district, North Tapanuli regency, North Sumatra which was a superior commodity fruit. Propagation of the Sipahutar pineapple plant was conducted vegetatively using bud stems and crown shoots, but the amount was limited to be planted in large areas. Therefore, propagation through tissue culture techniques could be used as an alternative solution.
Objective: Explants source were taken from plantlets *in vitro* aged 3 months were grown on Murashige and Skoog (MS) basic medium with the addition of *Indole Acetic Acid* (IAA) 1 ppm.
Methods: Sipahutar pineapple tissue culture work has been done in YAHDI Plant Tissue Culture Laboratory, Medan. The study design used a factorial completely randomized design. The first factor is coconut water with concentration of 0%, 10%, 20%, and 30%, second factor is *Benzyl Amino Purine* (BAP) with concentration 0 ppm, 2 ppm and 4 ppm, so there were 12 combination with 3 repetition.
Result: The results showed that the addition of coconut water on MS base medium with IAA of 1 ppm gave a significant effect on the number of leaf yield and shoot height. The provision of BAP on basic MS medium with IAA of 1 ppm gave a significant effect on the number of shoots produced, the number of leaves produced, the length of the leaves, the width ofleaves and the length of shoots. The interaction of coconut water and BAP addition on MS base medium with IAA of 1 ppm gave significant effect on leaf length, leaf width and shoot length as well.
Conclusions: MS + IAA basic media combined with coconut water + BAP could stimulate the growth of Sipahutar pineapple *in vitro*.

Keywords:*Coconut Water; BAP; Pineapple Ananas comosus L. from Sipahutar; In Vitro; Organogenesis; Multiplication*

1. Introduction

Pineapple (*Ananas comosus* (L.) Merr.) belongs to the *Bromeliaceae* family is a tropical plant originating from Brazil, Bolivia and Paraguay. This fruit in English is called pineapple because its shape is like a pine tree. This plant is one of the fruit commodities that can grow in all seasons. Pineapple in addition as a consumed fruit, is also processed into a variety of foods and drinks, jams, cocktails in drinks and others. In addition, pineapple contains high and complete nutrition. Traditionally pineapple fruit is believed to be efficacious for health, as a cure for constipation, urinary tract disorders, nausea, and flu and blood defficiency. Not only the flesh, pineapple skin can also be processed into a drink or extracted liquid for animal feed.
In Indonesia, the potential pineapple producing centers are West Java, East Java, North Sumatera, South Sumatera and Riau. Central of pineapple producer in North Sumatera is Sipahutar Subdistrict of North Tapanuli Regency. Pineapple Sipahutar has a characteristic that is large, red and has a sweet taste. Pineapple from Sipahutar is one of the superior commodities with production reached 144,210 tons or 78.72% of pineapple production in North Sumatera Province. Sipahutar pineapple has been exported to Hong Kong by 705 boxes or 8.4 tons in August 2016.The obstacle faced by pineapple farmers in Sipahutar is the difficulty in getting the pineapple seeds. At this time, Sipahutar pineapple farmers use seeds derived from bud shoots and crown shoots, while the number is very limited (Rukmana, 1996). An alternative method is needed to overcome these obstacles are applying the tissue culture method.
Tissue culture is one of the methods of *in vitro* plant propagation that utilizes the plant totipotential nature to obtain pineapple seeds in large quantities and in a short time (Yusnita, 2004; Harahap, 2011). The success of *in vitro* culture techniques is influenced by the use of media and growth regulators. The growth regulator substances added to *in vitro* culture media are capable of controlling organogenesis and morphogenesis in plants in shoot and root formation. The growth regulator substances commonly used in tissue culture were from auxin and cytokines groups. The addition of natural growing regulators such as coconut water could also be used. The addition of coconut water indicated to contain cytokines is expected to induce plant shoots (Harahap, 2011).The purpose of this study was to determine the effect of coconut water and BAP addition to the growth of Sipahutar pineapple.

2. Material and methods

2.1. Location and materials

This research has been conducted in YAHDI Plant Tissue Culture Laboratory, Medan from March to October 2017. The explants used were buds derived from Sipahutar pineapple plantlets produced from *in vitro* multiplication of 3 months old.

2.2. Method

This study used a complete randomized design. The treatments used basic MS medium with 1 ppm IAA combined with coconut water at concentrations of 0%, 10%, 20%, and 30% and BAP at concentrations of 0 ppm, 2 ppm and 4 ppm. There were 12 treatment combinations with 3 repetitions for each research unit. Each one research unit consisted of 1 culture bottle with 1 explant per culture bottle.

2.3. Statistical analysis

Observations were conducted weekly from week 1 to week 12 with parameters observed including: shoot time, shoot number, leaf number, leaf length, leaf width, shoot height and plantlet performance. Data analysis from observation results used an Analysis of Variance. If the results of the Analysis of Variance showed significantly differently, then it was followed by Duncan Mutiple Range Test (DMRT) test with 5% of error level.

3. Results

3.1. Number of buds

Number of buds observed from week 1 to week 12. Observation data on the number of buds taken by counting the newly emerging buds. The result of analysis of variance showed that the addition of coconut water and its interaction did not give a significant effect to the number of shoots, while the provision of BAP significantly affected the number of buds.

Table 1: Influence of Addition of BAP to Number of Buds

BAP Concentration (ppm)	Average Number of Shoots
0	0.91[a]
2	1.10[a]
4	1.43[b]

Description: the value followed by the same letter in the same column is not significantly different in the DMRT test P <0.05

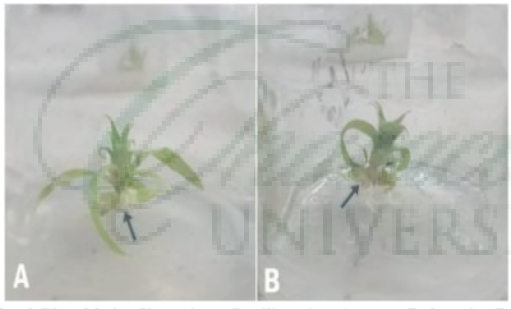

Fig. 1: Blue Marks Showed on Swelling that Appears Before the Bud Grows on Treatment (A) coconut water 10% + BAP 2 ppm and (B) coconut water 0% + BAP 4 ppm.

3.2. Number of leaves

The number of leaves observed from week 1 to week 12. Observation data on the number of leaves taken by counting the newly emerging leaves. The number of leaves at the beginning of the study was 4 leaves on each individual. The result of analysis of variance showed that the addition of coconut water and BAP had a significant effect on the number of leaves, while the interaction of coconut water + BAP had no significant effect on the number of leaves.

Table 2: Effect of Coconut Water and BAP Concentration on Number of Leaves

Treatment	Number of Leaves
Coconut water 0%	15.56[b]
Coconut water 10%	17.33[b]
Coconut water 20%	10.78[a]
Coconut water 30%	16.22[b]
BAP 0 ppm	14.00[a]
BAP 2 ppm	12.67[a]
BAP 4 ppm	18.25[b]

Description: the value followed by the same letter in the same column is not significantly different in the DMRT test P<0.05

The effect that gives the highest number of leaves was the addition of 10% coconut water and 4 ppm BAP (Table 1). In the addition of coconut water 20% and BAP 2 ppm there was a decrease of leaf number at week 12 (Fig. 2). The results showed that the dead leaves started with a change of leaf color from green to yellow and then died.

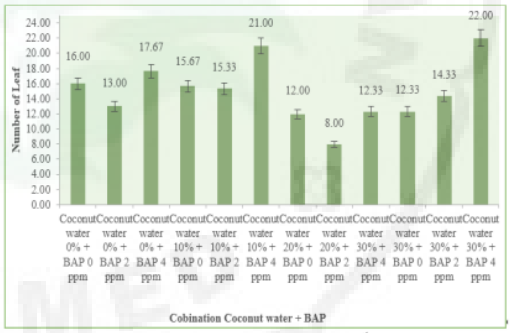

Fig. 2: Average of Leaf Number on 12th Week.

3.3. Leaf length

Leaf length observation was only done at 12th week by taking one longest leaf and placed on millimeter paper. The result of analysis of variance showed that the addition of coconut water did not give effect to leaf length. Meanwhile, the addition of BAP and coconut water + BAP interaction gives a significant influence on the length of leaves of Sipahutar pineapple plantlet.

Table 3: Effect of Addition of Coconut Water + BAP Combination Against Leaf Length

BAP (ppm)	Coconut Water (%)			
	0	10	20	30
0	3.63[ab]	5.80[bc]	7.63[c]	3.83[ab]
2	2.80[a]	2.00[a]	2.93[a]	3.00[a]
4	3.00[a]	3.73[ab]	2.50[a]	3.10[a]

Description: the value followed by the same letter in the same column is not significantly different in the DMRT test P <0.05

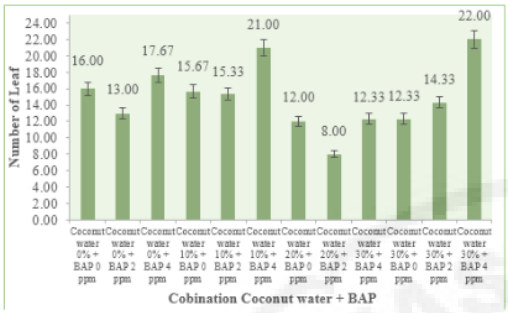

Fig.3: Average of Leaf Length on 12th Week.

3.4. Leaf width

Leaf width observation was only done at 12th week by taking the same leaf blade with leaf length measurement. The result of analysis of variance showed that the addition of coconut water, BAP and coconut water interaction + BAP gave significant effect to the width of Sipahutar pineapple.

Table 4: Effect of Coconut Water Combination + BAP on Leaf Width

BAP (ppm)	Coconut water (%)			
	0	10	20	30
0	0.53^{abc}	0.73^{cde}	0.83^{e}	0.63^{bcd}
2	0.40^{a}	0.53^{abc}	0.40^{a}	0.60^{abc}
4	0.73^{cde}	0.77^{de}	0.47^{ab}	0.60^{abc}

Description: the value followed by the same letter in the same column is not significantly different in the DMRT test P <0.05

Treatment of addition of coconut water 20% + BAP 0 ppm showed the highest leaf width of 0.83 which was significantly different from other treatment. The results of the measurement of the leaf length and width, the addition of coconutwater 20% + BAP 0 ppm stimulated the leaf length and leaf width higher, followed by treatment of coconut water 10% + BAP 0 ppm compared with other treatments (Fig. 4).

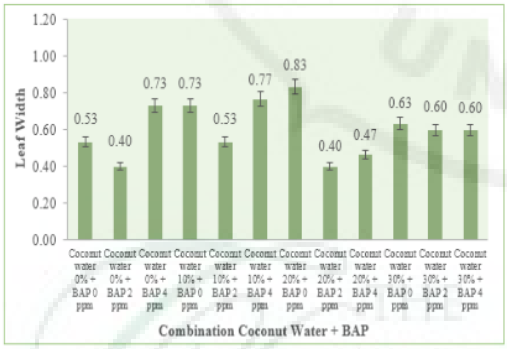

Fig.4: Average of Leaf Width on 12th Week.

3.5. Bud length

Observation of bud length was done as the measurement of leaf length was only done at week 12. The result of analysis of variance showed that the addition of coconut water, BAP and coconut water interaction + BAP had a significant effect to bud length.

Table 5: Effect of Addition of Coconut Water + BAP Combination to Bud Length

BAP (ppm)	Coconut water (%)			
	0	10	20	30
0	4.03^{ab}	8.37^{d}	9.87^{e}	5.43^{bc}
2	3.60^{a}	3.10^{a}	3.03^{a}	3.10^{a}
4	3.27^{a}	3.57^{a}	3.07^{a}	5.73^{c}

Description: the value followed by the same letter in the same column is not significantly different in the DMRT test P <0.05

Treatment of addition of coconut water 20% + BAP 0 ppm showed the highest yield of 9.78 cm which was significantly different with other treatment in bud length. The bud length corresponded to the leaf length, this was due to the measurement of bud length was measured on the longest leaf. The interaction between coconut water addition treatment of 20% + BAP 0 ppm showed the highest average compared to other treatments in shoot length (Fig.5).

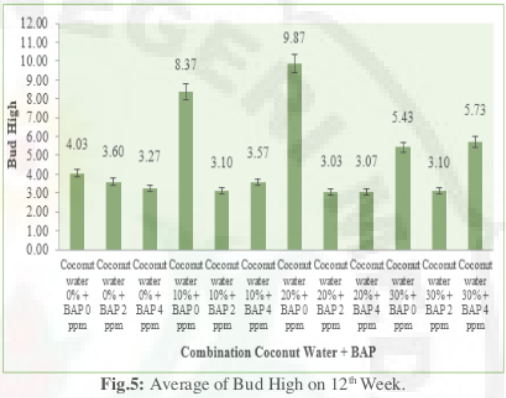

Fig.5: Average of Bud High on 12th Week.

3.6. Plantlet performance

Plantlet performance was observed for 12 weeks. Shoot performance in all treatment of coconut water + BAP addition showed fresh, sturdy shoots and wide leaves formation (Fig. 7). In the addition of coconut water 0%, 10%, 20% and 30% + BAP 0 ppm was able to stimulate root growth, while in another treatment showed no root growth. In the addition of coconut water 20% + BAP 2 ppm, Sipahutar pineapple plantlet was stagnant (Fig. 6). Leaves formed from each treatment tend to be green which light green to dark green. In the treatment of coconut water 0%, 10% and 20% + BAP 2 ppm of short shoots, the leaves formed were light green, the size of long leaves but the small width (Fig. 7 G and K). In addition, at this treatment swelling occurs faster but until the 12th week the leaves did not appear. In addition, 10%, 20% and 30% coconut water + BAP 0 ppm showed longer and wider length and width (Fig. 7 B, C, and D). In the addition of coconut water 0 ppm + BAP 2 and 4 ppm the length and width of the leaves were also long and wide, but not the same as the addition of coconut water (Fig. 7 E and I). Treatment of addition of coconut water 0% + BAP 0 ppm visible growth of length and width of leaves as generally not significant (Fig. 7 A).

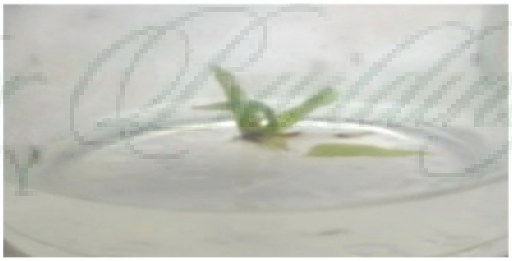

Fig. 6: Explant Pineapple Sipahutar that stagnated on the Addition of coconut water 20% + BAP 2 ppm.

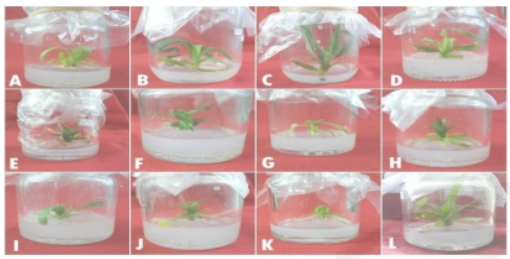

Fig. 7: The Condition of Pineapple Plantlet at 12th Weeks (A) Treatment of Coconut Water Addition 0% + BAP 0 ppm; (B) Coconut Water 10% + BAP 0 ppm; (C) Coconut Water 20% + BAP 0 ppm; (D) Coconut Water 30% + BAP 0 ppm; (E) Coconut Water 0% + BAP 2 ppm; (F) Coconut Water 10% + BAP 2 ppm; (G) Coconut Water 20% + BAP 2 ppm; (H) Coconut Water 30% + BAP 2 ppm; (I) Coconut Water 0% + BAP 0 ppm; (J) Coconut Water 10% + BAP 4 ppm; (K) Coconut Water 20% + BAP 4 ppm; (L) Coconut Water 30% + BAP 4 ppm

4. Discussion

4.1. Effect of addition of coconut water and BAP to bud growth

Growth of buds occurs due to the effects of endogenous hormones found in the Sipahutar pineapple plantlet as well as growth regulators contained in the media (Harahapet al., 2015; Nursetiadiet al., 2016). Visually, explants that were experiencing swelling look shaped like a green-colored buds. The buds, slowly began forming the leaves one by one and eventually would form new shoots (Fig. 1). The addition of high concentrations of cytokinens should lead to swelling of the plantlets before buds appear, but their bud growth may also be inhibited (Harahap *et al*., 2015). The addition of low doses of BAP made the swollen escalation could not develop into new shoots. This is due to an indication of increased cleavage and cell differentiation resulting in the appearance of buds but inhibited in the differentiation process so that buds can not appear (Harahap, 2011). The addition of high concentration BAP can stimulate bud growth (Harahap *et al*., 2014). In addition, the addition of auxin also gives effect to the growth and development of buds. This can be seen in the control of buds can still be due to the presence of endogenous cytokines in the explants. Harahap&Nusyirwan (2014) stated that the increasing dose of BAP without the addition of auxin will not provide an increase in the number of buds. The role of equilibrium auxin and cytokines will affect the number of shoots produced. This is in line with the study of Samudin (2009) stated the addition of growth regulator substances auxin-cytokines with optimal concentration will cause the occurrence of cell division that stimulates shoot formation. However, the addition of too high cytokines does not trigger the formation of shoots, such as research conducted by Santoso& Sobir (2013).

4.2. The effect of addition of coconut water and BAP to leaf growth

The addition of coconut water indicated to contain high cytokines that can stimulate leaf growth in optimal concentration, if too high or too low leaf growth is not formed (George & Sherrington, 1984; Gunawan, 1992; Harahap, 2011). Similar to coconut water, BAP can also affect the increasing number of leaves. The addition of BAP to the media will push the cells in explant to divide and develop into shoots and form leaves. The number of leaves is positively correlated with the number of shoots produced. The addition of high concentrations of cytokines with the ability to stimulate shoot growth is accompanied by an increase in the number of leaves (Harahap *et al*., 2014). In control, plantlet pineapple was able to grow leaves, auxin added to the media also encouraged the growth of leaves by assisting the development of young leaf prospective meristem (Sinulingga&Harahap, 2014). At week 7 there is a decrease in the number of leaves, the leaves are yellow at week 8 to week 12 and begin the growth of new leaves. Leaf color changes may be caused by aging leaves and the chlorophyll content of the leaves may decrease (Yusnita, 2004; Harahap, 2011). The interaction of coconut water additions and BAP looks good in increased leaf growth. Not all interactions can trigger the increase of leaf length growth, it can be seen that only coconut water interaction is 20% + BAP 0 ppm and coconut water 10% + BAP 0 ppm which has leaf length is higher than other treatments. Santoso&Sobir (2013) stated that the addition of cytokines affects the growth of leaf length but if given with high concentration will inhibit the length of the leaf. This was similar to the results of Nursetiadi *et al*. (2016) suggested that with the addition of high concentrations of cytokines can inhibit the growth of leaf length. Growth of the length of the leaf affects the width of the leaves produced. The longer the leaves the wider the leaves, and vice versa. The process of growth in the number and size of the leaves is influenced by growth regulators such as auxin and cytokines. The addition of auxin is able to stimulate the growth of leaves, especially the length of vascular tissue. The addition of cytokines is able to spur cell division in leaf primordial thus supporting the growth of leaf size (Santoso&Sobir, 2014). In addition to the addition of auxin and cytokines, organ formation is also determined by the balance of plant growth regulators present in plant tissues (Gunawan, 1992).

4.3. Plantlet performance

In auxinary media containing auxin added coconut water can increase auxin in tissues and meet the growth and morphogenesis needs of pineapple plantlets. Pisesha (2008) stated that the addition of coconut water can respond to root growth on media combined with auxin. However, if the cytokines concentration is higher than root formation is inhibited. This was in accordance with the results of research Pamungkas (2015) stated that with the addition of BAP concentration is too high to inhibit the formation of roots. This was indicated that the addition of cytokines would close the activity of the auxin.

Explant growth on basic medium MS + IAA 1 ppm combined coconut water + BAP showed good shoot growth, but at treatment of coconut water 20% + BAP 2 ppm plantlet was stagnated. Plantlets that were stagnant only in one single repeat. Stagnant was explant state not experiencing death but not grow from start planting until certain period (Yuliarti, 2010). The cause of stagnant can be caused by media used or caused by explant. Based on the observation of the explants caused stagnant originating from explant. Sipahutar pineapple explants did not show the growth of buds, leaves and roots. This was because the cells in the explants were not able to express totipotent so as not to experience growth and development (Smith, 2013). The same thing also happened in research conducted by Syabana *et al*., (2017) that growth of callus of Stevia plant (*Stevia rebaudiana Bertoni* M.) stagnant which allegedly came from media and explant. Yuliarti (2010) stated that to avoid stagnant conditions is by planting explant having meristematic tissue because the beginning of growth of explant starts from young cells that actively divide. The effect of auxin also contributes to the growth of Sipahutar pineapple explants. The addition of auxin with low doses can produce the highest number of leaves, height of plant and root formation. The larger the width and length the greater the effect on the growth of the shoot height (Sulistiyorini *et al*., 2012). However, if followed by administration of cytokines, root stimulation does not appear. This is because the effect of cytokines responds more to the plantlet to stimulate shoot formation (Harahap *et al*., 2015).

5. Conclusion

MS + IAA basic media combined with coconut water + BAP could stimulate the growth of Sipahutar pineapple in vitro. With the addition of 4 ppm BAP added was able to produce the highest

number of shoots. Addition of coconut water 10% and BAP 4 ppm could produce the highest number of leaves. Meanwhile, the treatment of coconut water 20% + BAP 0 ppm was able to produce the length and width of leaves and height of shoots was higher than the other treatments.

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