In Vitro Propagation of Pineapple (*Ananas comosus*L.) Shoots from Sipahutar North Sumatera Indonesia

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In Vitro Propagation of Pineapple (*Ananas comosus*L.) Shoots from Sipahutar North Sumatera Indonesia

Yuliana Fernando[1], Fauziyah Harahap[2*], Diky S. Diningrat[2], Rosmayati[3]

[1]Biology Education of Postgraduate School UniversitasNegeri Medan, Jalan Willem Iskandar Pasar V Medan Estate, North Sumatera 20221, Indonesia.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences UniversitasNegeri Medan, Jalan Willem Iskandar Pasar V Medan Estate, North Sumatera 20221, Indonesia.
[3]Departement of Biology, Faculty of Mathematics and Natural Science, Universitas Sumatera utara, Jalan Bioteknologi No.1, North Sumatera 20155, Indonesia

[*]Corresponding Author: fauziyahharahap@gmail.com

Abstract. Pineapple is a plant that needs to be developed on a plantation scale because the fruit is of economic value. The purpose of this study was to determine the best media combination on the growth of in vitro pineapple. This study used CRD with 12 treatments with 3 replications. Observation parameters consisted of the number of shoots, leaves and roots. Data analysis techniques used ANOVA followed by DMRT test. The results showed that administration of BA and vitamins could influence the propagation of in vitro pineapple shoots. The highest number of shoots was 8.00 shoots and 19.00 leaves produced MS + Vit 2 ppm + BA 2 ppm media. The highest number of roots 3.67 produced MS + Vit 0 ppm + BA 0 ppm media.

1. Introduction

Pineapple (*Ananas comosus* L.) is originally known as a yard plant, now a plantation crop. It needs to be developed on a plantation scale because the fruit is of economic value, market demand, the third export commodity in the world after the Philippines and Thailand [1]. Pineapple is one of the horticultural commodities that has the potential to be developed. The value of Indonesian pineapple exports reaches US $ 139 million per year [2].

At present, pineapple from Indonesia is the third export commodity in the world after the Philippines and Thailand. Sipahutar Pineapple has been planted by farmers in the Sipahutar area, North Tapanuli, North Sumatra, Indonesia. This pineapple has advantages because it tastes sweeter, the water content is low, the texture is denser, the color is yellow and is likeable by the public. This fruit is one of the leading horticultural crop commodities in North Tapanuli, but its production is very limited [3].

Sipahutar pineapple is a type of pineapple that is popular in the world's pineapple market freshly. This variety is included in the best hybrid pineapple that has a high commercial value in the market because of its unique color, aroma and flaccid compared to other pineapple varieties [4], For the development of these crops, needed planlets in large quantities and uniforms. One alternative to solve this problem is going through the tissue culture techniques. This technology has been widely used to

obtain the uniform seedlings especially on horticulture crops [5] . This is to prevent the occurrence of personal variation, which is not desired in the mass propagation [6].

[7] plant tissue culture is a technique for growing cells, tissues or slices of plant organs in the laboratory on an artificial media that contains aseptic (sterile) nutrients to become a whole plant. In vitro culture is widely used to continue or improve traditional/conventional breeding methods and to make modifications to plants and plant improvement [8].

Plant growth regulators which are added in the media will partly enter the plant cells either by diffusion or through active absorption [9]. [10] state that the effectiveness of auxin and exogenous cytokinin growth regulators depends on the concentration of endogenous hormones present in plant tissues.

Hormones work optimally at certain concentrations and cells generally contain enough or almost enough hormones to lengthen normally. [11], if the cytokinin conditions are suboptimum, an exogenous growth regulator is needed with the appropriate concentration to obtain the right balance between exogenous and endogenous growth regulators. The use of liquid media using one shoot produces 200 shoots in 12-15 weeks. Each shoot can be sub-cultured again to produce even more shoots. It can be speculated that one fruit crown may produce about 10,000 plants in 9 months [12].

[13,14], explant size influences the success of tissue culture. Larger explants have a greater ability to live and grow faster. Explants that are smaller in size are more easily sterilized and do not require large space for growth. [15] state that the appropriate BAP concentration would work optimally in certain plants in terms of shoot induction. Root growth in explants can be controlled by administering auxin growth regulators.

2. Materials And Methods

This research has been carried out in the Yahdi's Tissue Culture Laboratory, Perum Pelabuhan Jalan Lambung No. 18 Tanah 600 Medan Marelanfrom May to September 2019.

The devices used in this study are erlenmeyer, Laminar Air Flow Cabinet (LAFC), volume pipettes, measuring cups, glass funnels, autoclaves, pH meters, stirring rods, spatulas, tweezers, scissors, bunsen, culture bottles, analytical scales, petri dishes, millimeter paper, and culture rack. Propagation research with factorial treatment is the provision of MS basic media with the addition of 3 vitamin concentrations, namely Vitamin (0, 1, 2) ppm and 4 concentrations of BA (0, 1, 2, 3) ppm. The combination of media can be seen below:

Table 1. Combinations of pineapple shoots propagation media (*Ananas comosus*L.)

BA (ppm)	VIT (ms)		
	Vit_0 x media ms	Vit_1 x media ms	Vit_2 x media ms
Benzyl adenine 0	BA_0Vit_0 x	BA_0Vit_1 x	BA_0Vit_2 x
Benzyl adenine 1	BA_1Vit_0 x	BA_1Vit_1 x	BA_1Vit_2 x
Benzyl adenine 2	BA_2Vit_0 x	BA_2Vit_1 x	BA_2Vit_2 x
Benzyl adenine 3	BA_3Vit_0 x	BA_3Vit_1 x	BA_3Vit_2 x

Research using CRD (Completely Randomized Design). There were 12 media combinations in each treatment with 3 replications for each experiment. Each experiment consisted of one explant per culture bottle. The explants used were in vitro pineapple from Sipahutar. Conducting research includes equipment sterilization, making media, planting, and observing pineapple growth. Observation parameters consisted of observing plant growth starting at one week after planting (1 WAP) with the

observation parameters of the number of roots, number of leaves, number of shoots, observed 1 WAP to 12 weeks.

Data obtained from observations were analyzed with ANOVA. If the results showed a real difference, then the analysis continued using the DMRT test (Duncan's Multiple Range Test).

3. Results And Discussion

3.1. Number of shoots

Statistical test results showed that MS + Vitamin + Benzyl adenine media, on observation 12 weeks after planting (WAP) showed a significant effect on the increase in the number of pineapple buds (F_{count} 3.96) sig (P) 0.176), presented in Table 2.

Table 2. ANOVA test results on the number of shoots at 12 weeks after planting (WAP)

The Effect of Variant	Df	F_{count}	F_{table}	Sig.
Treatment	33	3.96	3.28	0.176

Based on Table 1 obtained $F_{count} = 3.96 \geq F_{table} = 3.28$ it showed a significant effect on the increase in the number of pineapple shoots using MS + Vitamin + Benzyl adenine. The average number of shoots at 12 weeks after planting (WAP) is presented in Table 3.

Table 3. Average number of shoots at 12 weeks after planting (WAP)

No	Treatment	Average
1	Media Ms+Vit0+Ba0	2.66
2	Media Ms+Vit0+Ba1	2.00
3	Media Ms+Vit0+Ba2	2.33
4	Media Ms+Vit0+Ba3	3.00
5	Media Ms+Vit1+Ba0	1.00
6	Media Ms+Vit1+Ba1	0.33
7	Media Ms+Vit1+Ba2	2.00
8	Media Ms+Vit1+Ba3	0.66
9	Media Ms+Vit2+Ba0	0.33
10	Media Ms+Vit2+Ba1	2.33
11	Media Ms+Vit2+Ba2	8.00
12	Media Ms+Vit2+Ba3	3.33

The results of data analysis showed that the treatment of MS + Vit2 + Ba2 media produced the highest number of shoots of 8.00 shoots at 12 WAP observations. The lowest number of shoots was obtained from the treatment of MS + Vit1 + Ba1 and MS + Vit2 + Ba0 with an average number of shoots of 0.33 buds. The average shoot growth responds to an increase in the number of shoots (Figure 1).

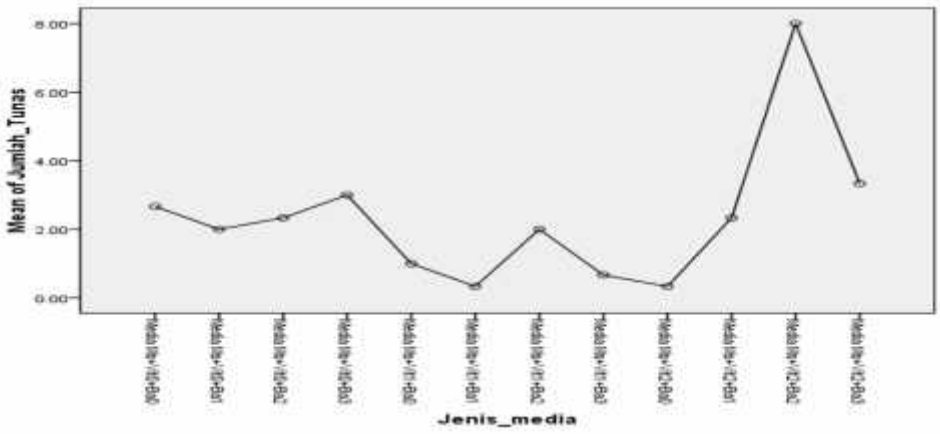

Figure 1. Average number of shoots in 12 WAP treated with Ms + Vitamin + Benzyl adenine

Addition of auxins to media containing cytokinins will increase the number of shoots, but if added cytokinins without combined with auxins do not spur the number of shoots [20]. the dose increases, but if no auxinsare added (IAA), it will not respond to an increase in the number of shoots. This is in line with research that has been done [21] on the propagation of pineapple clones (*Ananas comosus*(L.) Merr.). [22] stated that, shoot growth is determined by exogenous plant growth regulators given into the media and its balance with endogenous plant growth regulators found in explants. If auxins and cytokinins do not occur the right balance then the treatment is not able to grow shoots. [23] also stated that the improper addition of plant growth regulators tends to inhibit bud regeneration.

3.2. Number of Leaves

Statistical test results showed that the media Ms + Vitamin + Benzyl adenine, on observations 12 weeks after planting (WAP) did not show any significant effect on the increase in the number of pineapple leaves (F_{count} 0.625; sig (P) 0.790, presented in Table 4.

Table 4. ANOVA test results on the number of leaves at 12 weeks after planting (WAP)

The Effect Variant	Df	F_{count}	F_{table}	Sig.
Treatment	33	0.625	3.28	0.790

Based on Table 3, F_{count} = 0.625 <F_{table} = 3.28 showed that there was no significant effect on the increasing number of pineapple leaves by using MS + Vitamin + Benzyl adenine. The average number of leaves observed at 12 weeks after planting (WAP) is presented in Table 5.

Table 5. Average leaves counts at 12 weeks after planting (WAP)

No	Treatment	Average
1	Media Ms+Vit0+Ba0	16.33
2	Media Ms+Vit0+Ba1	14.00
3	Media Ms+Vit0+Ba2	16.00
4	Media Ms+Vit0+Ba3	15.33
5	Media Ms+Vit1+Ba0	10.66
6	Media Ms+Vit1+Ba1	9.33
7	Media Ms+Vit1+Ba2	7.33
8	Media Ms+Vit1+Ba3	12.33
9	Media Ms+Vit2+Ba0	15.33
10	Media Ms+Vit2+Ba1	13.66

11	Media Ms+Vit2+Ba2	19.00
12	Media Ms+Vit2+Ba3	11.33

The results of data analysis showed that the treatment of MS + Vit2 + Ba2 media resulted in the highest number of leaves of 19.00 leaves on 12 WAP observations. The lowest number of leaves was obtained from the treatment of MS + Vit1 + Ba2 media with an average number of leaves of 7.33 shoots. The average shoot growth responds to an increase in the number of leaves (Figure 2).

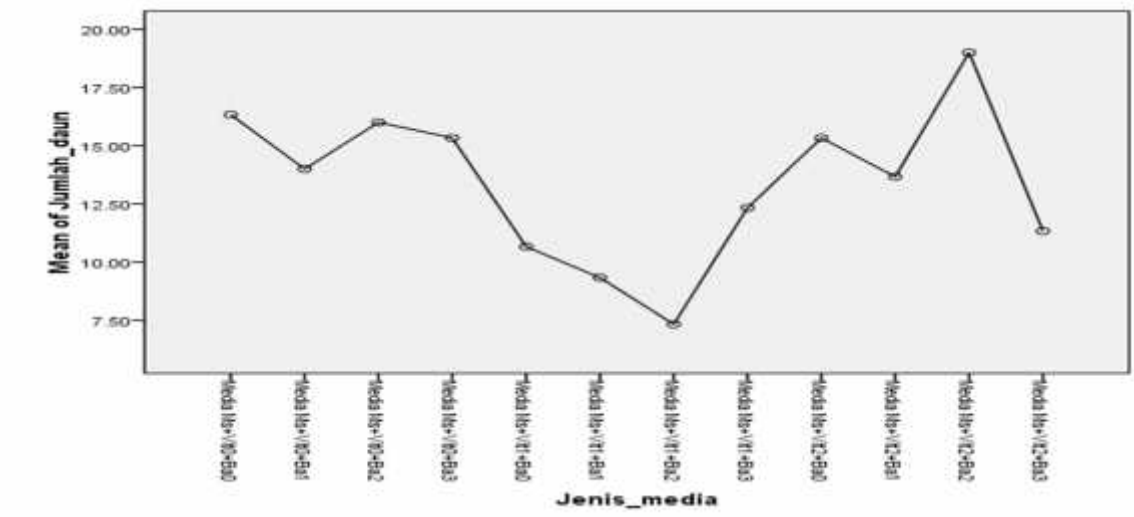

Figure 2. Average number of leaves in 12 WAP treated with MS + Vitamin + Benzyl adenine

Certain concentrations are needed to spur growth in numbers [24].

3.3. Number of Roots

Statistical test results showed that the medium of Ms + Vitamin + Benzyl adenine, at the observation of 12 weeks after planting (WAP) did not show any significant effect on the increase in the number of pineapple roots (F_{count} 0.625; sig (P) 0.790), presented in Table 6.

Table 6. ANOVA test results on number of roots at 12 weeks after planting (WAP)

The Effect of Variant	Df	F_{count}	F_{table}	Sig.
Treatment	33	0.625	3.28	0.790

Based on Table 5, F_{count} = 0.625 <F_{table} = 3.28 shows that there was no significant effect in increasing the number of pineapple roots using MS + Vitamin + Benzyl adenine. The average number of shoots at 12 weeks after planting (WAP) is presented in Table 7.

Table 7. Average number of roots at 12 weeks after planting (WAP)

No	Treatment	Average
1	Media Ms+Vit0+Ba0	3.66
2	Media Ms+Vit0+Ba1	2.00
3	Media Ms+Vit0+Ba2	2.66
4	Media Ms+Vit0+Ba3	1.00
5	Media Ms+Vit1+Ba0	3.33
6	Media Ms+Vit1+Ba1	3.33

7	Media Ms+Vit1+Ba2	1.33
8	Media Ms+Vit1+Ba3	1.33
9	Media Ms+Vit2+Ba0	1.33
10	Media Ms+Vit2+Ba1	1.66
11	Media Ms+Vit2+Ba2	2.33
12	Media Ms+Vit2+Ba3	0.33

The results of data analysis showed that the treatment of MS + Vit0 + Ba0 media resulted in the highest number of roots of 3.66 shoots on 12 WAP observations. The lowest number of roots was obtained from the treatment of MS + Vit2 + Ba3 media with an average number of roots of 0.33 shoots. The average root growth responds to an increase in the number of roots (Figure 3).

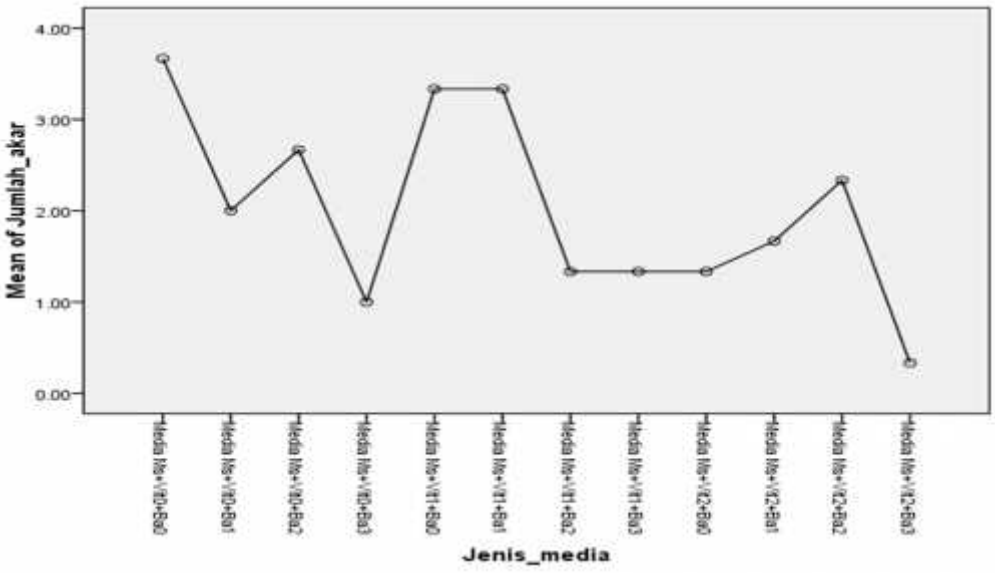

Figure 3. Average number of roots in 12 WAP treated with MS + Vitamin + Benzyl adenine

This can be understood because the roots will be formed if the media contains auxins higher than cytokinins [25].

4. Conclusion

1. In shoot propagation, treatment of MS + Vit2 + Ba2 media produced the highest number of shoots of 8.00 shoots on 12 MST observations. The lowest number of shoots was obtained from the treatment of MS + Vit1 + Ba1 and MS + Vit2 + Ba0 with an average number of shoots of 0.33 buds.

2. The number of leaves shows that the treatment of MS + Vit2 + Ba2 media produced the highest number of leaves of 19.00 leaves in the observation of 12 MST. The lowest leaf yield was obtained from the treatment of MS + Vit1 + Ba2 media with an average number of leaves of 7.33 shoots.

3. The number of roots showed that the treatment of MS + Vit0 + Ba0 media produced the highest number of roots of 3.66 shoots at 12 MST observations. The lowest root number was obtained from the treatment of MS + Vit2 + Ba3 media with an average number of roots of 0.33 shoots.

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