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EFFECT OF PLANT GROWTH REGULATOR ON TISSUE CULTURE MEDIA FOR TRIGGER OF KEPOK BANANA

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ABSTRACT

Kepok banana (*Musa acuminata* x *Musa balbisiana*) can be grown in a wide area of Indonesia and is still cultivated regularly in rural areas. The need for commercial-quality of Kepok banana seedlings is still an obstacle to supporting export success. Seed propagation using micropropagation techniques is an alternative to be developed in order to meet the needs of appropriate, high-quality, and potential seeds for high productivity. The use of growth regulators and suitable growth media will promote the success of in vitro culture. The method used is the media Murashige-Skoog (MS). The design used was completely randomized and arranged by factorial. Factor 1 is a type of growth regulator with a concentration of 2 mg/L. Factor I is Z1 = Gibberellins; Z2 = IAA; and Z3 = Auxin. Factor II is 50 mg of the growth medium, namely: M1 = Agar medium; M2= Potato medium; M3 = Medium with coconut; M4 = Media Aloe vera. Data analysis used F 5% test and 5% BNT test. The use of a gibberellin growth regulator showed a better shoot formation time of 8 days, the number of shoots was 13.24 seeds, the length of the shoot was 1.25 cm, the root length was 1.52 cm, and the number of shoots was 77.48%. The growing medium using potatoes and young coconut showed a root length of 0.43 cm and a root diameter of 0.34 mm. Young coconut media showed better results in the growth of the number of plantlet shoots as much as 12.53%. The function of growth regulators, gibberellins, IAA, and auxin gave a rapid response to plantlet meristematic changes and shoot formation. While the use of agar, potato, young coconut, and aloe vera growing media gave a slow response to plantlet meristematic changes and the formation of Kepok banana shoots.

Keywords: musa acuminata x musa balbisiana, growth regulators, tissue culture.

INTRODUCTION

Kepok banana is the one of local varieties from Indonesia, popular and in great demand in Malaysia and Japan. Ministry of Agriculture 9.8 tons of kepok bananas in the Puspa Agro area of Sidoarjo, East Java (Ministry of Agriculture, 2019). The demand for bananas is increasing along with the development of food diversification. The increase in many consumers must of course be balanced with the provision of quality banana plants and productivity. Bananas have a high enough potential to be developed as an export commodity. The high volume of banana exports from Indonesia needs to be balanced with a good cultivation technique so that the fulfillment of banana needs can be met.

Commercial banana cultivation must be supported by the provision of uniform, fast, precise, and high-productivity seeds. Companies engaged in horticulture, especially banana plants, need quality seeds. This situation is due to the limited number of seed producers who can provide quality, uniform, and large quantities of banana seeds. Banana plants in seed propagation are still relatively slow, the generation time is long (10-18 months) and requires large land (UNCTS, 2007).

Conventional propagation using macropropagation by planting suckers (saplings) directly in the field has not been able to minimize commercial needs. An alternative that can be done is by means of micropropagation of tissue culture techniques.

Micropropagation techniques are generally carried out by in vitro culture. In vitro culture is a technique to produce banana seeds in large quantities, uniformly, and in a short time. Micropropagation is done by cutting the tissue into small sizes and planting it on artificial media aseptically. Through the micropropagation technique, clones of plants with uniform genetic characteristics will be obtained.

The development of kepok banana seedlings with tissue culture techniques is expected to be able to overcome the need for seeds quickly and the purity of clones or varieties. Propagation by tissue culture technique has several advantages and disadvantages, including 1). The number of seeds produced is large and at the same time; 2). Can produce disease-resistant seeds; 3). Seeds obtained according to the parent; 4). It takes skill and is a bit complicated; 5). Vulnerable to failure in its implementation; 6). Need capital; and 7). The Place must be aseptic.

Growth regulators have an important role in stimulating shoot and root growth. The use of growth regulators in tissue culture depends on the goals to be obtained (Lestari, 2011). Some of the growth regulators that are often used are cytokinins, namely Benzyl Adenine (BA), and kinetin. Growth regulators are needed to stimulate shoot and root growth. Benzyl Adenine1 mg/L in MS medium is best for shoot multiplication of kepok banana seedlings (Supriati et al., 2006). Benzyl Adenine 5 mg/L is as good as kinetin 7 mg/L in producing banana plantlets (Avivi, 2004).

Micropropagation of red kepok banana was successfully carried out usingMurashige-Skoog (MS) treated with auxin growth regulators combined with

cytokinins. The shoot initiation stage can be carried out on MS media with the addition of 0.2 mg/L of Idole Acetic Acid (IAA) and *Benzyl Adenine*5 mg/L.

RESEARCH METHODS

The research was conducted in the laboratory and in the field for analysis of the description of local kepok banana seedlings. Plant material is the young shoots that grow on the weft of the Kepok banana. After cleaning, the weevil that has buds is taken by slashing 5 x 5 cm, 1 cm thick. Furthermore, the explant material that had buds was sliced 1x1 cm, then soaked in the fungicide Dithane-M45 2 g/L for 1 hour, and the bactericide Agrept 2 g/L for 1 hour. The media must be sterilized with 70% ethanol for 1 minute and Bayclin (active ingredient NaOCl) 30% for 30 minutes. The explants were then rinsed with distilled water and then the skin was cleaned until the white apical bud tissue appeared. The medium to be used was a modified MS (Murashige & Skoog, 1962) enriched with 2 mg/L glycine, IAA auxin (indole acetic acid) 0.2 mg/L, and 20 g/L sucrose as the base medium. Planting material or explants are expected to grow shoots in the tested medium. The shoot multiplication medium used was MS media which was treated with Gibberellin (Z1 = 2 mg/L), IAA (Z2 = 2 mg/L), and Auxin (Z2 = 2 mg/L) as growth regulators. The tested media was added with agar medium (M1 = 50 mg), Potato Media ((M2 = 50 mg), Coconut Water Media (M3 = 50 mg), and Aloe Vera Media (M4 = 50 mg). used was a completely randomized design arranged in a factorial manner. Each treatment was repeated 3 times. Each petri dish was filled with 15 explants and the combination per treatment consisted of 3 buds initiation media. Observations were made until the green color was visible on the growing shoots. The cultures were maintained in an incubation chamber at 25^{o}C under a TL lamp with a light intensity of about 20 mol photons (equivalent to about 1000 lux). The data were analyzed using the F 5% test, 5% BNT.

RESULTS

The results of the analysis showed that the administration of growth regulators and growth media did not show a significant interaction with the variables of shoot growth time, number of plantlet shoots, shoot length, percentage of plantlets sprouted, root length, and root diameter of banana kepok plantlets. However, separately, the treatment with various types of growth regulators and growth media showed a significant effect on growth variables, as presented in Table-1.

The results of the analysis that micropagation using a growth regulator Gibberellin 2 mg/L showed a faster growth time of 8 days when compared to Indole Acetic Acid which took 18 days and Auxin 14 days (Graph 1.). Saad and Elshahed (2012), reported that MS media contained nitrate, ammonium, calcium, and other macro and micro elements that could affect the growth of explants. A Variable number of shoots that managed to grow using Gibberellin showed better yields (13.24 seeds) and was significantly different from IAA (11.21 seeds) and auxin (10.35 seeds) (Graph 2).

Table-1. Growing time and number of banana kepok planlet shoots.

Treatment	Shoot length (cm)	Plantlet shoot root length (cm)	Root diameter (mm)	Plantlets sprout (%)
Gibberellin 2 mg/L	1.25 c	1.52 c	0.74	77.48 b
IAA 2 mg/L	1.18 b	1.38 b	0.76	75.27 a
Auxin 2 mg/L	1.06 a	1.27 a	0.63	74.76 a
BNT 0.05	0.05	0.08	NS	1.47
Agar agar 50 mg	0.51	0.24 a	0.21 a	10.24 a
Potato 50 mg	0.42	0.43 b	0.34 b	11.20 a
Young coconut 50 mg	0.45	0.42 b	0.32 b	12.53 b
Aloe vera 50 mg	0.13	0.31 a	0.26 a	11.35 a

*NS = not significant

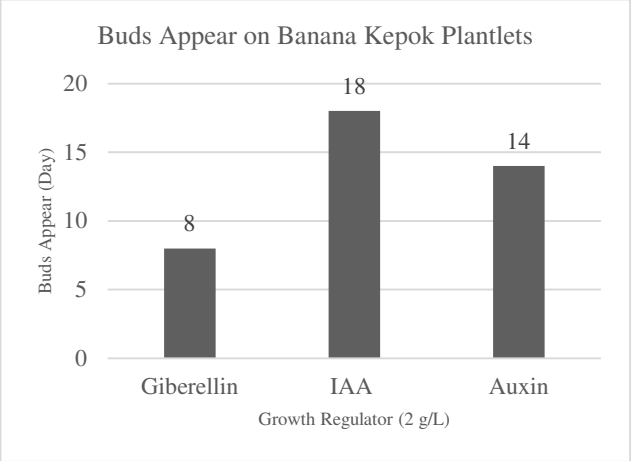


Figure-1. Application of growth regulators when shoots appear on banana kepok plantlets.

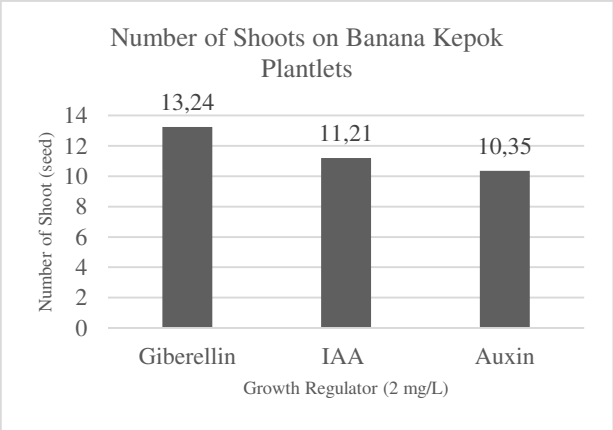


Figure-2. Application of growth regulators on the number of shoots of banana kepok plantlets.

Table-2. Changes in meristem cells and the formation of kepok banana shoots on the application of growth regulators and growth media.

Treatment	Plantlet meristematic changes	Forming buds
Gibberellin 2 mg/L	Fast (+)	Fast (+)
IAA 2 mg/L	Fast (+)	Fast (+)
Auxin 2 mg/L	Fast (+)	Fast (+)
Agar agar 50 mg	Slow (-)	Slow (-)
Potato 50 mg	Slow (-)	Slow (-)
Young coconut 50 mg	Slow (-)	Slow (-)
Aloe vera 50 mg	Slow (-)	Slow (-)

As a result of microorganisms, there are rotten plantlets and eventually, contamination occurs. Death is also caused by the material content of the growing media due to lack of sterility it triggers contamination. Death is also caused by a lack of additional nutrient intake in the growing media. Browning usually appears at the incision and then spreads to the newly growing shoots until it becomes infected and eventually dies and turns brown. (Table-3).

Table-3. The development of kepok banana plantlets during culturization.

Plantlet age (days)	Living plantlets (%)	Dead plantlets (%)	Contamination plantlets (%)
7	50	100	0
14	50	100	0
21	43	21.5	2.73
28	38	19	2.61
35	34	17	2.55
42	30	15	2.47
49	23	11.5	1.16
56	17	8.5	1.12

Table-4. Effect of temperature on emergence of shoots and browning of culture on kepok bananas.

Temperature (°C)	Shoots Appear (%)			Planlet Browning (tanaman)
	GA3	IAA	Auxin	
20	49,34 abc	38,57 a	40,21 a	3,67 e
21	52,76 bcd	42,64 b	40,85 ab	4,03 g
22	56,38 cd	41,85 bc	47,73 b	3,89 f
23	77,88 e	73,45 e	72,48 d	1,26 d
24	77,65 e	74,60 e	73,12 d	1,15 c
25	78,35 e	76,42 e	74,37 d	1,03 a
26	75,69 e	73,81 e	73,74 d	1,18 c
27	57,83 cd	53,39 d	52,98 c	1,07 b
28	57,34 cd	40,52 ab	45,01 b	4,45 i
29	45,47 ab	44,27 bc	42,87 ab	3,38 e
30	42,71 a	34,58 a	37,54 a	4,29 h
BNT 0.05	9,82	7,14	7,10	0,03

Tissue culture propagation influences the temperature of the growing medium very large on the success of plantlets to be able to live optimally. The growth of the emergence of kepok banana shoots on growing media that was given GA3, IAA, and Auxin showed different levels of shoot emergence. The lower the growing room temperature shows the results that the emergence of plantlet shoots is getting less and less. GA3 treatment at a temperature of 20 °C resulted in an average shoot emergence of 49.34% not significantly different from a temperature of 21 °C which was 52.76%, a temperature of 29 °C the number of shoots (45.47%), and 30 °C (42, 71%). Optimal shoot growth was shown in the temperature range of 23-26°C (the number of shoots emerging was 75.69 - 78.35 %).

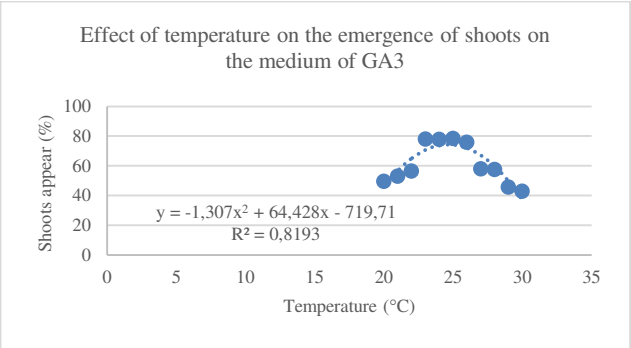
The IAA treatment at a temperature of 20 °C resulted in an average shoot appearance of 38.57%, not significantly different from a temperature of 30 °C which was 34.58%, and a temperature of 28 °C (40.52%). The growing room temperature of 21°C showed that the yield of 42.64% of shoots was not significantly different from the temperatures of 22°C (41.85%) and 29°C (44.27%). Optimal shoot growth is shown in the temperature range of 23-26°C (73.45-76.42%).

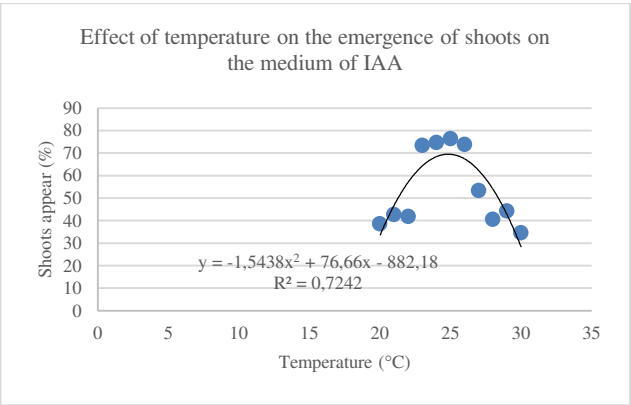
Auxin treatment at a temperature of 20 °C resulted in an average shoot emergence of 40.21%, not significantly different from the temperature of 21 the number of shoots was 40.85 %; temperature 29 °C number of shoots was 37.54%; and a temperature of 30 °C the number of shoots appeared 42.87 %). Optimal shoot growth was shown in the temperature range of 23-26°C (72.48 - 74.37 %).

The appearance of browning on banana kepok plantlets was also influenced by the high and low temperatures in the plantlet growing media. Growing room temperatures of 20 -23 °C and 27-30 °C showed an increase in browning attack on plantlets ranging from 3.38 to 4.45%. In-room conditions at room temperature between 24 - 26 °C, the appearance of browning can be suppressed to the range of 1.03 - 1.18%.

Death is also caused by a lack of additional nutrient intake in the growing media. Browning usually appears at the incision and then spreads to the newly growing shoots until they finally die and turn brown. Death of shoots on plantlets can be caused by contamination by fungi or bacteria which causes spoilage, usually caused by not optimal sterilization.

Browning begins with a brownish discoloration of the part affected by the incision or which was injured during subculture, then spreads to the explant organs as new shoots emerge. The longer the explant will turn brown in all parts of the explant which causes stunted growth and then dies.





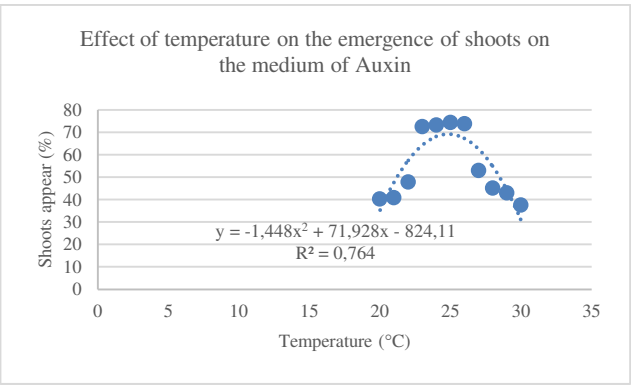
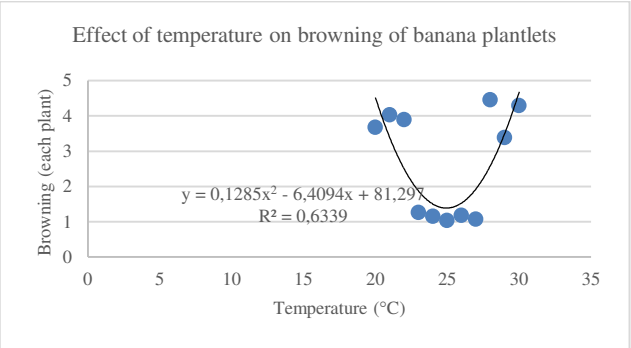


Figure-3. Effect of temperature on the emergence of shoots on the medium of GA3, IAA, Auxin.



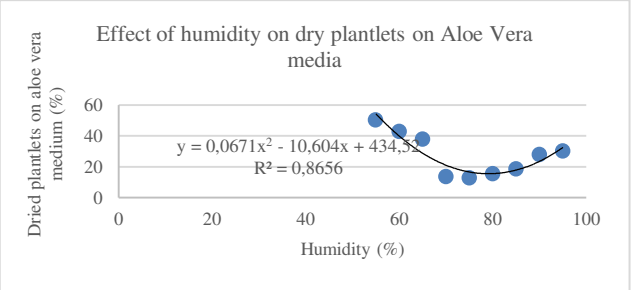
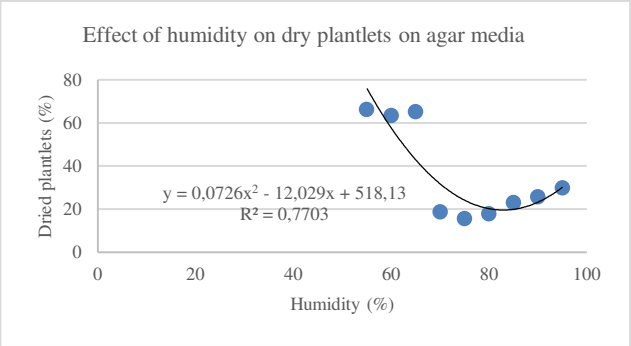
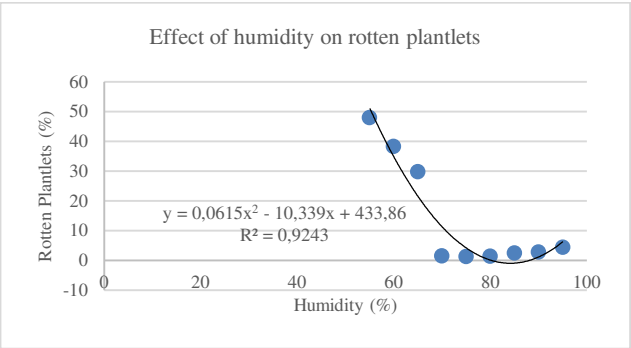


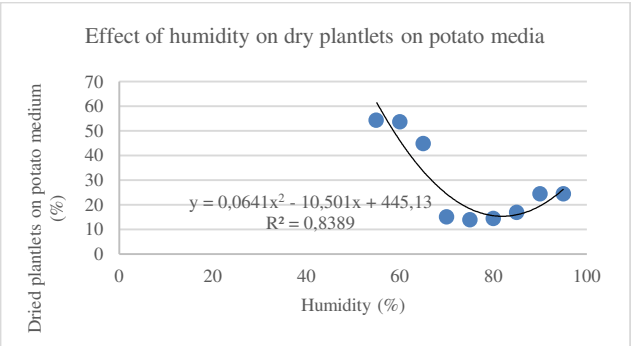
Figure-4. Effect of temperature on browning.

Table-5. Effect of humidity on growth and physical disturbances of banana kepok plantlets.

Humidity (%)	Dried plantlet shoots (%)				Rotten / Dead plantlets (%)
	Agar	Potato	Young coconut	Aloe vera	
55	66,25 d	54,28 d	47,29 f	50,36 g	47,93 g
60	63,40 d	53,63 d	32,61 d	42,86 f	38,27 f
65	65,18 d	44,75 c	23,72 c	37,93 e	29,72 e
70	18,72 a	14,92 a	14,38 a	13,62 a	1,45 a
75	15,69 a	13,82 a	11,74 a	12,73 a	1,24 a
80	17,82 a	14,31 a	12,32 ab	15,48 ab	1,34 a
85	23,04 b	16,84 a	15,62 b	18,52 b	2,35 bc
90	25,67 b	24,28 b	32,37 d	27,85 cd	2,65 c
95	29,94 c	24,37 b	37,12 e	30,19 d	4,28 d
BNT 0.05	4,16	4,28	3,35	3,47	0,45







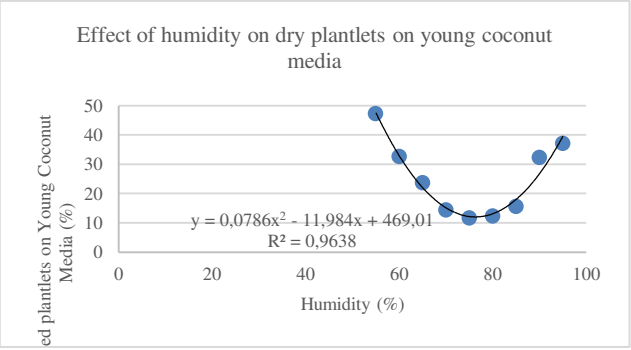


Figure-5. Effect of humidity on fry plantlets on different media.

DISCUSSIONS

This situation was also shown in the growing shoot length parameter that the administration of gibberellin 2 ml/L was able to increase shoot elongation as high as 1.25 cm and was significantly different from IAA administration which was able to extend shoots to 1.18 cm and auxin as high as 1.06 cm. The increase in plantlet root length with gibberellin also showed better results (1.52 cm) and was significantly different with IAA reaching 1.38 cm, while auxin was 1.27 cm. However, the root diameter of plantlet shoots did not show a significant difference from the administration of the three growth regulators observed. The results of the percentage of shoot growth of each observed growth regulator showed that gibberellin was able to produce 77.48% significantly different from IAA as much as 75.27% and auxin 74.76%. This situation is due to the content of compounds in gibberellins capable of regenerating meristematic cells in banana kepok plantlets. Development of shoot multiplication, MS medium with IAA 0.5 mg/L and Benzyl Adenine5 mg/L gave better results in shoot propagation and elongation, with the number of 6 - 17 shoots per explant. MS media can stimulate root growth, and together with *Naphthalene Acetic Acid* (NAA) 1 mg/L

the number of roots formed is 3-16 roots per plantlet (Fitramala E, *et al.,* 2016).

A medium like agar, potato, young coconut, and aloe vera was tested and showed no significant differences in the variables of budding time, number of shoots, and shoot length on banana kepok plantlets. This is because each growth medium does not contain many growth compounds and more carbohydrates and glucose so it is less able to encourage cell division in meristematic's. Micropropagation treatment with potato growing media was able to produce plantlet shoot root lengths of 0.43 cm and young coconuts of 0.42 cm. This situation was significantly different from the other two treatments, namely 0.24 cm agar and 0.31 cm aloe vera. This is because the potato and young coconut media contain lots of carbohydrates and glucose. So that it can be used for the needs of cell propagation and growth of plantlet shoot root diameter. The use of young coconut growing media showed a significant difference when compared to other media such as agar-agar, potato, and aloe vera, which showed a better percentage of shoots that appeared at 12.53%. This situation is due to the fact that young coconut media contains a lot of glucose as well as carbohydrates and protein so that it can help multiply cells that will form shoots.

Micropagation using growth regulators Gibberellin, IAA, and axin 2 mg/L showed meristematic changes and plantlet shoot formation was faster. This situation in young plantlets, especially in parts that are about to become buds, tends to show changes in cell regeneration. The use of coconut water in in vitro propagation with a concentration of 15% gave the best results in stimulating shoot growth (Kristina and Shahid, 2012). The use of coconut water in in vitro propagation with coconut water concentration 20% + BAP 2 mg/l on MS base media gave the best results on root length, number of shoots, and number of shoots. (Fate et al., 2008). Meristem changes and the formation of new shoots were seen slowly in the application of growing media. This situation is due to the fact that the growth media used are not many compounds that can help accelerate the occurrence of cell division, so it does not show good results. Cell division is influenced by the availability of protein or ATP and several activating substances for cell division to occur. The success of the development of tissue culture media is largely determined by the materials used. The selection of materials and the aseptic level of the media also contributed greatly to the achievement of the successful growth of kepok banana plantlet shoots. Various techniques were applied to produce a plantlet shoot with the addition of certain elements to make it more effective.

In vitro culture that has been developed has many factors that are limiting factors for success, including the availability of suitable media to provide the required nutritional intake and including explants taken as plantlets. The Murashige-Skoog (MS) propagation technique that has been developed in general still requires the addition of inorganic salts, organic compounds, sugars, hormones, and vitamins. The provision of nutrition in tissue culture has also used a lot of nutrients that are sold freely, this is done to reduce costs that are too expensive. Commercial foliar fertilizer is an alternative source of inorganic salts for seedling growth in in vitro culture (Yusnitawati and Triwahyuningsih, 2002). This addition aims to enrich the media with elements needed by plants such as macronutrients (N, P, K, S, Ca, and Mg) and micronutrients (B, Co, Fe, Mn, and Zn).

The effect of the humidity of the growing media is very influential on the success of plantlets to be able to grow optimally. The kepok banana culture developed on agar, potato, young coconut, and aloe vera growing media showed a degree of dryness due to the humidity conditions of the growing media. The lower the humidity in the growing room, the higher the number of plantlet shoots that died.

Treatment of agar medium with a humidity level below 55 - 65% showed that the dead plantlet shoots were 63.40 - 66.25%. The media humidity of 95% indicated that the plantlets were rotten and eventually died by 29.94%. Optimal humidity ranged from 70-80% showing that the dry plantlet yields were not significantly different, ranging from 15.69-18.72 %.

Potato media treatment with humidity levels below 55 - 60% showed dry plantlet shoots, namely 53.63 - 54.28 %. The media humidity was 90 - 95%, indicating that the dry dead plantlets were 24.28 - 24, 37 %. The optimal humidity ranged from 70-85%, which showed that the dry plantlet yields were not significantly different, ranging from 13.82 to 16.84%.

Treatment of young coconut media with humidity levels below 55 - 65% showed dry plantlet shoots ranging from (47.29; 32.61 and 23.72 %). The media humidity of 85, 90, and 95% showed rotten plantlets and eventually dried up (15.62; 32.37, and 37.12 %). Optimal humidity ranged from 70-80% dry plantlet yields were not significantly different, which ranged from 11.74 to 14.38 %.

One of the factors that influence the success of plant propagation in tissue culture is the composition of the media used. Plants need a number of very complex nutrients such as vitamins, amino acids, and growth regulators, to overcome this, coconut water and its fruit are often used.

Aloe vera media treatment with humidity levels below 55 - 65% showed dry plantlet shoots ranging from (37.93 - 50.36 %). The media humidity of 90 - 95 % indicated that the plantlets were rotten and eventually died (27.85 - 30.19 %). The optimal humidity ranged from 70-80%, showing that the dry plantlet yields were not significantly different, ranging from 12.73-15.48%. Humidity 70-80% showed no significant difference in the number of rotten or dead plantlets ranging from 1.24 to 1.45%. Humidity below 55 - 65% showed that the dead plantlets ranged (29.72 - 47.93 %). In high humidity, i.e. 85-95%, the number of plantlet deaths ranged from 2.35 to 4.28%.

Environmental conditions are one of the important factors that can determine success in in vitro culture. The incubation room environment is the result of

the interaction of planting material, culture bottles, and the external environment. Several environmental factors that influence the growth and development of in vitro cultures include temperature, humidity, light, carbon dioxide (CO2), oxygen (O2), and ethylene (C2H4) (Zulkarnain, 2009).

The daily temperature range of the incubation room obtained ranged from 22–22.8 °C, with an average daily temperature of 22.5°C. The temperature is suitable for the growth of banana plants in vitro culture. Temperature can affect plant growth and development both in vitro and in vivo. Plantlets have sensitive tissue so the temperature has a direct influence on the development of cells, tissues, and organ-forming plants (Zulkarnain, 2009).

CONCLUSIONS

The application of the use of gibberellin growth regulators showed better results when compared to the use of IAA and Auxin at the same concentration, namely 2 mg/L on plantlet shoot growth, plantlet shoot length, plantlet root diameter, and number of plantlet shoots, plantlet shoot root length, and many plantlet shoots. The use of young coconut and potato growing media gave a good influence on root length and root diameter of plantlet shoots, but the success of shoot growth was shown by young coconut media.

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