Akta Agrosia

Effect of Several Types and Concentrations of Complex Organic Compounds on Growth of Pineapple *In Vitro*

Mangara W Sianipar, Rustikawati*, RR Yudhy Harini B, Catur Herison, and Mukhtasar
Department of Agriculture, Faculty of Agriculture, University of Bengkulu
Jl. WR Supratman, Kandang Limun, Bengkulu 38120

ARTICLE INFO

Keywords:
Pineapple
in vitro
organic compounds

Article history:
Received: Mei 12, 2019
Accepted: Jun 26, 2019

*Corresponding author:
E-mail: rustikawati@unib.ac.id

ABSTRACT

Pineapple plants are usually propagated vegetatively by the basal stem of the fruits (ratoon), stem buds (sucker), fruit buds (slip) and crown. Plant propagation by any parts of the plant is become less effective to satisfy the large number of the needs as they produced a small amount and requires long time. An alternative to produce high amount of planting materials in relatively shorter time is by tissue culture techniques. This study aims to obtain the best types and concentration of complex organic compounds for the growth of pineapple shoots in vitro. The research was conducted in June to October 2017 in the tissue culture laboratory of Department of Crop Production, Faculty of Agriculture, University of Bengkulu. A completely randomized design with three replications was used to allocate nine treatments of complex organic compounds. The basic media was the Murashige and Skoog, and the treatments were 1) coconut water 100 ml/L, 2) coconut water 150 ml/L, 3) coconut water 200 ml/L, 4) banana pulp 100 g/L, 5) banana pulp 150 g/L, 6) banana pulp 200 g/L, 7) mung bean sprout extract 100 g/L, 8) mung bean sprout extract 150 g/L and 9) mung bean sprout extract 200 g/L. The results showed that at 16 weeks after transplanting, MS media with the addition of 100 g/L banana pulp exhibited the highest plant height with an average of 3.45 cm. The addition of 200 ml coconut water resulted in the highest shoot height of 1.5 cm and followed. The addition of 100 ml/L of coconut water was suitable for the growth of pineapple roots. The addition of mung bean sprout extract at any concentrations tended to reduce growth rate of pineapple plantlet *in vitro*.

INTRODUCTION

Pineapple plants (*Ananas comosus* L. Merr) are horticultural plants that have high nutritional value because pineapple contains vitamins A and C, calcium, phosphorus, magnesium, iron, sodium, potassium, dextrose and sucrose (Kurniawan, 2008). Pineapple production was increasing every year, for example, in 2014 pineapple production in Indonesia amounted to 448,134 tons, remembering to be 472,720 tons in 2015 (BPS, 2015). Pineapple has a sweet taste with a little acid which has many health benefits such as antioxidants, preventing cataracts, and preventing stress (Kurniawan, 2008).

Pineapple plants are usually propagated vegetatively by the basal stem of the fruit (ratoon), stem buds (sucker), fruit buds (slip) and crown. Propagation of plants by utilizing parts of the plant is considered to be less effective to fulfill the large amount demand because they produce small amount of planting material and require a long time (Rumondor *et al*., 2013). The alternative pineapple propagation to increase the amount of planting material is by tissue culture techniques. Tissue

ISSN: 1410-3354 / e-ISSN:2615-7136

Cited this as: Sianipar, M.W., Rustikawati, R.R.Y.H. Bertham, C. Herison, and Mukhtasar. 2019. Effect of several types and concentrations of complex organic compounds on growth of pineapple *in vitro* . Akta Agrosia 22(1):22-28 .

culture technique is be able to produce large number of propagule in a relatively short time with small plant source material by micro propagation technique. This technique is carried out by isolating plant parts such as protoplasm, cells, tissues or organs of plants which are then grown in sterile conditions so that parts of the plant can multiply and regenerate into complete plants (Henuhili, 2013). The tissue culture method is an appropriate multiplication technique to be applied because it can grow cells originating from the parent plant on an artificial media aseptically to get new shoots or plants.

The commonly used tissue culture media, the MS (Murashige and Skoog) composition, consists of macronutrients and micronutrients. Complex organic compounds are usually added to the media to improve plantlet responsiveness. Oktaviana *et al*. (2015) succeeded in growing pineapple shoots in vitro by shoot meristem on pineapple crowns with MS media added organic compounds from tomatoes and Sari *et al.* (2012) with MS media suplemented by IBA. Syafarudin *et al.* (2010) stated that pineapple plants grown in vitro with the addition of 0.01 mg/L Thiadiazuron to MS media have a good influence on the time of growing shoots. Aditiani (2006) added banana pulp 100 g/L + coconut water 150 ml/L in the medium to increase the growth of the number of leaves in Dendrobium sp. Addition of 150 g/L of banana pulp gave a significant influence on the induction of orchid roots in vitro compared to the treatment without the addition of banana pulp (Utami *et al*. 2016).

In addition to banana pulp, coconut water was also used to increase shoot production in vitro. Kristina and Syahid (2012) stated that young coconut water contained ZPT kinetin of 273.62 mg/L, zeatin 290.47 mg/L, and IAA 198.55 mg/L. The results of Tiwery's (2014) research on the volume of coconut water 250 ml/L increase plant height and the number of mustard leaves (*Brassica juncea* L.). Kasutjianingati and Irawan (2013) stated that 150 ml/L coconut water on Vacin and Went media was able to promote the formation of PLB (protocorm-like bodies) orchids. Bey *et al*. (2006) stated that a single treatment of coconut water could accelerate the emergence of PLB in Phalaenopsis orchids. Efra *et al*. (2012) found that coconut water of 225 ml/L to MS media resulted in the highest growth of the best orchid seedlings. Addition of coconut water with a concentration of 150 ml/L gave the best results on the formation of leaf number, root number, plantlet height and weight of chrysanthemum plantlets (Mustakim *et al*. 2015).

Other organic compounds that can increase shoot growth in vitro are mung bean sprouts. Hadi (2006) found that Vitabloom 2 g/L + bean sprout extract 37.5 g/L produced the most significant number of Kanayao Dendrobium orchid shoots at 8 MST. Likewise according to the results of Amilah and Astuti (2006), supplementation of several concentrations of bean sprouts extract in the Vacint and Went media can increase the growth of orchid sprouts in *Phalaenopsis amibilis* L. at a level of 150 g/L. The combination treatment of 10% mungbean sprouts extracts and 10 ppm BAP concentrations showed the earliest shoot formation and the highest number of shoots in black orchid plants (Saputri *et al*., 2015). The objective of this study was to obtain the type and concentration of the best complex organic compounds in the growth of pineapple shoots in vitro.

MATERIALS AND METHOD

This research was conducted from June 2017 to October 2017 at the Agronomy Laboratory of the Division of Biotechnology and Plant Tissue Culture, Department of Agriculture, Faculty of Agriculture, University of Bengkulu. The research was arranged in a completely randomized design with three replications. The basic media was the Murashige and Skoog (MS), and the treatments were 1) coconut water 100 ml/L, 2) coconut water 150 ml/L, 3) coconut water 200 ml/L, 4) banana pulp 100 g/L, 5) banana pulp 150 g/L, 6) banana pulp 200 g/L, 7) mung bean sprout extract 100 g/L, 8) mung bean sprout extract 150 g/L, 9) mung bean sprout extract 200 g/L. Each treatment was repeated three times so that there were 27 experimental units.

This research used the MS media combined with several types and concentrations of organic compounds. The organic compounds were bean sprouts, Ambon banana and coconut water. Bean sprouts and Ambon bananas are first mashed according to the treatment and coconut water is measured according to the procedure and added to the MS media solution and then sugar is added as much as 30 g/L. The pH measurement was carried out after all ingredients were mixed and stirred. The pH of the media was adjusted to 5.8 - 6.0 by HCl. The solution was then added to and cooked while stirring until boiling. After boiling, put it

in a culture bottle of about 25 ml per bottle which then sealed with clear and heat resistant plastic, and tied with the rubber band. The media was autoclaved at 121°C for 15 minutes.

Explants in this experiment were pineapple plants at one month old after subculture on MS media. The explants were chosen uniformly in the size of 0.5 cm - 1 cm with 2 leaves. Planting equipments were sterilized in an autoclave at 121°C for 30 minutes, then bottles containing sterile water and 96% alcohol were put into the LAC. The equipment was immersed in 96% alcohol then burned over bunsen before use. Explants uniformly selected were then planted with one explant in each bottle. After planting the bottle was sealed with sterile plastic and tied with rubber band wrapped with plastic wrap. Maintenance was carried out by maintaining the environment of the culture room, which was to provide light and room temperature around 20-24°C for 24 hours every day for 16 weeks after planting.

Observation of pineapple growth was carried out at weeks 12 to 16 after planting. Variables observed included plantlet height, number of shoots, shoot height, number of leaves, fresh weight of plants, and number of roots. The data were analyzed statistically using the F test at the level of 5%, and if there were significant differences between treatments, the mean comparison was performed by the Duncan Multiple Range Test (DMRT) at α=5%.

RESULTS AND DISCUSSION

Types and concentrations of complex organic compounds were significantly different in variable plant height and shoot height and did not significantly affect the number of shoots and number of leaves (Table 1). With respect to plant height, in general, there were three different plant growth patterns, which were prolonged, slow, and growth tends to be fast. The pattern of prolonged growth, which was of no more than 1 cm and almost no increase in plant height. This growth pattern occured in the addition of mung bean sprout extract. Growth patterns that tend to be slow where a significant increase in plant height but was slower every week was exhibited in the addition of organic compounds of coconut water of 150 ml/L. The third pattern of growth was fast growth pattern where average increase in plant height of about 0.5 cm. Plant height on the addition of 100 ml/L coconut water had an average plant height of 12 weeks after planting of 1.5 cm and grew 2.25 cm at 16 weeks after planting. Addition of coconut water 200 ml/L has a rapid increase from 14 weeks after planting to 16 weeks after planting, which is 2.25 cm to 2.45 cm (Figure 1.a).

Pineapple plants in the media with the addition of bean sprouts at all concentrations did not reveal high growth of more than 1 cm from week 12 after planting to 16 MST (Figure 1.b). This was probably be due to too high levels of auxin and cytokinin contained in the mung bean sprouts. According to the finding of Suparjo *et al*. (2016), high concentration of auxin or cytokinin reduced the growth of binahong plants and may also occur in pineapple plants. Extract mung bean sprouts of 12.5% increased the growth of aloe vera plants (Rita *et al*., 2017).

The highest plant height of all types and concentrations of organic compounds was found in the addition of 100 g/L banana pulp with an average plant height of 3.45 (Figure 1.c). In accordance with the research of Aziz (2016), the best medium for the growth of peppermint cuttings was the MS media with the addition of banana pulp but at a concentration of 150 g/L. Nitrogen content in bananas was considered to have an essential role in increasing plant height because N is one of the primary or macronutrients.

Pineapple young shoots were formed on the media with the addition of coconut water organic compounds with concentrations of 100 ml/L or 200 ml/L, and in the media with the addition of 100 g/L banana pulp. Different from the other types and concentrations of shoots that did not form young shoot up to 16 weeks after planting. The shoots on media with the addition of 100 ml/L of coconut water were established at 14 weeks after planting with an average of 0.5 but in addition to banana pulp 100 g/L and coconut water 200 ml/L buds formed at the beginning of the observation or 12 MST.

The highest number of young shoots was found in the addition of 100 g/L banana pulp

Tabel 1. The F-values of ANOVA on the effect of kinds and concentrations of some complex organic compounds on growth and multiplication of pineapple shoots in vitro at 16 weeks after transplanting

Variables	F-value
Plant height	6,74 *
Number of shoots	0,12 ns
Shoot height	3,43 *
Number of leaf	1,43 ns

Note : * = significant difference at α=5 %, ns = non-significant difference at α=5 %

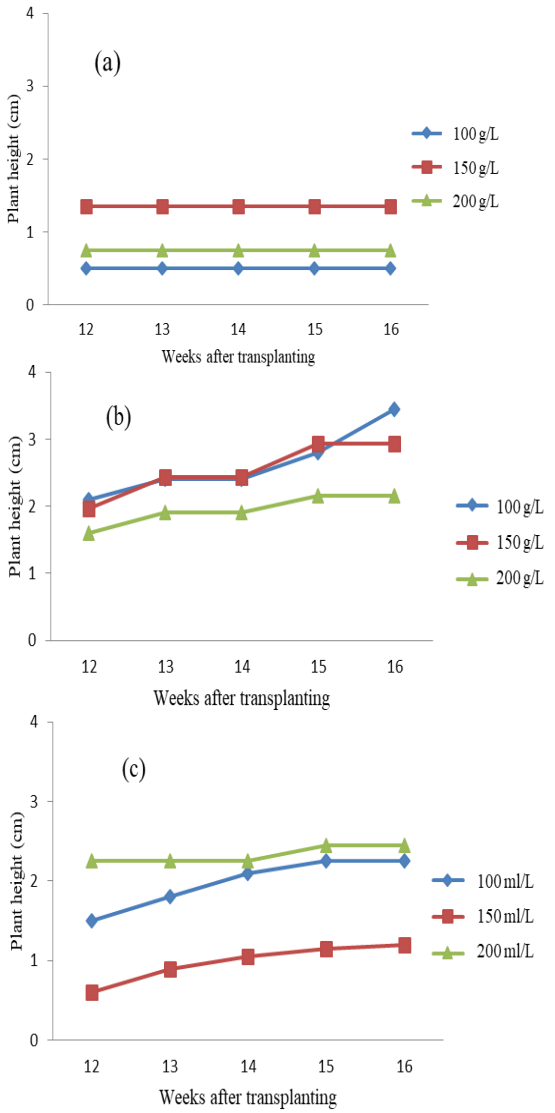

Figure 1. Pineapple plant height in vitro at 12 to 16 weeks after transplanting in (a) mung bean sprout extract, (b) banana pulp, and (c) coconut water

with an average of 4 shoots at 16 MST (Figure 2c) followed by the addition of coconut water 200 ml/L (Figure 2a). In line with the Kasutjianginati and Irawan (2013), addition of coconut or banana water to tissue culture media was able to produce a large number of shoots and was equivalent to the addition of ZPT BAP as much as 2 g/L. Coconut water contained kinetin (cytokinin) 273.62 mg/L and zeatin 290.47 mg/L, while the content of IAA (auxin) was 198.55 mg/L (Kristina *et al*., 2012) which was considered to accelerate the growth of shoots in the pineapple plant.

The shoot height was only measured on the plants in the media with the addition of coconut water organic compounds with a concentration of 100 ml/L, 200 ml/L and banana pulp 100 g/L media. The highest shoot height was found in the addition of organic coconut water of 200 ml/L with an average of 1.5 cm, followed by that of in the addition of 100 g/L banana pulp with an average height of 0.9 cm and coconut water of 100 ml/L which had plant height of 0.1 cm (Table 2).

Kristina *et al*. (2012) mentioned that coconut water contained IAA and cytokinin. The content was assessed both in plant growth and in the growth of new shoots. Surachman (2011) found that the use of MS media plus 10% coconut water in patchouli propagation in vitro resulted in an average percentage of shoots life of 100%. At the addition of bean sprouts extract, there was no growth of shoots in all concentrations, presumably due to the growth of the plants were also hampered and there was no bud formation until 16 weeks after transplanting.

The number of leaves formed in each type and the concentration of organic compounds varied. At 12 weeks after transplanting, the number of leaves was in a range of 4-8 leaves. However, it increased quickly at 16 MST in line with the growth of young shoots reaching a range of 5-18 leaves (Figure 3.). It was different from that of the addition of the type of mung bean sprout extract which was in range of leaves of 5-6 leaves through after the

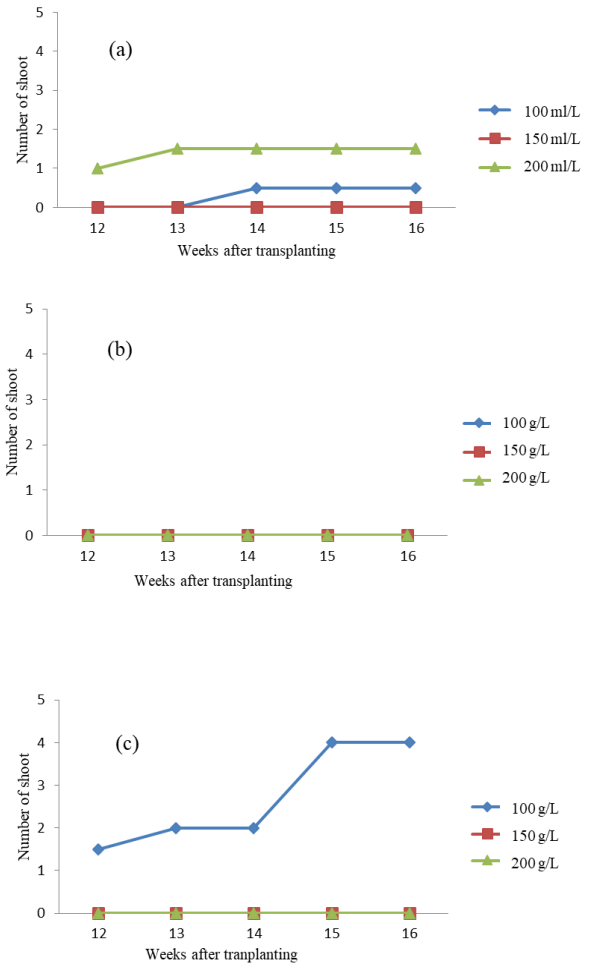

Figure 2. Number of young shoot of pineapple in vitro at 12 to 16 weeks after transplanting in (a) coconut water, (b) mung bean sprout extract, and (c) banana pulp

experiment. The addition of banana pulp with all concentrations dominated the number of leaves from the beginning to the end of the observation with a range of 8-21 leaves in line with the number of formed young shoots.

In this study, the highest number of leaves was found in the addition of 100 g/L banana pulp. In line with the results of Hadi (2006), the combination of Vitabloom 2 g/L + banana pulp 100 g/L was able to increase the number of leaves of Dendrobium orchid. The level of 100 g/L produced the highest number of leaves of Phalaenopsis orchid. Sugar and carbohydrates contained in banana pulp was the primary key in the metabolic process of the plants. Metabolic reactions in plants produced thousands of compounds to form organs such as leaves, stems, roots, and other structures found in plants (Djadjanegara, 2010).

Table 3. Fresh weight and number of root of pineapple plantlet in vitro at 16 weeks after transplanting

Treatments	Fresh weight (g)	Number of roots
MS + 100 ml coconut water	0,26	4,00
MS + 150 ml coconut water	0,19	0,00
MS + 200 ml coconut water	0,18	0,00
MS + 100 g mung bean sprout extract	0,02	0,00
MS + 150 g mung bean sprout extract	0,03	0,00
MS + 200 g mung bean sprout extract	0,02	0,00
MS + 100 g banana pulp	0,38	0,00
MS + 150 g banana pulp	0,21	0,00
MS + 200 g banana pulp	0,28	3,00

In this study, the addition of 100 g/L banana pulp had the highest plant height with an average of 3.35 cm, but it was not significantly different from the concentration of 150 g/L and 200 g/L with an average of 2.25 cm and 1.16 cm, respectively. The treatment was also not significantly different from the coconut water of 100 ml/L and 200 ml/L, with an average of 2.25 cm and 2.45 cm, respectively (Table 2). However, the treatment was significantly different from the extract bean sprouts of 100 g/L only had an average plant height of 0.2 cm. Aziz (2016) showed that banana pulp produced the highest plant height with a concentration of 150 g/L in peppermint plants. Fresh bananas contain elements of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), manganese (Mn) and iron (Fe) (Paull and Deuarde, 2011). The content of N in bananas have an active role in the growth of pineapple plants. According to the study of Elfiani and Aryati (2012), the application of nitrogen fertilizer to pineapple plants increased plant height, the number of leaves and fresh weight.

With respect to the height of young shoots, the addition of 200 ml/L coconut water showed the highest shoot height with an average of 1.5 cm although it was not significantly different from the addition of 100 g/L banana pulp with an average of 0.9 cm. At the same concentration, mung bean sprout extract did not produce young shoots until 16 weeks after transplanting, as well as 200 g/L banana pulp (Table 2). According to Kristina *et al*. (2012), young coconut water contained cytokinin of 273.62 mg/L and zeatin 290.47 mg/L, then auxin of 198.55 mg/L. Auxin is a growth regulating agent that is good at shoot formation or shoot meristem. Surachman (2011) showed that the addition of 100 ml of coconut water had the highest yield on variable young shoot height of patchouli. However, in this study, MS media with the addition of 200 ml of coconut water was the best media for young shoot height of pineapple, which was 1.5 cm at the end of experiment. The addition of mung bean sprout extract on MS media with various

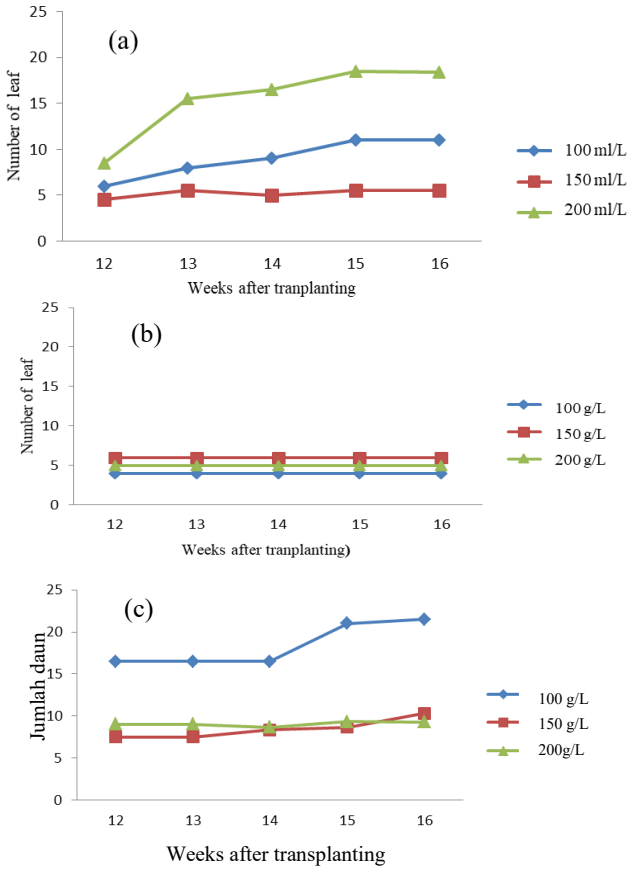

Figure 3. Number of leaft of pineapple in vitro at 12 to 16 weeks in (a) coconut water, (b) mung bean sprout extract, and (c) banana pulp

concentrations was not able to stimulate the growth of young shoots of pineapple.

The highest fresh weight of the plant which was of 0.38 g was found in the addition of 100 g/L banana pulp and followed by the concentration of 200 g/L of 0.28 g (Table 3). At the same level, coconut water shows the fresh weight of the plant at 0.26 g and mung bean sprout extract of 0.021 g. In number of roots, the addition of 100 ml/L coconut water produced the highest number of roots (4 roots), which was then followed by 200 g/L of banana pulp (3 roots) (Table 3). Zasari (2016) also showed that addition of 100 ml/L coconut water had a significant effect on the number of roots in orchid seedlings. Young coconut water contains macro, micro, sugar, sugar alcohol, vitamins, amino acids, organic acids, phytohormones (such as auxin and gibberellin cytokines) which are thought to be essential roles in pineapple growth especially in the formation of the root.

Table 3. Fresh weight and number of root of pineapple plantlet in vitro at 16 weeks after transplanting

Treatments	Fresh weight (g)	Num-ber of roots
MS + 100 ml coconut water	0,26	4,00
MS + 150 ml coconut water	0,19	0,00
MS + 200 ml coconut water	0,18	0,00
MS + 100 g mung bean sprout extract	0,02	0,00
MS + 150 g mung bean sprout extract	0,03	0,00
MS + 200 g mung bean sprout extract	0,02	0,00
MS + 100 g banana pulp	0,38	0,00
MS + 150 g banana pulp	0,21	0,00
MS + 200 g banana pulp	0,28	3,00

CONCLUSION

MS media with the addition of 100 g/L of banana pulp was the best media for pineapple in vitro. MS media with the addition of 100 ml of coconut water could increase the number and length of pineapple roots. MS media with the addition of mung bean sprouts with various concentrations were not able to stimulate shoot growth of pinapple.

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