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ORIGINAL RESEARCH

Root induction of *Phalaenopsis amabilis* with various species and concentrations of the banana extract by in vitro

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Abstract. Root induction is an important step in plant propagation in vitro. Root stimulation can occur in the presence of the hormones auxin and gibberellins which can be obtained from other plants, one of which is bananas. The research aims to determine the effect of various species and concentrations of the banana extract on *Phalaenopsis amabilis* root induction in vitro. The research was conducted in the Laboratory of Tissue Culture Alifa Agricultural Research Center (AARC), Medan, North Sumatera, from May until July 2020. The research used a Factorial Completely Randomized Design (CRD) with 2 factors, the first factor was the type of banana extract with 3 levels, consisting of Ambon, Raja, and Kepok. The second factor is the concentration of banana extract (C) with 4 levels, consisting of 50 g/l, 100 g/l, 150 g/l, 200 g/l. The results showed that the concentration of banana extract had a significant effect on the number of roots, but various species of bananas and the interaction of the species of bananas and concentration banana extract had no significant effect on root induction.

Keywords: *Phalaenopsis amabilis*, banana extract, concentration, in vitro

INTRODUCTION

Phalaenopsis amabilis, family Orcidaceae, genus phalaenopsis. In Indonesia, this orchid is very well known and very popular so it has the potential to be developed, because it has a high commercial value with a unique flower shape such as a butterfly, long blooming time, and does not wilt easily. However, one of the problems is the limited availability of seeds, where generative propagation is difficult because the seeds do not have an endosperm as a food reserve and at an early age orchid plants have the potential to be infected with viruses. Effective and efficient cultivation is needed in the development of lunar orchids to increase their productivity [1].

Tissue culture is the most suitable technique for producing plant seeds in a short time with large numbers and the same characteristics as the parent. However, in vitro propagation techniques still have challenges in terms of the slow growth of plantlets, low rates of multiplication, difficulty in rooting, and somaclonal variation [2]. Isda and Siti [3] stated that the rooting process is one of the most important stages in the in vitro plant growth phase.

Stimulation of shoots and roots can occur due to cell division and the process of cell differentiation where this can occur with the help of the hormones auxin and gibberellins which can be obtained from the banana extract. Kasutjianingati and Rudi [4] conducted a study on plantlets of dendrobium orchids with ambon banana extract treatment on the parameters of observing root length, plant height, the number of roots produced a good effect. This is because, in Ambon banana there is a potassium (K) content for photosynthesis and respiration, as activating enzymes, phosphorus (P) and iron (Fe) play a role in plant metabolic processes, thus giving a positive influence on the growth of orchid plants [5].

The results of Utami et al research [6] also said that the administration of plantain extract, in general, had an effect on root induction and shoot growth of *D. laianthera* with 150 g/l planta in extract capable of inducing more root formation than other treatments. This is because banana extract contains thiamine which is useful in root meristems to accelerate cell

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division and also contains auxin and cytokinin compounds which are useful in cell enlargement, elongation, and division.

Based on the above, researchers are interested in studying the effect of various species and concentrations of banana extracts on roots induction of *Phalaenopsis amabilis* in vitro.

METHODOLOGY

Banana extract

The bananas used to consist of 3 species, namely Ambon, Raja, and Kepok. Bananas are used in physiologically ripe conditions and good conditions, that are marked by soft flesh. The bananas are mashed with a blender, filtered, and then poured into a temporary cup [7].

Initiation culture

Explants were obtained from 1 year old *Phalaenopsis amabilis in vitro* with good condition and have 2 leaves are ready for root induction. Root induction using full MS medium with 3 species of banana extract as plant growth regulator (PGR). Turn on the Bunsen lamp and remove the explant from the old bottle, after that it is placed in a petri dish and cleaned of remnants so that those that are still attached and the roots that have grown must be cut. The explants were then separated and planted on new media (treatment). Two moon orchid plants in 1 jump jar bottle [8].

The parameters observed included the percentage of live explants, percentage of explants forming roots, number of roots per explant, and root length per explant.

Data analysis

This research used a Factorial Completely Randomized Design (CRD) with 2 factors, the first factor was the species of banana extract with 3 levels, consisting of M_1 = Ambon, M_2 = Raja, and M_3 = Kepok. The second factor is the concentration of banana extract (C) with 4 levels, consisting of C_1 = 50 g/l, C_2 = 100 g/l, C_3 = 150 g/l, and C_4 = 200 g/l.

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) v 23.0. The analysis of variance (ANOVA) procedure for a factorial experiment was used to test for a significant effect of treatments, followed by LSD test for comparisons of different means of different treatments.

RESULTS AND DISCUSSION

Percentage of live explants (%)

The results of statistical analysis showed that MS media treated with various species and concentrations of banana extract and the interaction had an effect on the percentage of live explants of *Phalaenopsis amabilis*. The mean percentage of live explants can be seen in Table 1.

Table 1. Percentage of live explants treated with various species and concentrations of banana extract

Treatment	The concentration of banana extract				Mean
	C_1	C_2	C_3	C_4	
	----------%----------				
M_1	100	100	100	100	100
M_2	100	100	100	100	100
M_3	100	100	100	100	100
Mean	100	100	100	100	100

Based on Table 1, it can be seen that the percentage of live explants treated with various species and the concentrations of banana extract reached 100% in all treatments. One of the characteristics live of the orchid plant is has the characteristics of green color (Figure 1).

Figure 1. Characteristics of live orchid plants

The use of basic media, namely MS, played an important role in the growth of explants because it contained high macro, micro, and vitamins so it has a good effect on the growth of *Phalaenopsis amabilis* plant. Pratama [9] states that the media used greatly influences the success in the propagation of tissue culture.

Complete macro and micronutrients and vitamins are found in MS media which can be used in various plants.

Percentage of explants forming roots (%)

The results of statistical analysis showed that MS media treated with various species and concentrations of banana extract and the interaction between the affected the percentage of explants forming roots. The mean percentage of explants forming roots can be seen in table 2.

Table 2. Percentage of explants forming roots on various species and concentrations of banana extract

Treatment	The concentration of banana extract				Mean
	C_1	C_2	C_3	C_4	
	----------%----------				
M_1	80.56	80.56	41.67	61.11	65.97
M_2	80.56	77.78	66.67	75.00	75.00
M_3	69.44	72.22	72.22	75.00	72.22
Mean	76.85	76.85	60.19	70.37	71.06

Based on Table 2, it can be seen that the highest mean percentage of explants forming roots was found at the M_1C_1, M_1C_2 and M_2C_1 treatments was 80.56%, while the lowest mean was found at the M_1C_3 treatment was 41.67%. This is cause M_1C_3 media is contaminated with fungal, where media contaminated with fungal will make it difficult for explants to grow because explants that are injured due to initiation will be attacked through their tissues and the media is the right host for the growth and development of fungal so that root growth will be disrupted. In line with the statement of [10] that fungal contamination that occurs in the affects on the growth of explants, as the day goes on, the growing media and explants will be covered by fungal microorganisms. A good substrate for the growth of fungal is the growth medium itself. The fungal will attack plant tissue through cuts caused by cutting during the initiation process.

Number of root per explant (unit)

The results of statistical analysis showed that MS media treated with various species of banana extract and treatment interactions had no significant effect on the number of roots per explant at the age of 1-6 weeks after planting (WAP), but the banana extract concentration treatment had a significant effect on the age of 6 WAP. The mean number of roots per explant can be seen in Table 3.

Based on Table 3, it can be seen that the concentration of banana extract significantly affected the number of roots per explant. The highest mean was found at the C_1 treatment was 3.17 cm, while the lowest mean at the C_4 treatment was 1.83 cm. Giving banana extract has a good effect on root growth, where the hormones gibberellins and auxins present in ripe bananas affect stimulating root growth. Lestari and Ni Wayan [11] stated that the hormones auxin and gibberellins are found in ripe bananas. Auxins in tissue culture, in addition to stimulating cell elongation, callus formation, chlorophyll, root and shoot growth, and embryogenesis.

Table 3. Number of roots per explant with various species and concentrations of banana extract at the age of 1-6 WAP

Treatment	Time of observation					
	1	2	3	4	5	6
	----------unit----------					
Type of banana						
M_1	0.13	0.67	0.79	1.58	1.75	2.38
M_2	0.33	0.67	1.02	1.67	2.00	2.29
M_3	0.21	0.58	1.08	1.58	2.08	2.42
Concentration						
C_1	0.28	1.00	1.39	2.11	2.61	3.17 a
C_2	0.28	0.61	0.83	1.50	1.89	2.50 ab
C_3	0.06	0.44	0.89	1.33	1.67	1.94 ab
C_4	0.28	0.50	0.75	1.50	1.61	1.83 b
Interaction						
M_1C_1	0.17	1.50	1.50	2.50	3.00	3.50
M_1C_2	0.33	0.67	0.83	2.00	2.17	3.17
M_1C_3	0.00	0.17	0.50	0.67	0.67	1.33
M_1C_4	0.00	0.33	0.33	1.17	1.17	1.50
M_2C_1	0.33	1.00	1.67	2.17	2.50	3.17
M_2C_2	0.33	0.83	1.00	1.33	2.00	2.50
M_2C_3	0.17	0.33	0.67	1.33	1.67	1.67
M_2C_4	0.50	0.50	0.75	1.83	1.83	1.83
M_3C_1	0.33	0.50	1.00	1.67	2.33	2.83
M_3C_2	0.17	0.33	0.67	1.17	1.50	1.83
M_3C_3	0.00	0.83	1.50	2.00	2.67	2.83
M_3C_4	0.33	0.67	1.17	1.50	1.83	2.17

Note: Numbers that are not followed by letters in the same row and column show no significant difference in the LSD test at the 5% and 1%.

The concentration of banana extract between 50, 100 and 150 g/l not significantly different in increasing the number of roots, so it is recommended to use a lower dose. In line with [12], if treatment with lower concentration but has the same effect as treatment with higher

concentration in increasing yields, the treatment the lower concentration is more better than higher concentration.

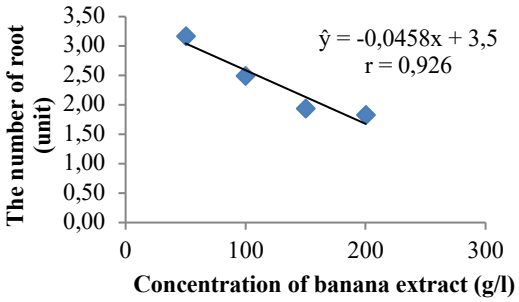

Figure 2. Graph of the relationship between the concentration of banana extract and the number of roots formed

Based on the negative linear equation diagram in Figure 3 shows the equation = -0.0458x + 3.5 and the value of r = 0.926. It can be said that there was a decrease in the number of roots when the banana concentration was given higher. This is presumably due to the dense of banana extract so excessive giving treatment of banana extract can cause explants to have disturbances in nutrient absorption. According to [13], plants grow maximally due to nutrient intake which is obtained. Besides, the dence of banana extract make difficult for plant roots to get oxygen. The results of Bakrie's research [14] show that the use of good growing media, namely media that has cavities and roots must be able to easily get oxygen for its development.

Length of root per explant (cm)

The results of statistical analysis showed that MS media treated with various species and concentrations of banana extract and the interaction between the two had no significant effect on root length per explant. The mean length of root per explant can be seen in table 4.

Table 4. Length of root per explant with various species and concentrations of banana extract

Treatment	The concentration of banana extract				Mean
	C_1	C_2	C_3	C_4	
	----------cm----------				
M_1	0.41	0.40	0.10	0.21	0.28
M_2	0.35	0.41	0.42	0.16	0.33
M_3	0.28	0.17	0.43	0.27	0.29
Mean	0.35	0.33	0.32	0.21	0.30

Based on table 4, it can be seen that the root length varied with the treatment of various species and concentrations of banana extract. The best banana type treatment was found at M_2, was 0.33 cm, the best concentration treatment at C_1 was 0.35 cm and the best interaction treatment at M_3C_3 was 0.43 cm.

The research results showed that in some culture media phenolic compounds were grown with phenolic compounds from the age of 3 WAP, which indicated by the appearance of a black color around the explants (Figure 3).

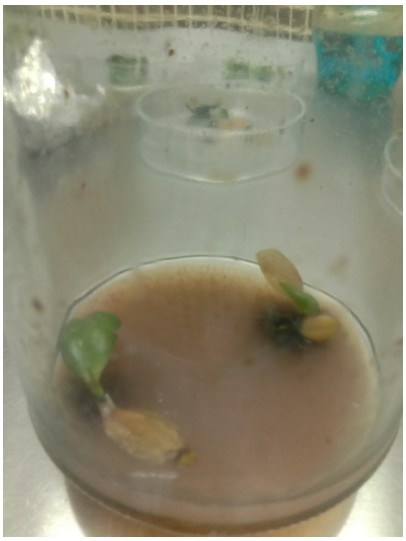

Figure 3. Phenolics in culture media

These phenolic compounds can affect plant growth and cause death because they are toxic to plants. According to [15], phenolics can grow in culture media will inhibit plant growth and sustainably the plant will die. In addition, [16] added that one of the causes of the formation of phenolic compounds is influenced by the plant species. Tropical plants have a higher phenolic content, one of which is orchids, which are oxidized when cells are injured or senescence occurs. As a result, plant tissue will turn brown to black and fail to grow. The browning that occurs is caused by the activity of the oxidase enzyme which contains institutions and toxic which over time causes the death of the plant.

There are several methods that often used as a pre-treatment of explants to avoid browning culture in vitro, namely, immersion and pre-conditioning on basic media [17]. The results of research by [18] reported that the best reduction of oxidative browning was with stirring the explants for 1 hour in an antioxidant containing ascorbic acid 100 mg/l and citric acid 1500 mg/l before planting in medium. The combination of these treatments with photo periodicity of 16 hours, was able to produce shoot growth of up to 100%.

CONCLUSION

The concentration of banana extract had a significant effect on the number of roots, but various species of bananas and the interaction of the species of bananas and concentration banana extract had no significant effect on *Phalaenopsis amabilis* root induction. Ambon banana extract 50g/l can be used to increase the number of roots *Phalaenopsis amabilis*.

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