

**Research Article****The Influence of Leaf Fertilizer Media on Multiplication of Barangan Banana (*Musa acuminata* L.) in vitro****Liberty Chaidir*, Dina Nur Mardiana, Ahmad Taofik, Yati Setiati Rachmawati***Departemen of Agrotechnology, Faculty of Science and Technology, UIN Sunan Gunung Djati Bandung. Jl. A.H. Nasution 105, Bandung 40614*

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ABSTRACT

*Barangan banana (*Musa acuminata* L.) is a plant that has the biggest contribution to national fruit production. The increasing demand for the banana requires the availability of seedlings, while conventional cultivation is unable to produce healthy, disease-free seedlings in a short time and large amount. In vitro culture is considered to be able to handle the constraints of the conventional seed supply. The purpose of this study was to determine the effect of foliar fertilizers on in vitro multiplication of barangan bananas. The method used was a single factor of Completely Randomized Design (CRD), the addition of leaf fertilizer consisting of five levels of media treatment, media MS (control), and 0.50 ml L⁻¹ (p1); 0.75 ml L⁻¹ (p2); 1.00 ml L⁻¹ (p3); 1.25 ml L⁻¹ (p4); 1.50 ml L⁻¹ (p5) of leaf fertilizer media repeated five times. The data were analyzed with Analysis Variance then followed by Duncan Multiple Ranged Test of 5%. The results showed MS media still gave the best results compared to other leaf fertilizer media. Meanwhile, the leaf fertilizer media used in this study still gave good results on the initial time of buds appearance (p3 treatment), the number of shoots and the number of leaves in the p5 treatment, as well as the height of the plant in the p4 and p5 treatments. Therefore, the leaf fertilizer can be used to substitute MS media.*

Keywords: Barangan banana; Leaf fertilizer; MS; Multiplication.**1. Introduction**

Banana is served as a staple food by millions of people around the world. It is rich in carbohydrate sources with a calorific value of 67/100 g of banana flesh. In addition, banana contains various vitamins and is used for various medicinal purposes (Maharana *et al.* 2017). Especially for Barangan banana, its demand is always increasing, especially in big cities, because of the superior and nutritious fruit. That's why Barangan banana is one of the banana plants with great potential and opportunity to be developed (Zebua *et al.* 2015). It is one of the specific fruits from North Sumatra which people highly favor because of its advantages over other bananas. Barangan banana tastes sweeter; its skin color is yellow with black spots when ripe; the flesh color is reddish, dry and has good smells (Balai Pengkajian Teknologi Pertanian Indonesia, 2009). Barangan banana is rich in dietary fibre, protein,

minerals such as sodium and potassium, fat, and yeast and produces sugar (Shankar *et al.* 2017). Therefore, it becomes a commercially superior commodity with increasing demand. Besides, it is known as red bananas that is widely cultivated in Southeast Asia.

The increasing demand for Barangan banana requires the availability of seedlings, but the conventional banana plant seedlings (macropropagation) cannot to produce a number of healthy and disease-free ones. Parida *et al.* (2017) state that traditional cultivation production costs for banana breeding spend high value. Addition of quality bananas needs to be initiated from the available provision of superior banana plant seedlings, so it is necessary to supply banana plant seedlings with *in vitro* multiplication techniques to address problems in conventional Barangan banana multiplication.

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The growing environment in tissue culture provides conditions optimized for the desired formation of organs or plantlets (Teixeira da Silva *et al.* 2019). The tissue culture technique has been widely exploited for large-scale propagation. Plant micropropagation has become a significant and informative technique for producing and maintaining plant availability. Banana micropropagation has been of interest in recent years because it produces profits and uniformity of plants and is free from disease. It also makes plants provide the best results and can be conducted in large quantities. However, tissue culture requires good quality planting material to produce good plants (Maharana *et al.* 2017). Generally, an explant originating from young tissue is the best explant because the tissue is still active in division like meristem tissue (Chaidir *et al.* 2018).

One important factor in the multiplication of Barangan banana shoots is growing media. It is very influential on the success of *in vitro* culture because the growing media contains nutrients and sources of nutrition needed by the explants. The basic media commonly used in *in vitro* culture is MS (Murashige and Skoog) media, this is frequently used because it has complete nutrients needed by plants. MS media with or without growth regulators is effective for the germination or shoot formation process (Pant dan Swar 2012). The use of MS media in the propagation of *in vitro* culture has been widely applied, but it requires a more expensive cost. The relatively high price of MS media demands efforts to replace some of the MS media components using simpler and cheaper media such as leaf fertilizer.

Leaf fertilizer is one type of fertilizer containing nutrients that are quite complete and considered able to provide a source of nutrition for seedling growth in *in vitro* culture. It is an option because it can provide nutrients for plants (Purwanto *et al.* 2017). Leaf fertilizer contains three main nutrients, namely nitrogen (N), phosphorus (P), and potassium (K) for various plants and growth conditions. Nitrogen increases leaf growth and forms proteins and chlorophyll. Phosphorus has roles for the growth of roots, flowers, and fruit development. Nutritional deficiencies cause plants to experience physical abnormalities, such as lack of nitrogen causing plants to turn yellow, lack of phosphorus causing plants to turn brown, and lack of potassium causing the plant's leaves to dry up to burn (Pritts 2018).

Application and modification of alternative media with leaf fertilizer in the production and propagation of high-quality Barangan banana plant seedlings is important to do. This relates to the efficiency of production costs that must be incurred. So in this study, leaf fertilizer media was an

alternative for the propagation of *in vitro* Barangan banana plant seedlings, especially for the multiplication stage carried out in supplying *in vitro* banana plant seedlings.

2. Material and Methods

The study was conducted at Tissue Culture Laboratory, UIN Sunan Gunung Djati Bandung, from January to July 2019. Barangan banana explants used were one month old from Biotrop Bogor. This research used the experimental method in Completely Randomized Design (CRD), which consisted of 6 treatments and five replications, so there were 30 experiment samples.

p_0 = Control

p_1 = Leaf fertilizer 0.50 ml L⁻¹

p_2 = Leaf fertilizer 0.75 ml L⁻¹

p_3 = Leaf fertilizer 1 ml L⁻¹

p_4 = Leaf fertilizer 1.25 ml L⁻¹

p_5 = Leaf fertilizer 1.50 ml L⁻¹

Data obtained were processed with Analysis of Variance, then further tested with Duncan Multiple Ranged Test (DMRT) in 5% level. The parameters observed included the first time the shoots appearance, the number of roots, the number of leaves, and the height of plantlets. The observations were conducted once every two weeks except at parameters of the initial time of the shoot and root appearances which were observed every day.

3. Results

The initial time of shoots appearance or forming is the easiest indicator to determine the success in doing tissue culture multiplication. The multiplication of Barangan banana generated different variations at the time of buds appearance, started on the 6th day after induction (DAI) up to the age of 14 DAI. Based on statistical analysis, any treatment applied did not significantly influence the initial time of bud appearance (Figure 1). In various levels of leaf fertilizer media used, the treatment of leaf fertilizer p_3 (1 ml L⁻¹) gave the fastest bud appearance time for six days after induction and in the treatment of leaf fertilizer p_4 (1.25 ml L⁻¹) gave the time of appearance of shoots at the latest on 14.4 days.

On observation, the number of shoots showed success in multiplication carried out. Shoots are very important in a study because as a support for other organs to grow and develop. Based on observations of the number of shoots on six treatments, the Analysis Variance (Anova) test results showed significant difference results.

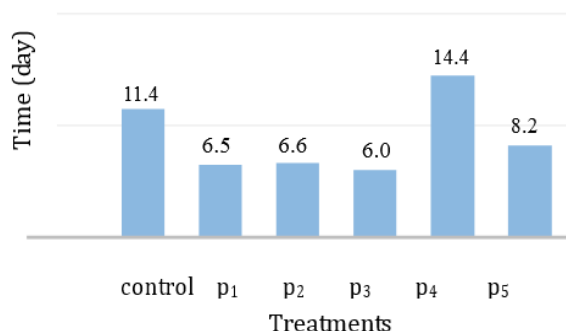


Figure 1 The initial time of bud appearance

In Table 1, the Anova result indicates influence on the number of shoots observation at eight weeks after induction, were control as the highest and p₁ (0.5 ml L⁻¹) as the lowest. The same figure also happened on the number of leaf parameters, the highest result was control and p₁ (0.5 ml L⁻¹) was the lowest. This shows that the treatment of leaf fertilizer concentrations given had not been able to match the MS media (control) in the growth of the number of shoots in the explants of Barangan banana plants.

Table 1. The Effect of Leaf Fertilizer on Number of Shoots and Number of leaves

Treatment	Number of Shoot/s		Number of Leaf /ves	
	6 DAI	8DAI	6 DAI	8 DAI
Kontrol	1.4 b	2.0 c	3.0 b	4.0 c
p ₁ (0.5 mL L ⁻¹)	0.4 a	0.4 a	0.0 a	0.0 a
p ₂ (0.75 mL L ⁻¹)	1.0 b	1.0 ab	0.0 a	0.0 a
p ₃ (1 mL L ⁻¹)	1.0 b	1.0 ab	0.0 a	0.6 a
p ₄ (1.25 mL L ⁻¹)	1.0 b	1.0 ab	1.0 a	2.4 b
p ₅ (1.50 mL L ⁻¹)	1.2 a	1.2 b	1.2 a	2.8 b

Note: numbers followed by same letter show non-significant difference based on Duncan Multiple Ranged Test of 5% level

From Figure 3, it can be seen that the growth of the best shoot was on banana explant in the media MS. At the age of 2 weeks, the explant began to rise to the buds and rose the leaf at the age of 4 weeks and became a complete planlet at the age of 8 weeks.

MS media was the best media that can grow the number of shoots of Barangan banana planlet

explant in *in vitro* culture, so that presume the nutrients contained in the treatment of leaf fertilizer had not been able to replace the nutrients contained in the MS media. Nitrogen nutrients in the MS media have a very important role in growing the number of shoots.

Based on observations, the shoots of Barangan banana planlets showed different results between control and leaf fertilizer media on various treatments.

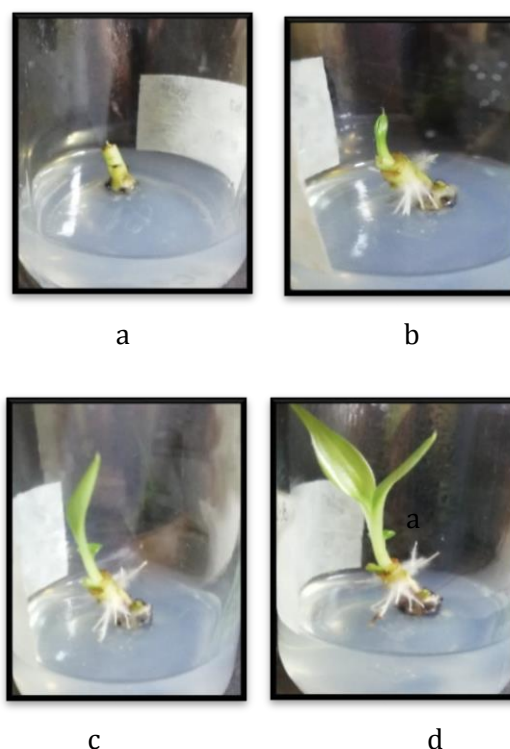


Figure 3. The growth of Barangan Banana shoot explant on MS media. (a) age 2 week after culture (WAC), (b) age 4 WAC, (c) age 6 WAC, (d) age 8 WAC.

Table 2. Influence of Leaf Fertilizer Media on Plantlet Height of Barangan Banana Explants

Treatments	Plantlet height (cm)			
	2 DAI	4 DAI	6 DAI	8 DAI
Control	0.28 a	0.60 a	1.70 b	2.58 b
p ₁ (0.5 mL L ⁻¹)	0.20 a	0.22 a	0.32 a	0.45 a
p ₂ (0.75 mL L ⁻¹)	0.14 a	0.14 a	0.14 a	0.14 a
p ₃ (1 mL L ⁻¹)	0.10 a	0.26 a	0.38 a	0.50 a
p ₄ (1.25 mL L ⁻¹)	0.13 a	0.22 a	1.22 b	1.78 b
p ₅ (1.50 mL L ⁻¹)	0.10 a	0.38 a	1.54 b	2.10 b

Note: numbers followed by same letter show non-significant difference based on Duncan Multiple Ranged Test of 5% level

Different concentrations of leaf fertilizer did not exert a different influence on 2 weeks after

induction and four weeks after induction observations. In the control treatment, p₄ (1.25 ml L⁻¹) and p₅ treatment (1.50 ml L⁻¹) gave different effects than other treatments (Table 2).

4. Discussion

The best shoots initiation appearance was known in p₃ (1 ml L⁻¹) treatment, which could grow shoots simultaneously at the age of 6 DAI (Figure 1). This was presumed by the availability of main macronutrients (Nitrogen (N), Phosphorus (P), Potassium (K)) in the leaf fertilizers that are able to meet the needs of young plants whose cells are still actively dividing. N and P elements are nutrients needed by plants in large quantities (Nofrianinda et al. 2017). The element of N has an important role in plant growth, including shoots initiation appearance. Nitrogen is a nutrient needed by young plants, especially in the formation of amino acids, protein, and nucleic acid.

The shoots initiation appearance was influenced by several factors, one of which was food availability. Food needed by Barangan banana plants was available on the media used to grow banana plants in the *in vitro* culture. Carbohydrates have a fundamental role in the process of shoot proliferation and affect the shoot growth and survival. The presence of sucrose in culture media in this study greatly influenced shoot growth (Chaidir et al. 2019). The response of buds initiation appearance was marked by the appearance of a bulge or swelling of explants to produce shoots formation finally. The buds initiation appearance in this study was known to vary for each treatment. The responses were fast and slow and even did not emerge buds. This is because each explant has different abilities in absorbing nutrients in the media (Elma et al, 2017).

The shoots growth was also influenced by other factors such as the media containing enough nutrients for explants growth. Hendrayono & Wijayani (1994) explain that the growth medium of *in vitro* culture greatly influences the explant's growth and development in the supply of plant seedlings.

In the treatment of p₁ in this study, the availability of nutrients given was too low compared to other treatments, it can be said that the available nutrients did not meet the explants' needs, so that the growth of the shoots was inhibited. Lakitan (1996) explains that a plant will grow optimally if the nutrients needed are available in sufficient quantities and in a suitable form so that plants can absorb it.

On the observation of the number of Barangan banana leaves, media control (MS) gave maximum

results compared to the other treatments (Table 1). Hence, it can be said that the nutrients contained in the treatment of leaf fertilizer media had not been able to totally replace the nutrients contained in MS media, especially for the element of N in growing the number of shoots. Besides that, N is essential in the growth and development of all-important plant tissues because it is a supporting organ for other organs to keep growing and developing. Each concentration of media given was different in result, it can be seen that the more concentration of leaf fertilizer was given, the greater the element content of N. The large concentrate of N generally has a higher number of leaves (Lakitan 1996). Garner (1991) said that the N element is very influential compared to other minerals on plant growth.

On the observation of Barangan banana plantlet height, the best result was still given by media control (MS) compared to other leaf fertilizer media (Figure 2). Plantlet height was also influenced by nutrients of N, P, and K, which were interrelated in the growth and multiplication of shoots, meaning that in this study, the nutrients needed by plants did not experience shortages. The nutrient of N, P, and K in the treatments of p₄ and p₅ could be interpreted as able to increase plantlet height growth better than the media p₀ (control). Other nutrients such as Ca (Calcium) could also influence plant growth in the formation of cell walls. Besides that, the nutrients of Na, I, Cu, Cl, Co, and so forth contained in leaf fertilizers are also likely to affect overall cell metabolism (Srilestari dan Sasmita 2015).

Growth is characterized by changes in plant morphology in the form of elongation and enlargement of tissues (Karjadi dan Buchory 2008). In the narrow sense, growth means cell division, which increases the number and enlargement of cells. As a result, cell size increases and causes an increase in shoots height marked by an increase in explants size. The increased shoots height in explants shows growth and the ability to add new organs around the shoot organs that have specific functions and are important for plant needs.

5. Conclusion

The multiplication of Barangan banana in tissue culture or in vitro by using leaf fertilizer media could not exceed the results gave MS media (control). However, the influence of leaf fertilizer treatment on the multiplication of Barangan banana occurred with the higher concentration, which affected the number of shoots, number of leaves, and plantlet height. The concentration of 1 ml L⁻¹ leaf fertilizer treatment was able to emerge the best buds initiation, the concentration of 1.25 ml L⁻¹ and 1.50 ml L⁻¹ were able to grow the best number of

leaves and plantlet height and able to increase the number of roots of Barangan banana plantlet.

6. Declaration of Conflicting Interests

The authors have declared no potential conflicts of interest concerning the study, authorship, and/or publication of this article.

7. References

- Balai Pengkajian Teknologi Pertanian Sumatera. 2009. Teknologi Penanaman Pisang Barangan Sistem Dua Jalur (Double Row). Medan.
- Chaidir L, Hasani S, Diana A, Subandi M, Wicaksana N. 2019. Effect of Sucrose on in vitro Bud Multiplication of Torch Ginger (*Etlingera elatior*). IOP Conf Ser Earth Environ Sci. 334:012015. doi:10.1088/1755-1315/334/1/012015.
- Chaidir L, Isnaini S, Hidayat C, Hasani S, Wicaksana N, Syantiara J. 2018. Sterilization Method of *Etlingera elatior* Explant on Tissue Culture. IOP Conf Ser Mater Sci Eng. 434(1):012292. doi:10.1088/1757-899X/434/1/012292.
- Elma T, Suminar E, Mubarak S, Nuraini A, Ariyanto NB. 2018. Multiplikasi tunas mikro pisang (*Musa paradisiaca* L.) 'raja bulu' secara in vitro pada berbagai jenis dan konsentrasi sitokinin. Kultivasi. 16(3):418-424. doi:10.24198/kltv.v16i3.14917.
- Gardner FP, Pearce RB, Mitchell RL. 1991. Fisiologi Tanaman. Jakarta: Universitas Indonesia Press.
- Karjadi AK, Buchory AKA. 2008. Pengaruh auksin dan sitokinin terhadap pertumbuhan dan perkembangan jaringan meristem kentang kultivar granola. J Hort. 18(4):380-384.
- Lakitan B. 1996. Fisiologi Tumbuhan : Pertumbuhan dan perkembangan. Jakarta: PT. Raya Grafindo Persada.
- Maharana K, Beura S, Sarathi Munsu P. 2017. A Fast Protocol for in vitro Cloning of Banana (*Musa acuminata*) cv. Amritpani. Int J Curr Microbiol Appl Sci. 6(10):586-594. doi:10.20546/ijcmas.2017.610.072.
- Nofrianinda V, Yulianti F, Agustina E. 2017. Pertumbuhan Planlet Stroberi (*Fragaria Ananassa* D) Var. Dorit pada Beberapa Variasi Media Modifikasi In Vitro di Balai Penelitian Jeruk dan Buah Subtropika (BALITJESTRO). Biotropic. 1(1):41-50.
- Pant B, Swar S. 2012. Micropropagation of *Cymbidium iridioides*. Nepal J Sci Technol. 12:91-96. doi:10.3126/njst.v12i0.6485.
- Parida SR, Beura S, Rout S, Beura R, Jagadev PN. 2017. Fast protocol for high frequency in vitro cloning of Banana (*Musa acuminata*) cv. Grande Naine. J Appl Nat Sci. 9(1):72-79. doi:10.31018/jans.v9i1.1153.
- Pritts M. 2018. Foliar Testing and Sampling in Berry Crops, Visual Symptoms of Deficiencies. New York: Cornell University.
- Purwanto P, Mujiono M, Tarjoko T. 2017. Effect of Foliar Liquid Organic Fertilizer to Increase Coconut Neera Production. Planta Trop J Agro Sci. 5(2):2015-2018. doi:10.18196/pt.2017.070.106-109.
- Shankar G, Jeevitha P, Shadeesh L. 2017. Nutritional Analysis of *Musa Acuminata*. Res Rev J Food Dairy Technol. 5(4):27-29.
- Slilestari R, Sasmita R. 2015. Perbanyakkan pisang raja bulu secara in vitro dengan menggunakan pupuk daun The in vitro multiplication of raja bulu banana using foliar fertilizer. Agrivet. 19(1):1-6.
- Teixeira da Silva JA, Zeng S, Godoy-Hernández G, Rivera-Madrid R, Dobránszki J. 2019. Bixa orellana L. (achiote) tissue culture: a review. Vitro Cell Dev Biol - Plant. 55(3):231-241. doi:10.1007/s11627-019-09969-3.
- Wijayani A, Hendaryanto DPS. 1994. Teknik Kultur Jaringan dan Petunjuk Perbanyakkan Tanaman secara Vegetatif Modern. Yogyakarta: Kanisius.
- Zebua D, Rahayu S, Hanum S. 2016. Induction of Barangan Banana shoot (*Musa acuminata* L.) from North Nias through Tissue Culture by giving 2,4-D and Kinetin. J Biosains. 1(2):1. doi:10.24114/jbio.v1i2.2780.