



The Growth Response of Banana cv. Barangan (*Musa acuminata* L.) from Tissue Culture with Organic Manure and Arbuscular Mycorrhizal Fungi

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10.18805/IJARE.A-633

ABSTRACT

Background: Acclimatization is the last stage of tissue culture that aims to adapt plantlets to environmental conditions. Nutrient availability needs to be considered in this critical phase. This research was conducted to determine the right type of organic manure and AMF dose to support the post-acclimatization growth of banana cv. Barangan.

Methods: The study using a completely randomized design (CRD) based on factorial with two treatment factors. The first factor is the type of organic manures: goat manure, cow manure, vermicompost and compost. The second factor is AMF doses: 0, 5, 10, 15 g/plant. Observed variables include the plant height, the pseudo-stem diameter, leaf number, leaf length and width and the percentage of mycorrhiza infections at the plant roots.

Result: The results showed an interaction between the type of organic manure and AMF dosage at the leaf length and width, but not on the plant height, the pseudo-stem diameter and leaf number. The application of various organic manures types has a significant effect on the plant height, the pseudo-stem diameter, leaf length and width, but no significant effect on the leaf number. The application of various doses of AMF has no significant impact on all of the observed variables.

Key words: Arbuscular mycorrhizal fungi, Banana cv. Barangan, Organic manure, Tissue culture.

INTRODUCTION

Banana cv. Barangan (*Musa acuminata* L.) is a banana that originates from North Sumatra and is commonly consumed in fresh and processed products. Banana production increased by 2.17% in 2017, while the increase in 2018 and 2019 was only 1.39% and 0.22% (BPS, 2019). This reduction causes less supply of bananas in the market because plant propagation is only done conventionally using banana sprouts that grow around the parent plant. Conventional propagation does not guarantee good quality due to the potential of bananas carrying pathogens causing various diseases (Deo *et al.*, 2020; Hussein, 2012). High quality planting material is needed to increase banana production (Bidhari *et al.*, 2018).

Tissue culture cultivation is a way to produce uniform plant seedlings in large quantities and free from disease. Acclimatization is the last and most crucial tissue culture stage that aims to get used to plantlet living in natural environments such as greenhouses and planting grounds than previously lived in controlled environmental circumstances (Duan *et al.*, 2020; Nasution *et al.*, 2020). The availability of nutrients needs to be considered because acclimatization is a critical and restrictive stage in plant micropropagation (Ferreira *et al.* 2017). Organic manures including compost as providers of macro and micronutrients can increase the pH and soil structure by improving organic matter and soil ability in maintaining groundwater (Nugroho *et al.* 2017; Samanhudi *et al.* 2018a;

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How to cite this article: Samanhudi, Widijanto, H., Muliawati, E.S., Favreta, V.F., Hidayanto, M. (2021). The Growth Response of Banana cv. Barangan (*Musa acuminata* L.) from Tissue Culture with Organic Manure and Arbuscular Mycorrhizal Fungi. Indian Journal of Agricultural Research. 55(5): 597-602. DOI: 10.18805/IJARE.A-633.

Submitted: 20-03-2021 **Accepted:** 16-06-2021 **Online:** 04-08-2021

Samanhudi *et al.*, 2017a). The addition of organic manure and compost will add plant nutrients to support growth and determine banana plantlet acclimatization success.

Arbuscular mycorrhizal fungi (AMF) is a hyphae that will penetrate soil, access water and nutrients inaccessible to the root hair (Li *et al.*, 2019; Muktiyanta *et al.*, 2018). AMF will assist plants in nutrient absorption, while organic manures as providers of macro and micronutrients (Samanhudi *et al.*, 2018b; Yunus *et al.*, 2018). Proper application of organic manures and AMF dose can help to meet plant nutrients' needs to support banana cv. Barangan's growth after acclimatization.

MATERIALS AND METHODS

This research was conducted from June - October 2019 at the Faculty of Agriculture's Experimental Field, Universitas Sebelas Maret. The analysis was done in the Soil Chemistry and Fertility Laboratory and Soil Biology and Biotechnology Laboratory, Faculty of Agriculture, Universitas Sebelas Maret. Materials used in this research include acclimatized banana cv. Barangan, soil, goat manure, cow manure, vermicompost, compost, AMF and water. Planting media contains soil and manure with a ratio of 2:1 based on volume and FMA according to the treatment on polybag size 25 × 30 cm.

The research method used is an experimental method with a completely randomized design (CRD) based on factorial with two treatment factors. The first factor is the type of organic manures: goat manure, cow manure, vermicompost and compost. The second factor is AMF doses: 0, 5, 10, 15 g/plant. The data obtained was statistically analysed using analysis of covariance (ANCOVA) and continued by Duncan's multiple range test (DMRT) at a 5% rate if significant.

RESULTS AND DISCUSSION

General conditions of research

Arbuscular mycorrhizal fungi (AMF) used is a product of MycoGrow with a carrier ingredient in zeolite and contains

33 spores per gram endomycorrhiza, 300 live propagules per gram and 18 kinds of amino acids. The alfisol soil used in this study had 0.18% total N (low), 2.04 ppm available P (very low), 0.11 mol(+)/kg exchangeable K (low), 1.26% organic carbon (low), 19.73 cmol(+)/kg CEC (moderate), 6.5 pH (somewhat acidic) and 8% moisture content. Alfisol has low soil fertility rates, low soil pH, low nutrient content such as N, P, K, organic carbon, Mo, Mg and low soil microbiological content, while high in mass density and erosion sensitivity (Kaharuddin *et al.* 2019; Soetedjo *et al.* 2019).

The results of the analysis of organic manures used in this study (Table 1) show that the pH in manures includes somewhat alkalis if it is in the range of 7.6-8.5 and alkalis if located >8.5. According to Fan and Li (2014), fertilizer with alkaline pH can increase soil pH significantly and keep soil pH in the range of 7-7.4. The highest organic carbon and CEC content (Table 1) is found in compost fertilizer, 21.92% and 53.79 cmol(+)/kg. Compost fertilizer has a high C/N ratio that can lead to decreased N's availability, limiting microbial metabolism, resulting in low demand for P microbes and high potential mineralization P (Chen *et al.* 2020; Gao *et al.* 2019).

Plant height

The interaction between organic manures and AMF doses has no significant effect, while organic manure exerts a significant impact on plant height. The dose of AMF in a single way has no significant effect on the plant height. The addition of organic manures might have released nutrients, especially P and limit the formation of symbiosis between AMF and host plants so that the positive effects of AMF are reduced (Kahiluoto *et al.*, 2000; Kahiluoto *et al.*, 2001; Richardson *et al.*, 2011). Fertilization should be considered when using AMF in increasing plant growth so that nutrient availability does not limit the positive role of AMF for host plants.

Table 2 shows that the addition of goat manure provides the best results than other manures increasing plant height

Table 1: Analysis result of organic manures.

Type of organic manures	pH	Moisture content (%)	Organic carbon (%)	CEC (cmol(+)/kg)	C/N ratio	N (%)	P (%)	K (%)
Goat manure	7.86	11.84	13.08	46.05	13.77	0.95	0.64	0.50
Cow manure	7.84	12.96	13.48	36.27	18.97	0.71	0.26	0.31
Vermicompost	7.86	11.05	14.50	28.49	16.67	0.87	0.21	0.10
Compost	8.62	10.16	21.92	53.79	28.47	0.77	0.41	0.44

Table 2: Effect of type of organic manures and AMF dose on plant height at 93 DAP.

Type of organic manures	AMF dosage (g)				Average (pieces)
	0	5	10	15	
Goat manure	53.83	44.10	52.17	42.90	48.25±6.62 c
Cow manure	37.67	40.43	44.10	44.33	41.63±4.59 b
Vermicompost	42.47	39.87	39.93	37.33	39.90±4.07 b
Compost	33.83	32.17	34.83	36.17	34.25±5.08 a
Average (pieces)	41.95±8.65	39.14±6.55	42.76±7.77	40.18±5.40	-

Description: (-) sign indicates no interaction between factors. Values followed by the same letter in the same column indicate no significant difference according to DMRT α 5%.

as also reported earlier (Prasetyo *et al.*, 2018). The analysis of nutrients in manures (Table 1) shows that goat manure contains higher N, P and K content so that the media with this manure application has more nutrient availability in the soil. Sufficient nutrient content can increase the distribution of roots so that the plant access to water sources is deeper and more stable (Polverigiani *et al.* 2018).

The lowest average plant height was recorded in banana with the compost application. Plant growth can be hampered by a decrease in NH_4^+ and NO_3^- due to microbial immobilization. Microbial immobilization occurs due to the use of manures with a high C/N ratio, while manures with low C/N ratios can increase mineralization (Baruah *et al.*, 2016).

Pseudo-stem diameter

Banana pseudo-stems form from piles of leaves that sit tightly and regularly to stand upright like plant stems. The interaction between organic manures and AMF doses has no significant effect. In contrast, the treatment of a single type of organic manure had significant impact on the banana pseudo-stem diameter. The AMF dose in a single way has no significant effect on the diameter of the pseudo-stem. This could be due to the moderate to high P in all treatments because of the application of organic manures that suppress the development of AMF in the soil and reduce AMF colonization with roots (Johnson *et al.*, 2013).

Table 3 shows that the goat manure application to the planting media results in a higher pseudo-stem diameter due to the chemical composition of the goat manure itself (Sebetha and Mashele, 2019). The high nitrogen content in goat manure forms nucleic acids, amino acids, proteins and cell enlargement so that the diameter of the stem increases (Purbajanti *et al.*, 2016). The lowest pseudo-stem diameter is seen in the compost application. This might be due to the

C/N ratio, which is still high 28.47 (immature) (Table 1). Immature compost will inhibit plant growth because the manure is contaminated with intermediate compounds (such as ammonia and organic acids) toxic to plants (Liu *et al.*, 2020; Phibunwatthanawong and Riddech, 2019).

Number of leaves

The interaction between the type of organic manure and the dose of AMF, also did not have a significant effect on the number of leaves.

Table 4 shows that the number of banana leaves are in the same range of about 6-7 pieces in all treatments. This is presumably because the N in the organic manures is in the same range 0.7-0.9%. N supply increases chlorophyll content, total protein, sugar content, protein, fat, auxin formation stimulant to soften the cell walls, improve plant ability in absorbing water and metabolites associated with photosynthesis (Bassi *et al.*, 2018; Dawiyah *et al.*, 2018; Permana *et al.*, 2018; Riyana *et al.*, 2018; Yunus *et al.*, 2018). Leaves that get a sufficient supply of N will form wider strands of leaves with higher chlorophyll to produce high amounts of carbohydrates to support plants' vegetative growth, including the number of leaves.

Leaf length

The interaction between the type of organic manure and the AMF dose significantly affects the banana leaf length.

Table 5 shows that the most massive leaf length is found in banana-with the application of goat manure without AMF, which is 29.65 cm, not significantly different from the 10 g dose and substantially different from the 5 and 15 g doses. This indicates that the length of the banana leaf can grow better if not combined with AMF. Organic manures increases the nutrients available in the soil, especially P. Smith *et al.* (2009)

Table 3: Effect of type of organic manures on pseudo-stem diameter at 93 DAP.

Type of organic manures	AMF dosage (g)				Average (pieces)
	0	5	10	15	
Goat manure	2.54	2.27	2.46	2.24	2.38±0.24 c
Cow manure	1.84	2.06	2.14	2.08	2.03±0.20 b
Vermicompost	2.06	2.05	2.01	1.95	2.02±0.11 b
Compost	1.71	1.58	1.62	1.79	1.67±0.20 a
Average (pieces)	2.04±0.37	1.99±0.32	2.06±0.36	2.02±0.23	-

Description: (-) sign indicates no interaction between factors. Values followed by the same letter in the same column indicate no significant difference according to DMRT α 5%.

Table 4: Effect of type of organic manures and AMF dose on the number of leaves at 93 DAP.

Type of organic manures	AMF dosage (g)				Average (pieces)
	0	5	10	15	
Goat manure	7.00	6.33	6.67	6.67	6.67±0.49
Cow manure	6.67	6.00	6.67	6.67	6.67±0.65
Vermicompost	6.67	6.00	6.33	6.33	6.33±0.49
Compost	5.67	6.67	6.33	6.33	6.25±0.62
Average (pieces)	6.50±0.67	6.25±0.45	6.50±0.52	6.67±0.65	-

Description: (-) sign indicates no interaction between factors.

Table 5: Effect of type of organic manures and AMF dose on leaf length at 93 DAP.

Type of organic manures	AMF dosage (g)				Average (cm)
	0	5	10	15	
Goat manure	29.65±1.20 f	24.85±2.26 e	29.10±3.09 f	22.99±1.87 cde	26.65
Cow manure	19.95±2.01 abcd	22.68±2.70 bcde	22.34±2.58 bcde	23.76±1.44 de	22.18
Vermicompost	23.79±1.93 de	21.63±1.46 bcde	21.90±2.51 bcde	21.12±3.50 bcde	22.13
Compost	18.29±2.65 ab	16.70±3.29 a	18.76±2.13 abc	19.80±1.07 abcd	18.38
Average (cm)	22.92	21.47	23.05	21.92	+

Description: (+) sign indicates an interaction between factors. Values followed by the same letter indicate no significant difference according to DMRT α 5%.

Table 6: Effect of type of organic manures and AMF dose on leaf width at 93 DAP.

Type of organic manures	AMF dosage (g)				Average (cm)
	0	5	10	15	
Goat manure	13.73±0.40 f	11.11±1.21 de	12.84±1.24 ef	10.29±0.77 bcd	11.10
Cow manure	9.02±0.82 bcd	9.92±1.09 bcd	9.92±1.72 bcd	10.58±0.56 cd	9.86
Vermicompost	10.49±1.25 bcd	9.28±0.09 bcd	9.67±0.97 bcd	9.25±1.38 bcd	9.67
Compost	8.39±1.32 ab	6.83±1.76 a	8.37±1.01 ab	8.9±0.58 bc	8.12
Average (cm)	10.4	9.29	10.2	9.76	+

Description: (+) sign indicates an interaction between factors. Values followed by the same letter indicate no significant difference according to DMRT α 5%.

Table 7: Percentage of root infected by mycorrhiza.

Combined treatment	Mycorrhizal infection (%)
Goat manure + 0 g AMF	16
Goat manure + 5 g AMF	96
Goat manure + 10 g AMF	92
Goat manure + 15 g AMF	96
Cow manure + 0 g AMF	16
Cow manure + 5 g AMF	100
Cow manure + 10 g AMF	96
Cow manure + 15 g AMF	88
Vermicompost + 0 g AMF	12
Vermicompost + 5 g AMF	80
Vermicompost + 10 g AMF	100
Vermicompost + 15 g AMF	100
Compost + 0 g AMF	24
Compost + 5 g AMF	100
Compost + 10 g AMF	96
Compost + 15 g AMF	96

Description: The percentage of root infected by mycorrhiza is based on Koch and Moawad's (1975) method.

explained that when AMF infects the roots, plants expect AMF to distribute nutrients to reduce roots from direct nutrient uptake. AMF cannot provide nutrients due to its limitations, so plant growth is hampered.

Leaf width

The results of the analysis showed that there was an interaction between organic manure types and AMF doses. The interaction that occurs shows that applying organic manure in the planting media has a meaningful relationship with the AMF, affecting the width of banana leaf.

Table 6 shows that the application of cow manure and vermicompost combined with AMF 0-15 g did not result in significantly different leaf widths of banana leaves. These results are in line with the findings of Begum *et al.* (2019) who reported that the addition of organic matter combined with AMF shows no significant different results because AMF effectively absorbs nutrients in low soil nutrient conditions.

Mycorrhizal infections

The use of mycorrhiza can increase microbes in the soil and increase the root branches so that the spread of roots is wider (Samanhudi *et al.* 2017b; Samanhudi *et al.* 2017c).

Table 7 shows that the roots of banana plants without AMF treatment appear to be infected with mycorrhiza in the range of 12-24%. Infections that occur in roots without AMF are caused by the absence of *indigenous* AMF from the planting media. Based on the observation, it can be seen that AMF infects the roots of banana in all treatments but does not significantly affect on all of the observed variables. This may be because the application of AMF has no significant effect on the vegetative period of banana. AMF helps plants absorb the application of AMF does not significantly affect the parameters of banana growth but could affect the time out of the heart/blossom and fruit weight per bunch.

CONCLUSION

Interactions occur between organic manures and AMF doses at the length and width of leaves, but not on the plant height, the pseudo-stem diameter and the number of leaves. The application of various organic manure types has a significant effect on the plant height, the pseudo-stem diameter, the number of leaves and the leaf length and width. The

application of various doses of AMF has no significant effect on the plant height, the pseudo-stem diameter, the number of leaves, the length and width of the leaves.

ACKNOWLEDGEMENT

The authors are grateful to the Universitas Sebelas Maret for funding this research through the scheme of Penelitian Unggulan UNS (PU UNS) of PNBP UNS for fiscal year 2019.

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