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Application of organic fertilizers and *arbuscular mycorrhiza* dosage to increase the growth of kepok banana seedlings (*Musa paradisiaca* L.) derived from tissue culture

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Abstract

Acclimatization is the last stage of the tissue culture technique which is crucial for the success of *in vitro* plant propagation. The use of organic fertilizers is known to improve the physical, chemical, and biological properties of the soil, while Arbuscular Mycorrhiza Fungi (AMF) is a well-known type of Mycorrhiza that benefits the host plant in nutrient absorption and to increase plant growth. This study aims to measure the growth response of the acclimatized Kepok Banana (*Musa Paradisiaca* L.) treated with a combination of various organic fertilizers and doses of AMF, expecting to support plant growth. This research was conducted in June- October 2019 and used a complete randomized design pattern (CRD) based on factorial (4 x 4) with two treatment factors. The first factor was the type of organic fertilizer: goat manure, cow manure, ver micompost, and compost. The second factor was AMF doses: 0, 5, 10, 15 g/plant. Variables observed included plant height, stem diameter, number of leaves, leaf length, leaf width, and mycorrhizal infectivity. The use of organic fertilizers significantly affected all growth parameters of kepok bananas. Goat manure gives a better result than other organic fertilizers on a plant's height, stem diameter, and length of leaves. The application of various doses of AMF either 5 g, 10 g, 15 g, or without AMF has not been able to exert a noticeable influence on the height of the plant, the number of leaves, the width of the leaves, the length of the leaves, and the diameter of the banana stem.

Keywords: vermicompost, goat manure, acclimatization, compost, *mycorrhiza.*

Introduction

Banana is a plant that is widely found in Indonesia, generally growing in tropical and subtropical areas. Among the tropical fruits found in Indonesia, bananas are widely consumed both in fresh form and processed into food products. Banana is an herbaceous fruit plant originating from Southeast Asia, including Indonesia. Bananas belong to the *Musaceae* family and come in a range of varieties with different appearance colors, shapes, and sizes. The superior banana varieties include Yellow banana, Ambon banana, Barangan banana, Rhino banana, Kepok banana, Milk banana, and Jackfruit banana (Wijayanto et al., 2013). World banana production from around 120 countries is estimated at over 68 million tonnes every year. Banana is the most important fruit type in Southeast Asia, including the first rank in fruit production in the Philippines, Indonesia, and Thailand, both in terms of land area and production (Suhartanto et al., 2012).

Kepok banana (*Musa paradisiaca* L.) is a herbaceous plant originating from the region of Southeast Asia, which thrives and has a uniform distribution throughout the territory of Indonesia. This banana has enough potential to be expanded in Indonesia because the demand for bananas is increasing both for food consumption and for industrial raw materials (Fitramala et al., 2017). This type of banana is popular because it has a delicious taste and contains sufficient nutrition. Also, Kepok bananas have several properties, including as a remedy for intestinal bleeding, mouthwash for tonsillitis, and can improve growth and black hair (Dinastutie et al., 2015). Because of its high commercial value, Kepok banana plants are widely planted. Planting on a large scale faces obstacles because these plants are susceptible to blood diseases caused by the *Pseudomonas solanacearum* bacteria (Supriati, 2010).

Conventional propagation of banana seedlings by using tillers or weevils takes a relatively long time, and it is difficult to get good-quality seeds in large quantities. In each year, mature banana plants only produce as many as 5-10 tillers. Tissue culture techniques are the right solution to overcome the obstacles. The advantages of procuring seedlings by way of tissue culture include obtaining prime plant matter in high quantities and uniforms, in addition to obtaining a sterile culture (parent stock) it can be used as a matter for further propagation (Lestari, 2011). Tissue culture is an attempt to grow cells, tissues, and plant organs on an artificial medium aseptically in a controlled environment. The procurement of seeds in this way is very suitable for large-scale banana businesses (industry). The final stage of plant propagation

Table 1. Effect of organic fertilizer and doses of AMF on the average height of plants at 85 DAP.

Organic fertilizer	Mycorrhiza dosage (g)				Average (cm)
	0	5	10	15	
Goat manure	57.86 de	48.40 c	64.66 e	51.76 cd	55.67 c
Cow manure	50.86 cd	50.96 cd	52.70 cd	51.50 cd	51.50 bc
Vermicompost	49.16 cd	48.93 cd	48.00 c	44.86 bc	47.74 b
Compost	37.83 ab	34.93 a	36.26 a	35.93 a	36.24 a
Average	48.92 a	45.80 a	50.40 a	46.01 a	-

Description: Values in each column and row followed by different letter showed results that differed from Duncan's 5% test.

using tissue culture techniques is plantlet acclimatization. Acclimatization is a process of adjusting the environmental transition from heterotrophic conditions to autotrophic environments in plan plantlets obtained through in vitro techniques. This stage will determine the success of planting in vitro.

Arbuscular mycorrhizal fungi are one type of fungi that are found in the soil and have widespread. Types of mycorrhizae that can affect symbiosis with plant roots without human intervention are called indigenous mycorrhizae. Arbuscular mycorrhizal fungi have a mutualistic symbiosis with the host plant. AMF obtains carbohydrates and other growth elements from the host plant. On other hand, AMF also provides benefits to the host plant in nutrient absorption, so that it can increase plant growth, as a biological barrier to defend root pathogens, increase water availability for plants and increase growth-promoting hormones (Sani and Farahani, 2010). AMF can help plants absorb both macro and micronutrients, especially inbound form and not fit plants. AMF makes plants to absorb more phosphorus from the soil and grow faster compared to plants that do not contain AMF (Nyimas et al., 2011). Nurmasyitah et al. (2013) stated that AMF has substantial potential in increasing the sustainability of agricultural ecosystems through its role in increasing the nutrient cycle of plants and the process of improving soil aggregates.

Organic fertilizer is fertilizer derived from nature, which is the remains of living organisms, both plant remains, and animal remains. Organic fertilizers contain both macro and micronutrient elements that are needed by plants so that they can flourish (Handayani et al., 2011). Organic fertilizers can be in the form of manure, compost, and liquid organic fertilizer. Compost is a solid organic fertilizer made from degraded dead plant remains. This fertilizer is widely sold in the market and can even be produced independently from household waste and other organic waste (Imelda et al., 2014). Improvement of soil properties such as biological, physical, and chemical properties of soil is needed to increase land productivity. Organic fertilizers are known to improve soil properties and increase the productivity of agricultural land. Nugroho et al. (2017) stated that organic fertilizers could improve soil structure by increasing the content of organic matter and increasing the ability of the soil to retain water content. The application of various dosages of AMF and organic fertilizers in this study is expected to support the growth of Kepok bananas resulting from tissue culture.

Results and discussions

Analysis of soil and manure content

The planting media used in the research were soil and fertilizers. The soil used is alfisols or red soil. The Alfisols have undergone intensive weathering and further development. Based on soil chemical analysis (Supplement 1), it is known that alfisol soil has a pH of 6.5 (slightly acidic) as explained in (Winarso, 2005), stating that in general, nutrients are easily absorbed by plant roots at a soil pH of around neutral because under these conditions the nutrients dissolve easily in water. The water capacity is 8.7%. The low water content is due to the texture of the soil at the research location, which is dominated by the fraction of loamy sand. According to (Bintoro and Widjajanto, 2017), soils with sandy or coarse fraction texture has difficulties to hold water, while soil that has a soil texture with clay or fine fraction will easily hold water. According to (Rosyidah and Wirosoedarmo, 2013) soils that have a coarse texture can hold small water. Therefore, plants planted on soil that contain a sandy texture tend to face drought easily. The C-organic found in this soil was 1.26% (low), and the CEC was 19.73 me% (moderate). The results of the analysis show that this soil contains 0.18% n-total (low). According to (Rahmah and Umar, 2014), the high Total-N is caused by the presence of organic matter, which contributes to the soil, 2.04 ppm available P (very low), and 0.11 cmol (+) / kg available K (low). Alfisols soil pH in seven locations in east java and central java showed reactions from acid to neutral, with low C-organic content, very low available P, medium to very high CEC, and high micro-elements (Fe and Zn) (Wijanarko et al., 2007).

Based on Supplement 2, the organic fertilizer that we used had a pH with a range of somewhat alkaline to alkalis. The highest pH condition is found in compost with a value of 8.62 or classified as alkalis. Goat manure, cow manure, and vermicompost have an average pH of 7.8 or tend to be somewhat alkaline. Manure contains many organic materials that can improve damaged soil conditions into fertile and have a diversity of soil microorganisms (Samanhudi et al., 2017a). Organic fertilizers as providers of macro and micronutrients (Samanhudi et al., 2018), enhance the volume of plant roots and increase the value of pH and organic materials of soil (Muktiyanta et al., 2018; Samanhudi et al., 2017b).

The highest water content was in cow manure, which was 12.96%, and the lowest was in compost, which was 10.16%. The highest macronutrient (NPK) was observed in goat fertilizer with a content of 0.95% N, 0.64% P, and 0.50% K. N supply increases chlorophyll content, total protein, sugar content, protein, fat, auxin formation stimulant to soften the cell walls, improve plant's ability in absorbing water, and metabolites associated with photosynthesis (Permana et al., 2018; Riyana et al., 2018; Yunus et al., 2018). The highest organic matter content is found in compost, which is 37.79%, while the lowest was found in goat fertilizer at 22.56%. The highest C/N ratio was found in compost, which was 28.47%. Goat manure, cow manure, and vermicompost fertilizer have a C/N ratio of 13.77%, respectively, 18.98%, and 16.66%. A high C/N ratio indicates that the organic material has not decomposed completely. The results of the analysis showed that the highest CEC was found in compost worth 53.79 me%, and the lowest was in vermicompost at 28.49 me%.

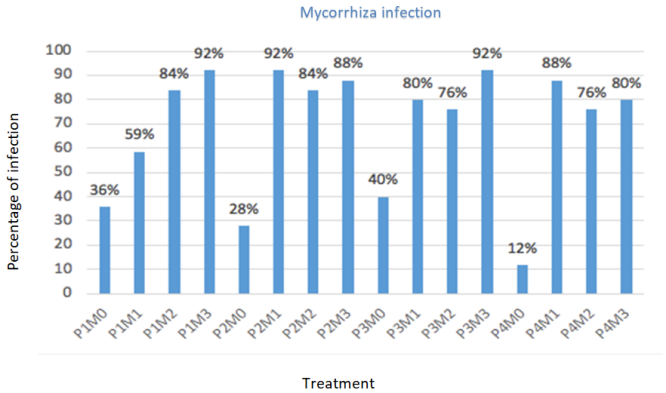

Figure 1. Percentage of mycorrhizal infection in the roots of kepok banana plants.

Table 2. Effect of organic fertilizer and doses of AMF on the average number of leaves at 85 DAP.

Organic fertilizer	Mycorrhiza dosage (g)				Average
	0	5	10	15	
Goat manure	7.33 cd	6.33 abcd	7.66 d	6.00 abc	6.83 b
Cow manure	6.33 abcd	6.33 abcd	6.66 abcd	6.66 abcd	6.50 b
Vermicompost	5.66 ab	6.33 abcd	5.66 ab	5.33 a	5.75 a
Compost	7.00 bcd	6.33 abcd	6.66 abcd	6.66 abcd	6.66 b
Average	6.58 a	6.33 a	6.66 a	6.16 a	-

Description: Values in each column and row followed by different letter showed results that differed from Duncan's 5% test.

Table 3. Effect of organic fertilizer and doses of AMF on average leaf length at 85 DAP.

Organic fertilizer	Mycorrhiza dosage (g)				Average (cm)
	0	5	10	15	
Goat manure	28.23 bc	24.56 b	30.08 c	27.21 bc	27.54 c
Cow manure	24.44 b	25.99 b	26.75 bc	27.71 bc	26.22 bc
Vermicompost	25.36 b	26.04 b	24.66 b	24.23 b	25.07 b
Compost	20.05 a	19.01 a	19.29 a	19.26 a	19.40 a
Average	24.54 a	23.90 a	25.19 a	24.60 a	-

Description: Values in each column and row followed by different letter showed results that differed from Duncan's 5% test.

Mycorrhiza analysis

Mycorrhiza density analysis was carried out to determine the number of spores contained in each gram. In this study, the mycorrhizae was branded Mycogrow, which has a zeolite as a mycorrhizal carrier. The analysis showed that the spore content was 20 spores/5 gram, dominated by the genus *Glomus*. The *Glomus* has an average H-shaped branching hyphae, arbuscular, oval-shaped vesicles, in which chlamydospores, spore colors are white, yellow, and brown (Pulungan, 2013).

Analysis of plant growing medium

Based on the chemical analysis in Supplement 3, the available N content in the growing media is very low. The average treatment composition containing available N was 0.01%. The treatment containing the largest available N was the treatment of cow manure without AMF, which was 0.03%. Nariratih et al. (2013) stated that the loss of N through washing generally happens on coarse-textured soils, with low organic matter content and low Cation Exchange Capacity (CEC) values. Prasetyo et al. (2018) added that deficiency of elemental N could cause several disturbances in plants. Some of them are stunted plant growth and disruption of the chlorophyll-making process, which results in the yellowing of the leaves.

The largest potassium content was found in the treatment of cow manure with 15 g/plant AMF, vermicompost with 15 g/plant AMF, and compost with 15 g/plant AMF, which was 0.31 ppm. The highest available P content was in the treatment of cow manure with 15 g/plant AMF, which was 19.99 ppm, while the lowest was in the treatment of vermicompost with 5 g/plant AMF of 11.68 ppm. Available P content includes moderate to high. The increase in available P content can be caused by giving AMF. Samanhudi et al. (2017c) stated that P nutrient greatly affects plant biomass, and the increase in absorption of this element is influenced by hyphae originating from mycorrhizae.

The average planting medium has a pH range of 6-7, so it is classified as neutral (Supplement 4). The highest pH conditions were found in goat manure treatment with 10 g/plant AMF, which was 7.25, while the lowest pH was in goat manure treatment and without AMF, which was 6.36. The highest organic matter content was found in compost treatment with 15 g/plant AMF worth 5.60%.

Plant height

Plant height is the most visible growth factor to show the vegetative growth of plants. Table 1 shows that the interaction between organic fertilizer treatment and AMF dose did not have a significant effect on the height of Kepok banana plants. The administration of various AMF doses has not been able to have a real effect. This was evidenced by the results that were not significantly different between each AMF dose. Pamungkas and Supijatno (2017) stated that the increase in plant height was due to the nitrogen content, which spurred the growth of the apical meristem, by which

plant grew longer. Leki et al. (2016) stated that compost experienced the slowest growth. It is suspected that there is a difference in the rate of nutrient absorption by plants. The absorption rate of nutrients is influenced by the speed of the decomposition process of each type of fertilizer treatment.

Table 1 shows that the growth of plant height using goat manure is significantly different from the results from the use of compost and vermicompost fertilizer. Goat manure gave the highest average height of the Kepok banana plant, which was 55.67 cm. Compost has the lowest average plant height growth, which is 36.34 cm. It is suspected that there is a difference in the rate of nutrient absorption by plants. Wahyudi (2007) adds that some of the raw materials used to produce compost are rich in nitrogen, but there are obstacles to maintaining it. During the first composting phase, nitrogen evaporates easily and, as a result, cannot be absorbed by the plants, so that plant growth is inhibited.

Number of leaves

The interaction between the two treatments did not have a significant effect on the number of plant leaves (Table 2). The administration of various AMF doses did not have a significant effect on the number of Kepok banana leaves, but the provision of various kinds of organic fertilizers had a significant effect on the number of Kepok banana leaves. Elpawati et al. (2016) stated that organic fertilizers have a good effect on soil physical properties. Adding topsoil has a very positive effect on the physical properties of the soil, maintains soil structure, which is filled with sufficient oxygen, increases water absorption, and can increase the availability of nutrients for plants that are safe for the environment.

The DMRT test (Table 2) shows that the number of leaves on vermicompost treatment was significantly different from other treatments, with the lowest average value of 5.75. Treatment of goat manure, cow manure, and compost showed no significant difference in the number of leaves. The highest average number of leaves was in the goat manure treatment, namely 6.83. Vermicompost has the lowest average number of leaves. This could be because the potassium content in the vermicompost is the lowest. Potassium deficiency is known to inhibit the photosynthesis process in leaves. This can affect the growth of the Kepok banana plant, including the number of leaves.

Leaf length

The analysis of variance showed that the interaction between various kinds of fertilizers and AMF dosage did not significantly affect the length of Kepok banana leaves. The treatment of various types of AMF did not have a significant effect. However, the application of various kinds of organic fertilizers could have a significant effect on plant leaf length. Syafruddin et al. (2016) explained that environmental factors could affect the development of mycorrhizae in infecting plant roots such as soil organic matter, nutrient availability, soil water content, pH, temperature, light intensity, metal brat, and fungicides.

DMRT test (Table 3) shows that goat manure treatment is significantly different from vermicompost and compost fertilizer. Cow manure treatment was not significantly different from goat manure or vermicompost fertilizer. The treatment that gave the longest average yield of leaves was goat manure, with an average of 27.54 cm. The shortest average leaf length was 19.40 cm with compost treatment. This could be caused by the C/N ratio in compost, which was the highest (28.47%). Theunissen et al. (2010) stated that a high C/N ratio causes a reduced biological activity that can produce lower quality.

Leaf width

The leaves function as a place for photosynthesis, respiration, and a regulator of the transpiration process. The greater the width and length of the leaves, the higher the leaf area, the more light the leaves can absorb to carry out photosynthesis. Table 4 shows that the interaction between AMF treatment combinations and organic fertilizers did not have a significant effect on the width of Kepok banana leaves. The treatment of various AMF doses also did not have a significant effect, but the treatment of various kinds of organic fertilizers had a significant effect on plant leaf width. Dawiyah et al. (2018) stated that the establishment of leaves by plants is strongly affected by the availability of macronutrients in the planting medium. The DMRT test (Table 4) shows that goat manure has the highest average leaf width with a value of 13.50 cm. The average leaf width in the treatment of goat manure and cow manure was not significantly different, but significantly different from the treatment of vermicompost and compost manure. The vermicompost and compost also had significantly different mean leaf widths. Compost has the lowest average leaf width (8.55 cm).

Stem diameter

The interaction between AMF and organic fertilizers did not have a significant effect on the diameter of the Kepok banana stem (Table 5). The administration of various AMF doses had not had a significant effect on the stem diameter Kepok banana plant, but the provision of various kinds of organic fertilizers in this study had a significant effect on the stem diameter acclimatized Kepok banana plant. DMRT test (Table 5) shows that cow manure produced an average diameter that is not significantly different from vermicompost, but significantly different from goat manure and compost. Goat manure produced the highest average stem diameter growth of 2.19 cm, while compost showed the lowest average diameter (1.50 cm). This is because goat manure has a higher macronutrient content than other fertilizers.

Arbuscular mycorrhiza fungi infection

Observation of mycorrhizal infections was carried out to determine the symbiosis between AMF and plant roots, which was indicated by the presence of fungal colonization in the roots of the Kepok banana plant. Djodi et al. (2014) stated that application of mycorrhizae may increase growth hormone, preventing infection of pathogens that attack roots and increasing nutrient absorption from the soil. The mycorrhizal doses given were 5 g/ plant, 10 g/plant, 15 g/plant, and without AMF.

From Figure 1, it is observed that the mycorrhizal infection rates for the respective treatments with dosages of 5 g, 10 g, and 15 g are 92%, 84%, and 88%, respectively. The highest percentage of mycorrhizal infections was 92%, namely in the combination of goat manure and 15 g dose of AMF, cow manure, and 5 g dose of AMF, and vermicompost and 15 g dose of AMF treatments. Treatment without AMF dose also showed a mycorrhizal infection in the roots of the Kepok banana plant. Goat manure without AMF showed the percentage of infection was 36%, 28%, 40%, and Compost without AMF 12%. This can be caused by the pathogenesis of the original AMF in the soil because the soil used is not sterilized first. Infected plant roots are characterized by the presence of spores of AMF, which are round and have a dark color. The spores of the genus *Glomus* are characterized

Table 4. Effect of organic fertilizer and doses of AMF on average leaf width at 85 DAP.

Organic fertilizer	Mycorrhiza dosage (g)				Average (cm)
	0	5	10	15	
Goat manure	14.44 ef	11.56 cd	15.34 f	12.64 cde	13.50 c
Cow manure	11.68 cd	12.73 cde	12.56 cde	12.98 de	12.49 c
Vermicompost	10.84 bc	11.24 bcd	11.25 bcd	9.28 ab	10.65 b
Compost	9.36 ab	8.35 a	8.34 a	8.14 a	8.55 a
Average	11.58 a	10.98 a	11.87 a	10.76 a	-

Description: Values in each column and row followed by different letter showed results that differed from Duncan's 5% test.

Table 5. Effect of organic fertilizer and doses of AMF on average stem diameter at 85 DAP.

Organic fertilizer	Mycorrhiza dosage (g)				Average (cm)
	0	5	10	15	
Goat manure	2.18 def	2.28 ef	2.24 f	1.98 cde	2.19 c
Cow manure	1.93 cd	1.79 bc	1.67 cde	1.90 cd	1.89 b
Vermicompost	1.79 bc	1.78 bc	1.91 cd	1.66 abc	1.79 b
Compost	1.65 abc	1.39 a	1.45 a	1.51 ab	1.50 a
Average	1.89 a	1.81 a	1.91 a	1.76 a	-

Description: Values in each column and row followed by different letter showed results that differed from Duncan's 5% test.

by a round and oval spore wall with more than one layer.

The administration of various mycorrhizal doses in this study did not affect all growth parameters of Kepok banana. This shows that each plant has a different response to AMF inoculation, both in terms of the type of AMF given and the dose of AMF given. This is directly proportional to the results of research by Muas et al. (2019), which states that the dose of AMF inoculant at a dose of 5-20 grams/polybag is still not optimal so that its effect in helping the growth of sticky banana seeds has not been observed. Contesa (2010) also reported that AMF (*Acaulospora* sp + *Glomus* sp) inoculation at a dose of 0-100 grams/polybags did not show significantly different results on the height, diameter, number of leaves, and dry weight of FHIA-25 cultivar banana plants aged 3.5 months after acclimatization. This shows that each plant has a different response to AMF inoculation, both in terms of the type of AMF and the dose of AMF given. The effectiveness of each type of AMF depending on the type of AMF itself is also very dependent on the type of plant and its environments, such as pH and soil fertility.

Materials and methods

Plant materials

This research was established in June – October 2019 in the experimental field of the Faculty of Agriculture Universitas Sebelas Maret, Surakarta, Indonesia. The AMF analysis was conducted at the Laboratory of Biology and Soil Biotechnology of UNS. Soil analysis and organic fertilizers are conducted at the soil chemistry and fertility laboratory of the faculty of agriculture UNS. We used 1-month-old Kepok banana plants that have been acclimatized using rice husk ash, humous, and sterile river sand with a ratio of 1:1:1.

Experimental design

The research method used is an experimental method with a completely randomized design of 2 factors. The first factor included goat manure, cow manure, vermicompost, compost. The second factor is without AMF (M0), 5 g/plant AMF (M1), 10 g/plant AMF (M2), 15 g/plant AMF (M3). There was 16 combinations of treatment with three replication, resulting in 48 trial units. The measured variables included plant height, stem diameter, number of leaves, leaf length, leaf width, and mycorrhiza infection.

Statistical analysis

The data obtained were statistically tested with covariance analysis (ANCOVA) based on the f test level 5%, when the real effect was carried out by using DMRT at a rate of 5% to find out the real difference between treatment combinations. The calculation of spores, as well as analysis of mycorrhizal infection, is done descriptively.

Conclusions

The use of organic fertilizers significantly affected all growth parameters of kepok bananas. Goat manure gives a better result than other organic fertilizers on a plant's height, stem diameter, and length of leaves. The application of various doses of AMF either 5 g, 10 g, 15 g, or without AMF has not been able to exert a noticeable influence on the height of the plant, the number of leaves, the width of the leaves, the length of the leaves, and the diameter of the banana stem.

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