Jurnal Kultivasi Vol. 23 (3) December 2024
ISSN: 1412-4718, eISSN: 2581-138x

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Correlation of arbuscular mycorrhizal dosage with level of colonization, nutrient concentration, and photosynthesis pigment of cavendish banana plant

Abstract. The commercial development of Cavendish bananas still faces many obstacles, including the availability of seedlings/suckers at low prices, so technological substitution is needed to stimulate the growth of Cavendish banana suckers in the form of arbuscular mycorrhizal (AM) biological agents. The research aims to determine the correlation between AM doses and colonization levels, tissue nutrient concentrations, and photosynthetic pigments in Cavendish banana seedlings/suckers. It is also a novelty in this research study. The research was carried out in Parepare city at coordinates 3°59'30.204" S; 119°38'42.936" E using four AM doses as independent variables, namely 0 g, 5 g, 10 g, and 15 g pot^{-1}. The variables observed (dependent variables) were the level of colonization, nutrient concentration in the tissue, and photosynthetic pigment content. Using Microsoft Excel software, statistical tests, regression, and correlation analyses were conducted to see the relationship between treatment (independent variable) and observed parameters (dependent variable). The research showed that AM dosage was positively correlated with the level of root colonization, the concentration of N, P, and K elements in the tissue, and the photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids). A dose AM of 15 g pot^{-1} gave a better effect on Cavendish banana suckers, which can be recommended for the development of Cavendish banana seedlings.

Keywords: Chlorophyll · Endomycorrhiza · Infection · Nutrients · Suckers

Submitted: 21 May 2024, Accepted: 27 November 2024, Published: 15 December 2024
DOI: https://doi.org/10.24198/kultivasi.v23i3.54832

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Akib MA, Ilmi N, Risnawati , Prayudyaningsih R, Syatrawati. 2024. Correlation of arbuscular mycorrhizal dosage with level of colonization, nutrient concentration, and photosynthesis pigment of cavendish banana plant. Jurnal Kultivasi, 23(3): 258-272

Introduction

Bananas, as a horticultural crop, are ranked fourth as the most essential food commodity in the world after rice, corn, and wheat (Awa et al., 2024); (Hariyanto et al., 2021); (Tan, 2022). The types of bananas in Indonesia are very diverse, including the Cavendish banana (*Musa acuminata* L.) discovered by William Cavendish (Hariyanto et al., 2021); (Hastuti et al., 2019). This banana comes from Mauritius (East Africa) and was first planted at Chatsworth House (England) in the 1830s with Joseph Paxton. In 1900, Cavendish banana cultivation became increasingly widespread in various places in the world (Bragard et al., 2021); (Justine et al., 2022); (Perrier et al., 2019).

Cavendish bananas have high economic value, especially as an export commodity (Acevedo et al., 2021); (Alho et al., 2021); (Veliz et al., 2022). In 2020, Cavendish bananas were exported to China, Hong Kong, Malaysia, the United Arab Emirates, and Pakistan, with a contribution of 6% to total fresh fruit exports from Indonesia (Nola et al., 2022; Sukmaya et al., 2022). According to Afzal et al. (2022) and Phillips et al. (2021), Cavendish bananas are mostly consumed as fresh fruit and as raw material for making banana flour (Lorenzo et al., 2024; Maseko et al., 2024; Sawant et al., 2023).

Some of the nutritional content found in 100 g of Cavendish bananas is an energy value of 110.15 kcal; Fiber 2.3%; Calcium 12.5 mg; Magnesium 41.5 mg; Iron 0.9 mg; Copper 0.3 mg; Zinc 0.2 mg; Manganese 0.1 mg; Vitamin C 18.5 mg; Protein 0.3 mg; Phosphorus 59.1 mg; Carbohydrates 26.2%; β-carotene 21–290 μg; potassium 400 mg, apart from that also found vitamin A, vitamin B6, riboflavin, folate, fathothanic acid and niacin (Huang et al., 2024; Mustakin et al., 2023; Phillips et al., 2021; Siriwardana et al., 2019; Zein et al., 2023).

Commercial development of Cavendish bananas still faces many obstacles, including providing superior sucker in large quantities at affordable prices. Cavendish banana plants can be cultivated conventionally by separating suckers from clumps or tubers (corm). Still, the number of fully grown banana suckers obtained is only 2 – 3 suckers per parent or 5 – 10 per year (Blende & Kurien, 2015); (Sorn et al., 2024). Another method that can be used to cultivate Cavendish bananas is by dividing the tubers (corm) according to the number of adventitious/axillary buds and plant roots, which is also commonly used as a biological agent (Sapheera et al., 2024; (Tumuhimbise & Talengera, 2018); (Wahab et al., 2023); (Weng et al., 2022). Farmers can easily use this method to get more and faster sucker of bananas. However, this method needs to be supported with technology that can stimulate the root growth of the Cavendish banana. One technology that can be applied is using biological agents.

Mycorrhiza is a form of association between fungi (Figueiredo et al., 2021; Wahab et al., 2023). Using biological agents such as arbuscular mycorrhizal (AM) which colonize plant roots can increase the growth of plant seedling/sucker roots in large quantities to increase nutrient absorption (Gough et al., 2020; Khan et al., 2022); Wahab et al., 2023). Indrawati & Suswati (2019) and Castillo et al. (2016) stated that the application of AM *Glomus* sp. and *Acauluspora* sp. had a positive effect on increasing the growth of the roots of Barangan banana seedlings/sucker cultured in vitro, although the wet root weight did not have a statistically significant effect. In Musa paradisiaca (local variety of Ardhapur), the percentage of mycorrhizal colonization reached 60 to 70%, and the mycorrhizal structures colonizing the root tissue were round and elongated vesicles and arbuscules (Wankhede & Mulani, 2023). These three studies have not stated what dose of AM can have a positive effect on AM activity and the physiological supporting components of Cavendish banana seedlings/sucker. This study aims to determine the correlation of AM dose with colonization level, nutrient concentration in tissue, and photosynthetic pigments in Cavendish banana seedlings/shoots. However, in this study, the hypothesis that can be raised is that there is a positive correlation between AM dose and dependent variables (colonization level, nutrient concentration in tissue, and photosynthetic pigments), which is also new in this study.

Materials and Methods

This research was conducted in the research area of Agroplastid Farm, Bukit Harapan District, Soreang Regency, Parepare City, South Sulawesi, at an altitude of 37 m above sea level with coordinates 3°59'30 "S and 119°38'43 "E.

Preparation of planting media. The sand and husk charcoal are cleaned and sifted before steaming for 8 hours. After cooling, the two-planting media are mixed in a 1:1 ratio and placed in a labeled culture pot.

ISSN: 1412-4718, eISSN: 2581-138x

Figure 1. Preparation of materials for propagating cavendish bananas using tubers.

Preparation of materials for propagating Cavendish banana. Cavendish banana axillary shoots were obtained from banana tubers by dividing the tuber 10 x 10 cm according to the number of axillary shoots. The axillary buds that have been separated are cleaned using running water and then soaked in a disinfectant solution for ± 2 minutes to kill pathogenic microorganisms.

Preparing the AM doses. The AM propagules are weighted according to the treatment. One gram of AM propagule contains carrier media (sand, zeolite, and biochar), root pieces of the host plant, and 80 – 100 units of spores.

The study used four AM doses as treatment (independent variable): without AM application (control), dose 5 g, 10 g, and 15 g pot^{-1} culture. Meanwhile, the variables observed were the level of AM colonization, the concentration of N, P, and K in the tissue, and the content of photosynthetic pigments (dependent variable) after the plants were 30 days old after being incubated with AM.

The level of AM colonization and analysis of AM-colonization roots were carried out at the Environmental Technology and Security (ETS) Section Laboratory, SEAMEO BIOTROP, Bogor. The technique for observing mycorrhizal colonization is root staining, which is done by selecting fine, fresh roots from the roots of sample plants. The roots were placed in a tube containing FAA (formaldehyde, alcohol, acetic acid) solution for 24 hours. The FAA solution is discarded, and the roots are washed until clean. Next, the roots are soaked again in 10% KOH solution for 24 hours. The KOH solution is then discarded, and the roots are washed clean. Next, the roots are soaked in a hot H_2O_2 solution for 24 hours and washed thoroughly. The roots that have been washed thoroughly are soaked in a 2% HCl solution for 24 hours. The HCl solution was discarded, and the fine roots were washed with running water. Next, the roots were soaked in 0.05% trypan blue solution for 24 hours. Then, one root sample with a length of 1 cm was taken from the colored roots and arranged on a glass slide. Root pieces on slides were observed at each angle. The percentage of root infection was calculated using the following formula (Brundrett et al., 1984).

$$\text{Root Colonization}(\%) = \frac{\sum \text{roots area colonization}}{\sum \text{roots area observed}} \times 100$$

The percentage of colonized roots was determined based on the criteria of Rajapakse & Miller (1992) as follows:

– <5% = very low
– 6 – 25% = low
– 26 – 50% = medium
– 51 – 75% = high
– >75% = very high

The N, P, and K concentrations in root, stem, and leaf tissue were analyzed at The Soil Chemistry and Fertility Laboratory, Department of Soil Science, Faculty of Agriculture, Universitas Hasanudin. The N concentration in the tissue was analyzed using the Kjeldhal method, while the P and K concentrations were analyzed using the wet ashing method with HNO_3 and $HClO_4$.

The total content of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) in leaves was analyzed in the Water Productivity and Quality Laboratory, Faculty of Marine Affairs and Fisheries, Hasanuddin University, using the spectrophotometer method with acetone as the solvent.

Data from laboratory tests were analyzed statistically using Microsoft Excel software. Statistical regression and correlation analysis tests were conducted to see the relationship between the treatment (independent variable) and the observed parameters (dependent variable).

Results and Discussion

The results of the analysis of the level of colonization in the root tissue of Cavendish banana seedlings based on the criteria of Rajapakse & Miller (1992) showed that the roots of Cavendish banana

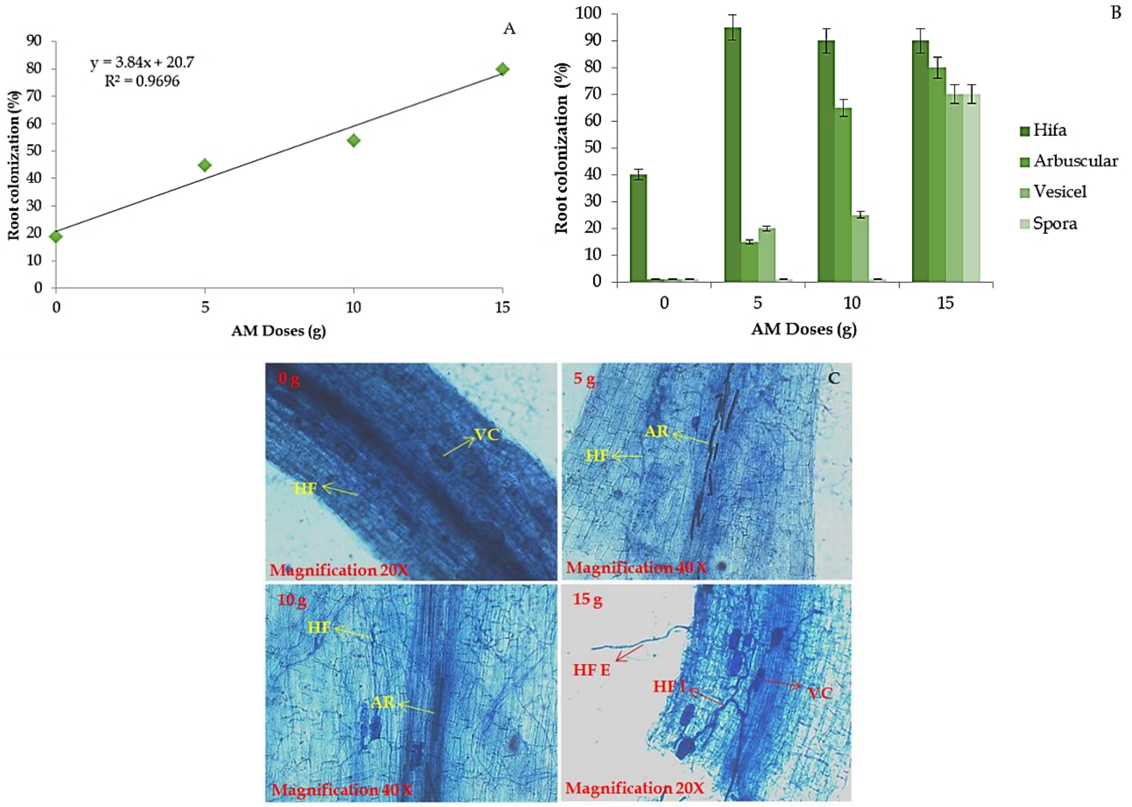

Figure 2. Relationship between AM dose and root colonization at 60 DAT (A), percentage of AM organelle colonization is the average value ± SE from 10 root samples examined; Vertical bars show the Standard Error (SE) value at the 5% level (B); and cross sections of roots of cavendish banana seedlings/sucker infected with AM at 30 DAP with 20 and 40 times magnification (HF, Hyphae; HFE, External Hyphae; HFI, Internal Hyphae; VC, Vesicles; AR, Arbuscula; SP, Spores) (C).

seedlings that were applied with AM at different doses showed AM colonization activity which was in the category of low (6% - 25%) to very high (>75%), and The results of regression and correlation analysis showed that the AM dose treatment had a very strong correlation (R=0.9847) with 97% of the variation in root colonization explained by the AM dose (R^2=0.9696) (Figure 2A). Application of doses of 5 g, 10 g, and 15 g pot^{-1} showed an increase in root colonization activity of 57.78%, 64.81%, and 76.25%, respectively, carried out by AM structures in the form of hyphae, arbuscular, cysts, and spores mainly when AM was applied at a dose of 15 g pot^{-1} (Figures 2B and 2C). The results of correlation analysis in Figure 2A and infection activity in Figures 2B and 2C are in line with the previous observations by Rashad et al. (2021) and Lin et al. (2021) that showed the level of AM (*Gigaspora macrocarpum*) infection reached 69-71% in the root tissue of banana plantlets where AM was applied to a planting medium. Meanwhile, treatment without AM showed the lowest results, namely 0%. This phenomenon occurs because the compatibility between the type of AM and the host plant (Cavendish banana) is not an inhibiting factor in colonizing Cavendish banana roots by AM hyphae. This colonization benefits both of them, namely that AM gets energy from carbohydrates in the form of glucose (simple sugar) from Cavendish banana. Cavendish bananas can increase nutrient absorption with the help of AM hyphae. Previous studies show that banana plants, as a highly mycotropic species, are plants that have a high level of dependence on AM, namely 37%-46% when the plant does not experience stress and increases to 59%-74% when the plant experiences stress (Avila et al. 2022; Dwiyani et al. 2024; Rashad et al. 2021; Ramirez-Silva et al. 2022).

The statistical analysis of nutrient concentrations in Cavendish banana seedling tissue showed a different phenomenon. The mycorrhizal dose factor has a very high/strong correlation (R=0.9502) for nitrogen (N) nutrients and a high/strong correlation (R=0.8186) for

ISSN: 1412-4718, eISSN: 2581-138x

Phosphorus (P) and Potassium (K) nutrients (R=0.8438). Mycorrhizal dose affected 90% of N concentration (R^2=0.9029), 67% concentration of P (R^2=0.6701), and 71% of K (R^2=0.7121) in Cavendish banana seedling tissue (Figures 3A, 3C and 3E). Applying AM doses of 5 g, 10 g, and 15 g pot^{-1} can increase the N concentration in cavendish banana seedling tissue by 8.59%, 9.46%, and 20.17%, respectively (Figure 3A). The highest N concentration was found in leaf tissue, followed by stem and root tissue, especially in the AM treatment dose of 15 g pot^{-1} (Figure 3B).

Doses of 5 g, 10 g, and 15 g pot^{-1} could increase the P concentration in Cavendish banana seedling tissue; respectively, the P concentration increased by 4.55%, 19.23%, and 14.29% (Figure 3C). The highest P concentrations were found in root and leaf tissue in all AM dose treatments (Figure 3D).

The concentration of element K in Cavendish banana seedling tissue increased by 24.12%, 25.33%, and 26.88% with the application of AM doses of 5 g, 10 g, and 15 g pot^{-1} (Figure 3E), and this element accumulates more in plants roots and stems (Figure 3F).

The phenomenon in Figures 3A and 3B is possible that AM performance can increase the N element in the tissue by increasing the number of hyphal structures (Figure 2). AM hyphae can intensify N-fixing microorganisms to provide a source of N in the soil (Dellagi et al., 2020; Ghorui et al., 2024; Jansa et al., 2019). AM reduces N nutrient loss through mineralization by abundant N-fixing

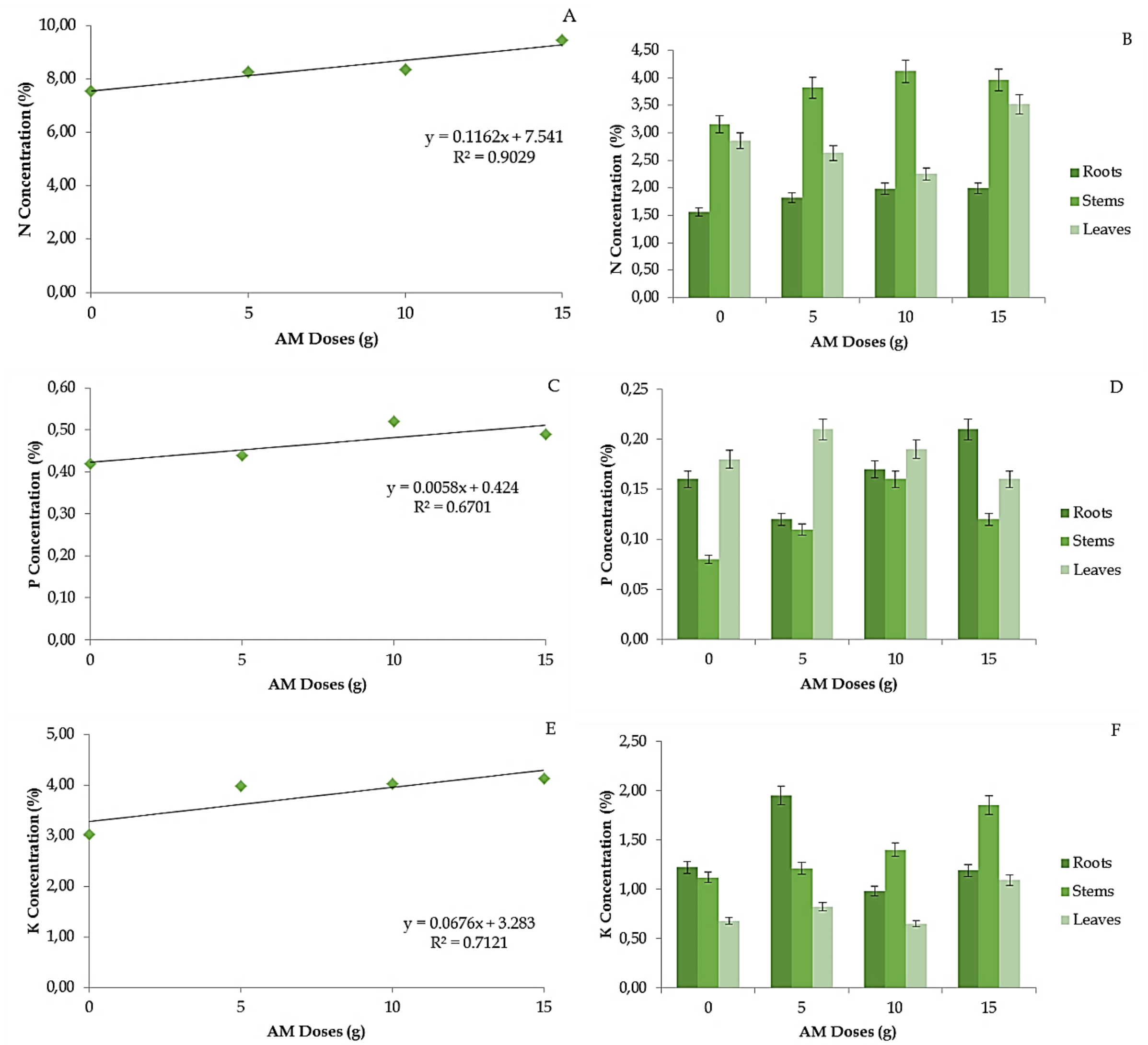

Figure 3. Relationship between AM dose and nutrient concentration in cavendish banana seedling/sucker tissue at 90 DAP (A, C, E). Nutrient concentration values are the average calues ± SE of the 12 samples examined. Vertical bars show the Standard Error (SE) value at the 5% Level (B, D, F).

bacteria due to improved soil structure by AM (Martin & van der Heijden, 2024; Santoyo et al., 2021). This results from the external hyphae of AM, which provide C as an energy source for N-fixing bacteria (Hawkins et al., 2023; Hoosein et al., 2023; Wang et al., 2024). The compatibility of AM with the roots of Cavendish banana seedlings allows the roots to expand the absorption of nutrients by the roots, so the growth of Cavendish banana seedlings can be promoted. Previous studies stated that the N is essential to all living cells (Grzyb et al. 2021; Govindasamy et al. 2023; Zayed et al. 2023)). In plants, N functions as the main constituent component of amino acids, proteins, hormones, chlorophyll a and b, vitamins, and enzymes, which are essential for plant life (Baslam et al., 2021; Fathi, 2022; Nieder & Benbi, 2022). Nitrogen makes up 40%-50% of the dry weight of cell protoplasm, which is the physical basis of living cells (Alharbi et al., 2022; Kerru et al., 2020; Parashar & Jain, 2020). Therefore, N is needed in large quantities to support the plant growth process (Anas et al., 2020; Leghari et al., 2016).

The increase in P concentration in Cavendish banana seedling tissue shown in Figures 3C and 3D is possibly caused by the activity of AM hyphae, which secrete phosphatase enzymes to dissolve P bound in the soil so that it can be available to plants. In plant cells, P is an essential element that plays a role in physiological and biochemical processes, especially in producing, storing, and transferring energy in the form of Adenosine diphosphate (ADP) and Adenosine triphosphate (ATP) (Javed et al., 2022); (Khan et al., 2023); (Xiao et al., 2024). ATP is necessary for plant growth because cell division and increasing the number of cells require energy in the form of ATP (Althaher & Alwahsh, 2023); (Braun, 2020). The research results of Soumare et al. (2021) and Wahome et al. (2023) showed that giving AM gave the best growth response to bananas from in vitro culture after acclimatization. Etesami et al. (2021); Kuila & Ghosh. (2022); Zhao et al. (2024) added that plants infected with AM could produce high P uptake. External hyphae from AM support the increase in P concentration by mycorrhizal infected plants through an increase in surface area, which is more effective in absorbing P elements from the soil to plant roots (Begum et al., 2019; Kuila & Ghosh, 2022; Wahab et al., 2023). Element P is an easily mobile plant nutrient (Doydora et al., 2020; Ibrahim et al., 2022; Khan et al., 2023; Pang et al., 2024). If there is a P deficiency, the P translocation originates from the soil solution, and the older leaves (translocation) go to the roots to be used in root formation (Dixon et al., 2020; Nadeem et al., 2022; Solangi et al., 2023; Zhao et al., 2021). Therefore, P accumulation will increase in the roots during the growth period (Bagyaraj et al., 2015; Chen et al., 2023; Liu, 2021). Several research results show that banana seedlings in symbiosis with AM have a higher growth response and P absorption compared to seedlings without AM. The effectiveness of AM infection inoculated on banana seedlings reaches 23.7%–71.7%, but each type of AM is effective—different infections for each banana seedling (Das & Sarkar, 2024; Etesami et al., 2021; Ferrol et al., 2019; Lin et al., 2021).

The K element in Cavendish seedling/sucker tissue has increased in concentration (Figures 3E and 3F); the increase in K concentration may be the effect of the enhanced P concentration in Cavendish banana seedling tissue. Several previous studies have proposed several hypotheses that increased K accumulation in mycorrhizal plants may be a partial consequence of increased P uptake (Alam et al., 2023; Chandrasekher & Ray, 2020; Luan et al., 2017) and the second hypothesis proposes that K becomes one of the primary counterions of polyphosphates that form K ions (Hashem et al., 2018; Micheluz et al., 2022; Pipatpolkai et al., 2021). Wilkes. (2021) and Kaba et al. (2021) shows that K accumulation is found in spores, hyphae, arbuscular, and AM vesicles and shows that high K concentrations are found in roots. Han et al. (2023) and Mdrid-Delgado et al. (2021) confirmed that the abundance and distribution of K are correlated with the abundance of P in spores at tissue root plants. In AM fungi, P is present mainly as polyphosphate (Petriglieri et al., 2022; Tarayre et al., 2016; Wahab et al., 2023), and it is thought that K acts as a counterion to P in polyphosphate (Christ et al., 2020; Herrmann et al., 2023; Muller et al., 2019) thereby stabilizing the molecule. Calcium polyphosphate deposits previously reported for the AM structure are now believed to be an artifact caused by chemical fixation (Chen et al., 2024; Hazzoumi et al., 2022). The spatial distribution of P and K in spores is closely related (Ji et al., 2022; Xue et al., 2017). No other elements are associated with P in this way, indicating a specific interaction between K and P (Luan et al., 2017; Xie et al., 2021). In addition, the K content tends to increase in spores when the P content increases. Elhindi et al. (2017); Srivastava et al. (2018); Yang et al. (2017) found high levels of P and K in AM vacuoles; their abundance appears to be interrelated. This may be due to the polyphosphate transport mechanism in the vacuolar system.

Mycorrhizal colonization on plant roots can expand the area of root absorption by the presence of external hyphae that grow and develop through root hairs. Hyphae involved in the root system can extend the root absorption range to 80 -100 mm compared to plants without AM (only 1 - 2 mm) and increase the nutrient absorption rate six times faster (He et al., 2020; Hou et al., 2021). Plants infected with AM can absorb higher levels of P fertilizer (10-27%) than plants without mycorrhizae (0.4-13%). Mycorrhizal fungi can replace approximately 27-50% of the use of phosphate, 40-50% of nitrogen and 20-25% of potassium (Prayogo et al., 2021; Rui et al., 2022; Yuwati et al., 2020). Chen et al. (2018) and Tran et al. (2020) explained that mycorrhizal fungi can help absorb nutrients and prevent leaching of nutrients.

Statistical tests on the content of photosynthetic pigments in the leaf tissue of Cavendish banana seedlings showed different phenomena. The AM dosage factor has a high/strong correlation with chlorophyll a pigment (R=0.7105) and a very high/strong correlation with chlorophyll b pigment (R=0.9190) and carotenoids (R=0.9505). Furthermore, the dose of AM affected 50% of the chlorophyll a content (R^2=0.5048), 84% chlorophyll b content (R^2=0.8445), and 90% carotenoid content (R^2=0.9035) in the leaf tissue of Cavendish banana seedlings/suckers (Figures 4A, 4B and 4C). The results of photosynthesis pigment analysis also show that chlorophyll a pigment ($C_{55}H_{72}O_5N_4Mg$) is more dominant than chlorophyll b ($C_{55}H_{70}O_6N_4Mg$) and carotenoids/carotene ($C_{40}H_{56}$) (Figure 4D).

The increase in photosynthetic pigments, as shown in Figures 4A, 4B, and 4C, may be related to the concentration of N in the leaves, the absorption of which is assisted by AM hyphae, thereby accelerating the absorption of light intensity and increasing the photosynthetic capacity of the leaves, which ultimately produces carbohydrates to support AM performance.

According to Begum et al. (2019); Delaeter et al. (2024); Saboor et al. (2021a); Wahab et al. (2023) the symbiosis of AM with roots can increase the chlorophyll content of plants in soil that lacks nutrients, including nitrogen (N). Several research results show that the application of AM (*Glomus mosseae*) can increase the chlorophyll content in *Geranium* (Amiri et al., 2017), *Xanthium italicum* (Shi et al., 2020) and *Zea mays* (Saboor et al., 2021b). Furthermore, Balestrini et al. (2020) and Mathur et al. (2021) explained that the presence of AM will help protect the photosystem II process in the light phase of photosynthesis, which plays a vital role in water absorption and nutrients. However, according to Bell et al. (2024) and Wahab et al. (2023), factors that reduce the photosynthetic capacity of plants will affect the function of AM because fungi in symbiosis are very dependent on the carbon produced by their host plants. Bell et al. (2024); Delaeter et al. (2024); Salmeron-santiago et al. (2022) suggest that AM receive carbohydrates in the form of simple hexose sugars ($C_6H_{12}O_6$) (examples of hexose sugars are glucose and fructose) from the host plant, around 12-27% or 1/3 of the total carbon produced by photosynthesis, and this causes changes in the distribution of photosynthesis—plants to other organs.

The photosynthetic pigment analysis results are dominated by chlorophyll a (Figure 4D), possibly because the leaves of Cavendish banana seedlings are still relatively young, so chlorophyll formation is incomplete. Ebrahimi et al. (2023); Gao et al. (2024); Mulay & Kokate (2019) explain that chlorophyll has not yet formed completely in new and young leaves but is still in the form of protochlorophyll (Protochlorophyll is a green pigment containing magnesium and found in etiolated leaves and seedlings grown in the dark). However, after the transformation of protochlorophyll into chlorophyll, the leaves will become green. Similar results have also been reported for the leaves of *Mangifera indica* (Niaz et al., 2024), *Eleutherine palmifolia* (Ekawati & Saputri, 2022), *Codiaeum variegatum* (Falcioni et al., 2023), *Musa Paradisiaca* (Saha & Zude-Sasse, 2022) and *Syzygium polyanthum* (Adriano et al., 2021). Zhu et al. (2024) and Zhao et al. (2020) stated that apart from genetic factors, chlorophyll formation is also influenced by the quality and quantity of light and Mg^{2+} nutrition as a form and catalyst in chlorophyll synthesis. In plants, about 5–35% of Mg is detected in chloroplasts, and Mg is the central element of the tetrapyrrole ring in chlorophyll (de Bang et al., 2021; Kan et al., 2022). All higher plants contain chlorophyll a and chlorophyll b. Chlorophyll a makes up 75% of the total chlorophyll (Juhaeti et al., 2020); (Veazie et al., 2020) and the re-chlorophyll formation is also influenced by the quality and quantity of light and Mg^{2+} nutrition as a form and catalyst in chlorophyll synthesis.

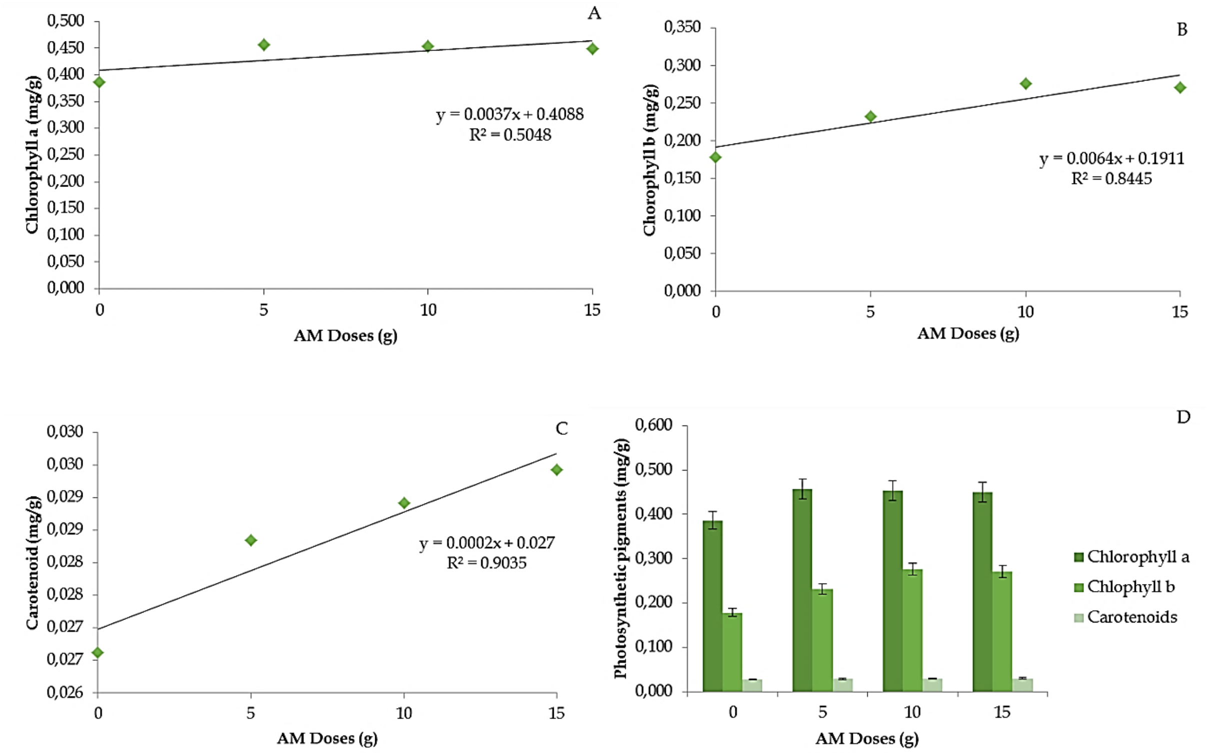

Figure 4. Relationship between AM dose and photosynthetic pigment content in cavendish banana seedling/sucker tissue at 90 DAP (A, B, C). Photosynthetic pigment content is the average value ± SE of the 12 leaf sections examined. Vertical bars show the Standard Error (SE) value at the 5% level (D).

In plants, about 5–35% of Mg is detected in chloroplasts, and Mg is the central element of the tetrapyrrole ring in chlorophyll (de Bang et al., 2021); (Kan et al., 2022) constructing 25% makes up chlorophyll b and other pigments. The chlorophyll content in green plants is around 1% of dry weight (Arshad et al., 2023; Veazie et al., 2020).

Conclusion

The AM dosage is positively correlated with the level of root colonization, the concentration of N, P, and K elements in the tissue, and the content of chlorophyll a, chlorophyll b, and carotenoid pigments as a means of photosynthesis. This has a better effect on the growth of Cavendish banana seedlings/shoots, especially at an AM dosage of 15 g pot^{-1}, so it can be recommended for plant breeders in the propagation of Cavendish banana seedlings.

Acknowledgments

The research team would like to thank the Ministry of Education, Culture, Research, and Technology for supporting research funding in 2023. This research is an inseparable part of the "AM Colonization Test in Different Dosage Forms on Various Plants of C3, C4, and CAM".

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