Scientific Bulletin. Series F. Biotechnologies, Vol. XXIX, No. 2, 2025
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND MYCORRHIZAL HELPER BACTERIA DOSES ON *IN VITRO* POTATO PLANTLET PROPAGATION

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Abstract

The procurement and distribution of certified seed potatoes, which have not met the demand, is one of the challenges in potato production. In vitro culture propagation is one of the methods that can produce high-quality potato seeds. Potato seedling propagation can be optimized through maintenance using biological fertilizers Arbuscular Mycorrhizal Fungi (AMF) and Mycorrhizal Helper Bacteria (MHB). The study aims to see the growth response of several doses of FMA and MHB in the propagation of potato seedlings in vitro. The experiment used a factorial completely randomized design (CRD) with 9 treatments repeated three times. AMF application consisted of doses without AMF, 2.5 g AMF, and 5 g AMF. MHB application consisted of doses without MHB, MHB 1 × 10^9 cfu ml^{-1}, and MHB 1 × 10^{11} cfu ml^{-1}. The result showed no interaction between AMF and MHB on plant height, number of leaves, and number of shoot buds. The doses of AMF and MHB given in the study were not effective because they could not increase plant growth.

Key words*: mycorrhiza, in vitro, Helper Bacteria, potato.*

INTRODUCTION

Potato is one of the most important agricultural commodities in Indonesia due to its numerous benefits and high economic value. It is a tuberous annual herbaceous vegetable with significant nutritional content in 100 grams of tuber, including energy, carbohydrates, protein, fiber, folate, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, vitamins A, C, and K, as well as sodium, potassium, calcium, iron, magnesium, manganese, phosphorus, and zinc (USDA, 2022). As a carbohydrate source, potatoes have great potential as a rice substitute. This is evidenced by the increasing variety of potato-based food products in Indonesia, leading to a growing demand for potato raw materials.

According to data from the Central Statistics Agency (BPS), potato production in Indonesia reached 1,282.77 tons in 2020, 1,361.06 tons in 2021, and increased to 1,504 tons in 2022. This continuous increase in production over the past three years is also supported by the rise in household consumption. In 2022, BPS recorded household potato consumption at 874,250 tons, an increase of 13.32% (102.79 tons) from the previous year's 771,460 tons. Therefore, improving potato productivity is necessary to maintain production stability.

Several challenges hinder potato production, such as the limited availability of land with suitable altitude and temperature (Wattimena, 2000). One proposed solution is to expand cultivation to medium-altitude areas, which range from 350 to 700 meters above sea level, with daytime temperatures reaching 35°C and nighttime temperatures around 25°C. However, high temperatures in such areas often result in smaller tuber sizes. Another major challenge is the insufficient supply and distribution of certified potato seed, primarily due to the high cost compared to farmer-produced seeds (Lestari et al., 2018).

Seed quality is a crucial factor in the production process. Therefore, proper selection

and specific treatment are essential to produce superior seed. The use of high-quality seed can enhance both yield quantity and quality. One effective method to produce high-quality potato seed is through in vitro culture propagation. In vitro culture is a technique used to grow and develop living cells or tissues into complete plants (Dwiyani, 2015). This method offers several advantages, including rapid and uniform growth, genetic consistency with the parent plant, and freedom from viruses (Putri et al., 2021). In addition to plant propagation, tissue culture is also used for secondary metabolite production (Fadilah, 2023). Potato seedling propagation can be further optimized through maintenance practices such as the use of biofertilizers.

Biofertilizers consist of living microorganisms that colonize plant tissues or the rhizosphere and, when applied to seeds, soil, or plant surfaces, enhance nutrient availability and promote plant growth (FNCA, 2006). These fertilizers improve nutrient use efficiency, supply macro- and micronutrients, enhance enzymatic activity, and ultimately promote plant growth and yield (Roupahim et al., 2016). Commonly used biofertilizers include Arbuscular Mycorrhizal Fungi (AMF) and Mycorrhizal Helper Bacteria (MHB).

AMF forms mutualistic symbioses with approximately 90% of plant species (Husna et al., 2021), including many horticultural crops. These fungi support plant growth by improving nutrient uptake and enhancing resistance to drought, diseases, and other stress conditions (Widhiantoro & Slameto, 2023). Additionally, AMF plays important roles in nutrient cycling, carbon transport in the root system, phytoremediation, and disease protection (Sianturi et al., 2015).

The mechanism involves infection of the root system and extensive hyphal development, which increases nutrient absorption capacity, especially for phosphorus (P), facilitated by phosphatase enzymes and organic acids secreted by AMF (Islamiyah et al., 2017). Furthermore, AMF in the rhizosphere enhances bacterial activity and supports the growth of bacteria associated with fungal hyphae (Hidayat et al., 2013). According to Barea et al. (2005), AMF in the rhizosphere interacts with various bacteria, including plant growth-promoting rhizobacteria (PGPR), nitrogen-fixing bacteria, and phosphate-solubilizing bacteria, with MHB among the microbes that stimulate hyphal growth.

Mycorrhizal Helper Bacteria (MHB) assist AMF in performing their functions. They promote root receptivity to AMF, facilitate the recognition process between AMF and plant roots, enhance spore germination and fungal growth, and mobilize nutrients in the rhizosphere (Rigamonte et al., 2010). A bacterium is considered an MHB if it resides within AMF structures and contributes to their function (Handayani, 2015). An example is Pseudomonas diminuta, a well-known MHB species (Rigamonte et al., 2010). These bacteria promote AMF growth and help plants absorb essential nutrients like phosphorus and nitrogen (Hidayat, 2013).

Currently, limited research exists on the application of AMF and MHB in in vitro propagation. Therefore, this study aims to investigate the growth response of in vitro potato seedlings treated with AMF and MHB.

MATERIALS AND METHODS

Time and Location

The research was conducted from January to May 2024 at the Tissue Culture Laboratory of Seed Technology and the Soil Biology Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor District, Sumedang Regency, West Java.

Materials and Equipment

Sterilization equipment included an autoclave and a hot air oven. Tools used to prepare the media solution included measuring cylinders, hot plates, analytical balances, beakers, pH meters, test tubes, glass stir rods, spatulas, test tube racks, magnetic stirrers, aluminum foil, plastic wrap, and rubber bands. Tools for plantlet transplantation included a Laminar Air Flow (LAF) cabinet, Bunsen burner, fire starter, media-filled test tubes, test tube racks, Petri dishes, scalpels, scissors, and sprayers. For culture storage, culture racks equipped with thermohygrometers and fluorescent lamps were used. Supporting tools included labeling paper, rulers, and documentation equipment.

The plant material used was Atlantic potato plantlets obtained from the Agricultural

Training Center (BBPP) Lembang. The media used was Modified Strullu Romand (MSR) medium (MSR - Declerck et al., 1998, modified from Strullu & Romand, 1986), made from stock solutions. Other materials included, sucrose ($C_{12}H_{22}O_{11}$) obtained from Merck KGaA (Darmstadt, Germany; Cat. No. 107651), sterile distilled water, 70% alcohol from Jayamas Medica Industri, Sidoarjo, East Java, Indonesia, is a registered facility with the U.S. Food and Drug Administration, 0.1 N NaOH from EMSURE is a registered trademark of Merck KGaA, Darmstadt, Germany, 0.1 N HCl from EMSURE Merck KGaA, Darmstadt, Germany, AMF (*Glomus* sp., *Gigaspora* sp.) with a density of 10 spores/g, and *Pseudomonas diminuta* MHB at concentrations of 1×10^{11} cfu ml^{-1} and 1×10^{9} cfu ml^{-1}, obtained from the Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran.

Experimental Design

The experiment employed a factorial Completely Randomized Design (CRD) consisting of two factors: AMF dosage (f) and MHB density (m). A total of 9 treatment combinations were tested with 3 replications each, yielding 27 plant samples.

Factor 1: AMF dosage (f):

f_0 = Without AMF (control);

f_1 = AMF 2.5 g;

f_2 = AMF 5 g.

Factor 2: MHB density (m):

m_0 = Without MHB (control);

m_1 = MHB 1×10^{9} cfu ml^{-1};

m_2 = MHB 1×10^{11} cfu ml^{-1}.

Equipment Sterilization

Glassware such as Petri dishes and test tubes were sterilized using a hot air oven. Before sterilization, equipment was wrapped in paper and heated at 160-180 °C for 1.5 to 3 hours. Liquids such as sterile distilled water and prepared media solutions were sterilized using an autoclave.

Media Preparation

The MSR (Modified Strullu Romand) medium was prepared using stock solutions. Chemical components were weighed and dissolved in sterile distilled water, then homogenized. The pH was adjusted to 5.6-5.8, followed by heating on a hot plate. Once the solution reached boiling, it was poured into test tubes and sterilized in an autoclave at 121 °C for 15 minutes.

Plantlet Culturing in Test Tubes

Plantlet transfer was conducted inside a LAF cabinet in an aseptic environment. Instruments such as scissors, forceps, and Petri dishes were disinfected using 95% ethanol and flame sterilized. Each test tube contained one potato plantlet measuring 1-3 cm, totaling 54 plantlets.

Inoculation of AMF and MHB

AMF and MHB were inoculated after plantlets had formed roots, approximately one month after transplanting. AMF was added according to the treatment (2.5 g and 5 g), and MHB was added at 1×10^{9} cfu ml^{-1} and 1×10^{11} cfu ml^{-1} in 3 ml volumes. Tubes were sealed with plastic wrap and rubber bands and then stored in the culture room. An illustration of the treatment setup is shown in Figure 1.

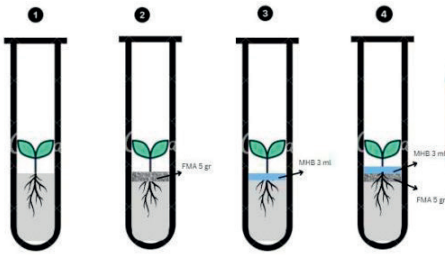

Figure 1. Treatment set up of Application of AMF and MHB *in vitro*

Observational Variables

Observations were divided into two types: primary and supporting. Primary variables were statistically analyzed and included plantlet height, number of leaves, and number of shoots, measured weekly from Week 1 to Week 3 after inoculation. Supporting observations included room temperature and humidity, phosphatase enzyme activity, indole-3-acetic acid (IAA) hormone concentration, and explant survival rate, also monitored weekly for three weeks.

RESULTS AND DISCUSSIONS

Phosphatase Enzyme Activity and Auxin Hormone (IAA) Content

Phosphatase enzymes and auxins play critical roles in plant growth and development. As

shown in Figure 2, AMF treatment resulted in the highest phosphatase activity (3.33 µg pNP g^{-1} h^{-1}) compared to other treatments. This is because AMF can produce phosphatase enzymes that convert complex organic phosphorus-unavailable to plants-into inorganic forms that can be absorbed by hyphae and translocated to plant tissues (Abbott & Robson, 1984; Prihantoro et al., 2023).

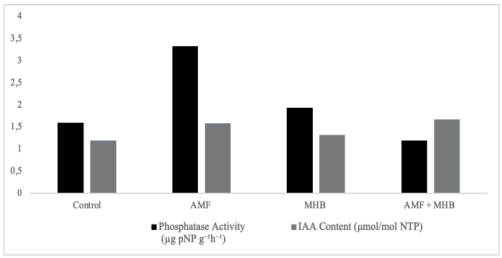

Figure 2. Phosphatase Activity and IAA Content Across Treatments

The plant hormone IAA is a type of auxin involved in promoting cell elongation and increasing DNA and RNA synthesis. Although the IAA content was similar across treatments, the highest concentration (1.67 µmol/mol NTP) was observed in the combination of AMF and MHB treatments. Wang et al. (2021) noted that AMF can increase IAA levels in tomato roots. Additionally, *Pseudomonas* bacteria are known to actively produce IAA (Nurbaity et al., 2024), which likely explains the elevated IAA levels in the combined treatment.

Survival Rate of Explants

The survival rate of explants is influenced by the ability of explants to absorb nutrients and hormones from the culture medium, as well as by contamination, which can cause plantlet mortality. The overall survival rate across all treatments was 62.9%, with 17 explants surviving. The highest survival rates (100%) were observed in the control, MHB 1 × 10^9 cfu ml^{-1}, and MHB 1 × 10^{11} cfu ml^{-1} treatments. The lowest survival rate (33.33%) occurred in the AMF 2.5 g, AMF 2.5 g + MHB 1 × 10^{11}, AMF 5 g, and AMF 5 g + MHB 1 × 10^{11} treatments (Figure 3).

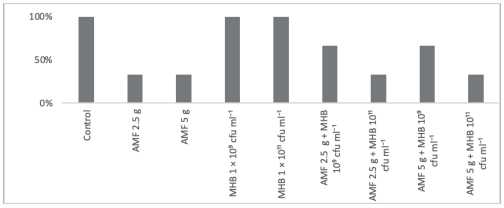

Figure 3. Explant Survival Rate Across Treatments

Plantlet Height

Plant height is a key indicator for assessing plant response to treatment, as it reflects changes in size and dimension. Based on the analysis of variance (ANOVA), no significant interaction was found between AMF and MHB treatments on plantlet height (Table 1).

Table 1. Independent Effects of AMF and MHB on Potato Plantlet Height

Treatment	Average Plantlet Height (cm)
AMF Dose	
f0 (Control)	5.79
f1 (2.5 g)	5.41
f2 (5 g)	4.66
MHB Dose	
m0 (Control)	5.10
m1 (1×10^9)	5.50
m2 (1×10^{11})	5.67

Note: Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Both AMF and MHB treatments resulted in lower plantlet heights compared to the control. Although independent effects were observed at 3 WAI, the control consistently showed greater height. A slight increase in height was observed in 7.8% at 1 WAI and 0.6% at 2 WAI and 11.1% at 1 WAI compared to no MHB.

The independent effect observed in the control may be attributed to MHB's ability to produce auxins, which stimulate plant growth. Hidayat et al. (2013) noted that MHB can produce or modulate concentrations of phytohormones such as gibberellins, cytokinins, ethylene, and indole-3-acetic acid (IAA), a primary auxin. This aligns with findings from Nurbaity et al. (2024), which demonstrated that MHB treatments yielded the highest IAA levels compared to AMF.

Lower plant height in AMF treatments may be explained by its role in increasing phosphatase activity (Muis et al., 2016). The elevated enzyme activity is likely a response to phosphorus solubilization, rather than a direct contribution to nutrient uptake for plant growth (Qin et al., 2019). Moreover, AMF may not have germinated optimally or formed effective symbiosis with the plantlet roots under sterile *in vitro* conditions (Nurbaity et al., 2024).

Unlike natural soil environments, sterile culture media lack microbial interactions essential for effective AMF functioning. According to Ralle et al. (2021), AMF effectiveness is influenced by environmental factors such as host interactions and microbial communities. The absence of these interactions in a sterile medium may reduce AMF efficacy (Miransari, 2011).

Thus, the applied doses of AMF and MHB in this study were not at optimal levels to promote significant increases in plantlet height.

Number of Leaves

The number of leaves was counted manually for each plantlet. Analysis of variance at the 5% level showed no significant interaction between AMF and MHB doses on the number of leaves from week 1 to week 3 after inoculation. However, a significant independent effect was observed in weeks 2 and 3 under the AMF factor, specifically in the control treatment (Table 2).

Table 2. Independent Effects of AMF and MHB on the Number of Leaves of Potato Plantlets

Treatment	Average Number of Leaves
AMF Dose	
f0 (Control)	11.2
f1 (2.5 g)	6.5
f2 (5 g)	5.8
MHB Dose	
m0 (Control)	9.9
m1 (1×10^{9})	7.5
m2 (1×10^{11})	7.9

Note: Means followed by the same letter in a column are not significantly different (Duncan's Multiple Range Test, $\alpha = 5\%$).

Both AMF and MHB treatments resulted in fewer leaves compared to the control.

Treatments with 2.5 g and 5 g AMF showed reductions of 47% and 53%, respectively, while MHB treatments at 1×10^{9} and 1×10^{11} cfu ml^{-1} showed decreases of 66% and 20% compared to the control.

The independent effect in the control may be attributed to reduced competition for nutrients in the absence of AMF. Since all organisms require nutrients for growth, limited nutrient availability may inhibit microbial growth (Hajoeningtijas, 2012). The MHB treatments showed better performance than AMF, consistent with the role of MHB in promoting plant growth and health (Yadav, 2015).

The decline in leaf number in AMF and MHB treatments may also be related to high IAA levels (Figure 2), which can inhibit leaf growth when exceeding optimal concentrations (Kou et al., 2022). Auxin-induced ethylene production can further inhibit growth (Abeles et al., 1992; Iqbal et al., 2017), as demonstrated in G. aparine, where auxin application increased ethylene and abscisic acid (ABA) levels, thereby suppressing growth (Hansen & Grossmann, 2000).

During the study, some plantlets-particularly those treated with AMF-showed signs of abnormality, such as yellowing and even death. Koukounaras et al. (2007) stated that ethylene accelerates leaf senescence, which is often characterized by chlorophyll degradation. This finding is supported by Giel and Bojarczuk (2002), who noted high levels of leaf chlorosis were associated with increased auxin concentrations.

Leaf yellowing reduces chlorophyll production, directly impairing photosynthetic efficiency. Plants lacking chlorophyll cannot effectively absorb sunlight, thus disrupting photosynthesis. Yama and Kartiko (2020) stated that higher chlorophyll levels enhance photosynthetic activity, which positively affects plant growth parameters such as height and leaf number.

Since photosynthesis produces assimilates that serve as energy for plant growth, impaired photosynthesis reduces the availability of assimilates, consequently limiting energy for growth (Apriliani et al., 2016).

Leaf number is also closely associated with plant height, as leaves develop from nodes along the stem. Taller plants tend to produce more leaves due to the presence of more nodes (Hartanti et al., 2019). This observation is consistent with the results of this study.

Therefore, the application of AMF and MHB doses in this study was not effective in increasing the number of leaves in potato plantlets.

Number of Shoots of Plantlets

The number of shoots per plantlet was counted manually. Based on the analysis of variance, there was no significant interaction between AMF and MHB on shoot number. However, a significant independent effect was found in the AMF factor, particularly in the control treatment.

The highest number of shoots was observed in control with a mean of 2.59 shoots, while the lowest was in 5 g AMF, as shown in Table 3. In general, higher AMF doses corresponded to lower shoot counts.

Table 3. Independent Effects of AMF and MHB on the Number of Shoots of Potato Plantlets

Treatment	Average Number of Shoots
AMF Dose	
f0 (Control)	2.59 b
f1 (2.5 g)	1.43 a
f2 (5 g)	1.36 a
MHB Dose	
m0 (Control)	1.96
m1 (1×10^{9})	1.74
m2 (1×10^{11})	1.68

Note: Means followed by the same letter are not significantly different (Duncan's Test, $\alpha = 5\%$).

The low shoot numbers may be attributed to auxin levels that inhibit growth at high concentrations. This inhibition may occur through auxin-induced ethylene synthesis (McGaw & Burch, 1995). Furthermore, high auxin concentration in the apical meristem can promote apical dominance, suppressing lateral bud outgrowth (Makmur, 2020). Although plant height and leaf number increased in some treatments, this did not translate into higher shoot production.

Shoot development is related to leaf formation and plant height. According to Banurea et al. (2017), the increase in branch number correlates with photosynthesis efficiency in leaves, and since shoots initiate branches, increased shoot number is influenced by improved photosynthesis.

Thus, excessive IAA at the shoot apex suppresses lateral shoot development, suggesting that auxin-induced apical dominance plays a key role. Furthermore, AMF and MHB doses used in this study were not efficient, as they did not result in better growth or yield than the control.

CONCLUSIONS

This study demonstrated that the application of Arbuscular Mycorrhizal Fungi (AMF) and Mycorrhizal Helper Bacteria (MHB), either individually or in combination, did not produce a significant effect on the height, leaf number, or shoot number of in vitro-propagated potato plantlets. Although AMF enhanced phosphatase enzyme activity and MHB contributed to auxin (IAA) production, these physiological responses did not translate into improved plantlet growth. The results suggest that the doses used, as well as the sterile in vitro environment, may not have supported effective microbial colonization or plant–microbe symbiosis. Further research is needed to optimize conditions for their beneficial interaction.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Education, Science and Technology Republic of Indonesia Research Grants (Penelitian Fundamental Reguler Kemenristekditi) 2024.

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