

## RESEARCH ARTICLE

# Interactive effect of arbuscular mycorrhizal fungi (AMF) and transplanting media improves early growth, physiological traits, and soil nutrient status of coconut ‘Bido’ under tropical monsoon climate

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## ABSTRACT

Support for the development of sustainable coconut (*Cocos nucifera* L.) plantations is important. The ability of arbuscular mycorrhizal fungi (AMF) to enhance plant growth has long been recognized. However, AMF's role in transplanting media for the early growth of coconut is not well researched. The purpose of this study was to investigate the effects of AMF and various transplanting media on plant growth and soil nutrient status. A factorial randomized block design was used, with a three levels dose of AMF inoculum as the initial factor. The second factor was the composition of the transplanting media, specifically the combination of manure (PK), sawdust (SG) and sand (P) at three different levels. The research was conducted in North Maluku, Indonesia, from 2019 to 2022. The measured plant attributes were plant growth from 6 to 30 m after planting (MAT), some physiology traits, and soil nutrient status. The results indicated that the combination of 4 g AMF inoculum seedling<sup>-1</sup> with media composition of PK:SG:P = 1/3:1/3:2/3 has resulted in significant improvement ( $p < 0.01$ ) in stem circumference, leaf midribs number, leaf midribs length, plant height and availability of soil nutrients in terms of N (214.2%), P (78.5%), and K (88.9%). The activity of leaf nitrate reductase also increased significantly ( $p < 0.01$ ) by 79.5%, as well as chlorophyll *a* ( $p < 0.01$ ) and total chlorophyll content ( $p < 0.01$ ) which increased by 40.6% and 49.1% respectively. The combination of AMF inoculation and transplanting media represent a sustainable agronomic technology that can enhance the optimal nutritional status and promote the early growth of coconut palms.

**Key words:** AMF inoculation, coconut, growth, physiology, nutrient status, transplanting media.

## INTRODUCTION

Planting media plays a crucial role in ensuring plant growth and optimizing production (Popescu and Popescu, 2016). However, suboptimal growth of coconut (*Cocos nucifera* L.) plantations in tropical regions, including Indonesia, is often faced with a challenge, primarily due to factors such as low soil fertility, inadequate nutrient management, and damaged plants (Santos et al., 2022; Kumar and Kunhamu, 2022).

Due to abiotic stress conditions such as low soil fertility (pH = 5.28) and land flooded when it rains due to poor drainage which leads to the leaching of nutrients, optimal coconut growth is facing significant

challenges in the North Maluku region. This area represents an important part of the coconut plantations, occupying approximately 61.7% of the total land area dedicated to plantation commodities (352.142 ha). It is worth noting that all coconut plantations in North Maluku are owned by smallholders (BPS-Statistics of Maluku Utara Province, 2019; Directorate General of Estate Crops, 2020). Additionally, Indonesia ranks as the world's fifth-largest coconut producer, trailing behind India, the Philippines, Sri Lanka, Thailand, and Malaysia. Coconut plantations in Asia and Southeast Asia together contribute to 80% of the global coconut production area (Pham, 2016).

Indonesia's coconut production, is facing challenges due to the decline in productivity. From 2013 to 2019, the coconut productivity decreased from 1150 kg ha<sup>-1</sup> in 2013 to 1114 kg ha<sup>-1</sup> in 2019 (Directorate General of Estate Crops, 2020). One of the factors contributing to this decline is the limited support for technological innovation in cultivation, particularly in small-scale plantations. Smallholders have a significant stake in 99.06% of coconut plantations in Indonesia according to Statistical of National Leading Estate Crops Commodity 2019-2021 (Directorate General of Estate Crops, 2020).

The decline in coconut productivity is also caused by pest factors such as the "coconut fruit scab" caused by the mite, *Aceria guerreronis* Keifer (Tzec-Simá et al., 2022). Likewise, the pests *Rhynchophorus* spp., *Cyrtotrachelus* sp., and *Oryctes rhinoceros*, as well as infectious diseases as bud rot disease (BRD) and nut fall disease (NFD) which are caused by *Phytophthora palmivora*, causing a decrease coconut productivity in North Sulawesi, Indonesia by 25.0% (Hosang et al., 2023).

This condition requires technological control efforts through integrated cultivation, such as intercropping, resistant cultivars, and increasing organic matter in the soil to maintain a good fertilization regime (Tzec-Simá et al., 2022). In addition, inoculation of arbuscular mycorrhizal fungi (AMF) is used as a biocontrol agent in controlling plant diseases (Dey and Gosh, 2022). Dey and Gosh (2022) stated that AMF improves plant immunity and control of plant pathogens through a mycorrhiza-induced resistance (MIR) mechanism. Thus, an increase in colonization reaching 41.50% in AMF inoculation of coconut growth (Sulistiono et al., 2020) will increase resistance to pests and diseases. This was confirmed by Nurzannah et al. (2022) that the application of AMF inhibits attacks of basal stem rot (BSR) disease caused by *Ganoderma boninense* in oil palm nurseries by 36.1%.

In order to address these challenges, there is a pressing need to implement technological innovations that can optimize growth and production. Technological innovations can help improve coconut production by selecting superior varieties and optimizing transplanting media treatments. According to Sulistiono et al. (2021), utilizing the technology of superior varieties improves physiological characteristics and early growth of robusta coffee.

Indonesia has several superior cultivars of coconut, including 'Bido' from Morotai Island, North Maluku. The 'Bido' coconut is well-known for its substantial fruit size (2.508 g per fruit), with an average of 9.47 fruits per bunch and 133 fruits per tree. Short stem length, closely spaced leaf marks, early fruiting, and high fruit production (Anonymous, 2017) are among the desirable features of this plant. However, the development of plantations has been limited, with most cultivation primarily limited to local planting locations.

The development of superior traits in coconuts across different environments relies on the influence of transplanting media and cultivation treatment. Research by Nirukshan et al. (2022) found that a dry condition application of arbuscular mycorrhizal fungi (AMF) resulted in increased early growth of coconuts and enhanced P absorption. Similarly, Sulistiono et al. (2020) report that the combination of AMF inoculation (commercial species) with NPK fertilizer during the transplantation of coconut seedlings, results in a 41.50% increase in AMF colonization, as well as improved nitrate reductase activity and root surface area under tropical monsoon climate, Indonesia. Additionally, AMF inoculation has increased the survival and growth of micro-propagated coconut plantlets (Gómez-Falcón et al., 2023). It was also reported by Senarathne and Ilangamudali (2018) that the application of AMF-based biofertilizer increased shoot growth (leaf and stem circumference) and roots (dry weight and volume) of coconut seedlings in the nursery.

The AMF inoculation was more effective in improving plant survival with a mixture of arbuscular mycorrhizal fungi than single-species inoculants. Gómez-Falcón (2023) stated that the use of commercial

AMF (consists of several genera) resulted in better growth in plant height, stem diameter, and leaf area of *C. nucifera* plantlets compared to control and native AMF.

However, the effect of AMF inoculation and transplanting media on the early growth of coconuts under field condition remains largely unknown and has not been the subject of numerous reports. So far, research in the field shows that fertilizing a combination of organic chemicals with organic fertilizer (vermicompost, green leaf fertilizer) increased coconut nuts per year (Shinde et al., 2021).

It is therefore essential to conduct studies on the use of superior coconut cultivars coupled with innovative cultivation technologies, such as AMF inoculation and optimal composition of transplanting media. The purpose of this research was to investigate the impact of these technological innovations on physiological traits, plant growth, and the nutritional state of the soil. The findings from this research could reveal new advances in coconut cultivation technology. Development of precise cultivation techniques is urgently required to rejuvenate existing plantations and enhance coconut productivity.

## MATERIALS AND METHODS

This research was conducted from January 2019 to January 2022 in the plantation area of the Assessment Institute of Agricultural Technology (AIAT) of North Maluku (0°41'38.17" N; 127°33'15.18" E), Indonesia. The soil type of the research site is Alluvial. This type of soil with loam to sandy loam texture, dark brown to yellowish brown soil color, rounded loamy soil structure, and very sticky soil consistency when wet. C-organic is classified as very low, and base saturation is low  $\leq 50\%$  (24.35%) (Gayo et al., 2022). These sites fall under the classification of tropical monsoon climate with humid conditions, as per Schmidt-Ferguson's classification ( $0.143 < Q < 0.333$ ). The 2021 monthly precipitation recorded was: January 306.6 mm, February 927.9 mm, March 839.4 mm, April 1154.5 mm, May 931.4 mm, June 621.8 mm, July 1119.2 mm, August 993.4 mm, September 632.5 mm, October 832.3 mm, November 861.2 mm and December 945.4 mm. In 2021, the average monthly temperature was 27.1 °C, with a maximum of 30.9 °C and a minimum of 24.0 °C, with an average sunshine duration of 43.53% (BMKG-Stasiun Geofisika Ternate, 2021).

### Environmental design and treatments

The experiment employed a factorial randomized complete block design (RCBD) arranged in a 3×3 factorial. The first factor consisted of three different doses of arbuscular mycorrhizal fungi (AMF) application: 2 (M1), 4 (M2) and 6 g seedling<sup>-1</sup> (M3). The second factor involved was three transplanting media compositions: Goat manure (PK), sawdust (SG), and sand (P) = 1/3:1/3:2/3 (T1), 2/3:1/3:1/3 (T2) and 1/3:2/3:1/3 (T3). Nine treatment combinations were applied using two seedlings per combination. To ensure reliability, the experiment was repeated three times. The macro nutrient content of goat manure is N = 0.70%, P = 0.40%, K = 0.25%, while C-organic = 31.00% (analyzed in Plant Soil laboratory of AIAT Yogyakarta).

### Seeds source and coconut growth germination in the nursery

'Bido' coconut (*Cocos nucifera* L.) seeds were obtained from selected mother trees in Bido Village, Morotai Island Regency of North Maluku Province, Indonesia. Initially, the seedlings were grown in a nursery, a shade house with a 40% light absorption capacity. Three months after sowing, polybag transplantation was performed on seeds with average germination growth using a polybag of dimensions 40 cm × 50 cm. The soil used to fill the polybags was obtained from the nursery environment and was not sterilized. The seedling polybags were placed triangularly with a spacing of 60 cm × 60 cm × 60 cm.

The transplanted coconut seedlings in the polybags were subjected to a 9 mo treatment period following the operational standards of coconut nursery cultivation. This treatment included regular watering, proper fertilization, weed control measures, and pests and disease management. After 9 mo of care and management, the coconut seedlings have reached the appropriate stage for transplantation to the field.

### Transplanting seedlings

The coconut planting system implemented in this study consisted of a mixed garden comprising coconut and nutmeg (*Myristica fragrans* Houtt.) trees, with a spacing of 7 m by 7 m as a modification from Tamil Nadu Agricultural University (2022). According to the guideline, the ideal spacing for tall coconut varieties is 7.5 m × 7.5 m, with hybrid varieties spaced at 8.5 m × 8.5 m and dwarf coconuts spaced at 6.5 m × 6.5 m. Since the 'Bido' coconut used in this research is a shorter plant type, this research employed a rectangular spacing of 7 m × 7 m.

The cultivation of the coconut plants followed the operational standards. Intensive maintenance practices were carried out for 3 yr to maintain the treatment effects (Figure 1).



**Figure 1.** *Cocos nucifera* 'Bido' performance under the influence of arbuscular mycorrhizal fungi (AMF) inoculation and various transplanting media in the Kusu experimental garden, located at AIAT\_Sofifi in North Maluku.

The transplanting hole used in the experiment was 50 cm deep with a diameter of 30 cm. The treatment media was carefully placed in the transplanting hole according to the designed environmental conditions. The AMF inoculation was performed 1 d after transplanting using granular zeolite media containing an average of 3.6 spores per gram. The identified AMF species included *Glomus* sp., *Funneliformis* sp., *Acaulospora* sp., *Gigaspora* sp., and *Scutellospora* sp.

### Measurements

The parameters observed included various parameters related to plant physiology, growth, and soil nutrient status. Plant physiological traits, including leaf activity of nitrate reductase (NRA), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and leaf total chlorophyll content (Chl), were measured at 24 mo after planting (MAT). Growth parameters, such as plant height, stem circumference, leaf midribs number, and leaf midrib length, were observed at 6, 12, 18, 24, and 30 MAT.

Data on soil nutrient status, including pH, C-organic, N-total, available K, and P<sub>2</sub>O<sub>5</sub>, were observed at 12 MAT. The pH (H<sub>2</sub>O) testing method used a pH meter 1:5, C-organic using the Walkley and Black method, N-total using the Kjeldahl method, K available using the Morgan-Wolf method and available P using the Olsen method (analyzed in Plant Soil laboratory of AIAT Yogyakarta).

The leaf nitrate reductase activity (NRA) and chlorophyll were measured by randomly selecting leaf blades from sample plants in each treatment. The Chl *a*, Chl *b*, and Chl levels were determined following the method described by Wintermans and de Monts (1965).

The leaf NRA content was measured using a modified version of Indradewa's method described by Song et al. (2017). These physiological trait observations were conducted at the Plant Production Laboratory, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia.

### Statistical analyses

The collected data were analyzed according to the observation intervals with ANOVA of a randomized complete block design  $3 \times 3$  factorial using the SAS 9 program for Windows (SAS Institute, Cary, North Carolina, USA). The interaction effects were further compared in the event of inter-factor interaction. If there was nonsignificant interaction, the treatment endpoints were compared using Duncan's Multiple Range Test at a significant of  $p < 0.05$ . Furthermore, the Principal Component Analysis (PCA) Biplot was used to analyze the physiological, soil nutrient status, and agronomic parameters to identify the key traits that determine plant growth, particularly plant height.

## RESULTS

### Plant height and number of leaf midrib

The interaction between the application doses of AMF inoculum and transplanting media influenced the height of plant and the number of leaf midribs at the age of 6-30 MAT (Tables 1 and 2). The highest plant height was observed when applying the transplanting media of a mixture of manure (PK), sawdust (SG), and sand (P) with a ratio of 1/3:1/3:2/3, and the seedlings were inoculated with 4 g AMF (M2T1). This treatment showed a significantly different plant height ( $p < 0.01$ ) in comparison with other treatment combinations at 18, 24, and 30 MAT (Table 1).

**Table 1.** Treatment combination effects of transplanting media (TM) and doses of arbuscular mycorrhizal fungi (AMF) on plant height at the ages of 6-30 MAT. Numbers followed by the same letters in the same column did not differ significantly at  $p < 0.05$  according to Duncan's multiple range test. T1: Manure:sawdust:sand 1/3:1/3:2/3; T2: manure:sawdust:sand 2/3:1/3:1/3; T3: manure:sawdust:sand 1/3:2/3:1/3; MAT: month after transplanting. \*, \*\*, <sup>ns</sup>Significant, very significant, nonsignificant, respectively.

AMF g seedlings <sup>-1</sup>	Transplanting media	cm				
		6	12	18	24	30
2 (M1)	T1	156.37 <sup>b-d</sup>	186.38 <sup>cd</sup>	210.35 <sup>cd</sup>	251.78 <sup>bc</sup>	276.12 <sup>bc</sup>
2 (M1)	T2	169.08 <sup>a-c</sup>	205.42 <sup>b-d</sup>	235.73 <sup>bc</sup>	241.82 <sup>c</sup>	267.48 <sup>bc</sup>
2 (M1)	T3	161.10 <sup>bc</sup>	252.43 <sup>ab</sup>	221.85 <sup>b-d</sup>	265.32 <sup>bc</sup>	292.97 <sup>bc</sup>
4 (M2)	T1	179.38 <sup>a-c</sup>	262.72 <sup>a</sup>	296.05 <sup>a</sup>	350.57 <sup>a</sup>	389.23 <sup>a</sup>
4 (M2)	T2	200.47 <sup>a</sup>	235.45 <sup>a-c</sup>	234.48 <sup>bc</sup>	255.43 <sup>bc</sup>	274.82 <sup>bc</sup>
4 (M2)	T3	189.53 <sup>ab</sup>	199.70 <sup>b-d</sup>	207.03 <sup>cd</sup>	254.60 <sup>bc</sup>	270.48 <sup>bc</sup>
6 (M3)	T1	150.17 <sup>dc</sup>	181.15 <sup>d</sup>	200.13 <sup>cd</sup>	233.03 <sup>c</sup>	257.37 <sup>bc</sup>
6 (M3)	T2	126.48 <sup>d</sup>	127.15 <sup>e</sup>	185.17 <sup>d</sup>	234.73 <sup>c</sup>	247.42 <sup>c</sup>
6 (M3)	T3	198.83 <sup>a</sup>	203.93 <sup>b-d</sup>	264.45 <sup>ab</sup>	295.87 <sup>b</sup>	313.87 <sup>b</sup>
Significance						
AMF		0.93 <sup>ns</sup>	0.29 <sup>ns</sup>	0.08 <sup>ns</sup>	0.95 <sup>ns</sup>	0.91 <sup>ns</sup>
TM		0.01 <sup>*</sup>	0.35 <sup>ns</sup>	0.31 <sup>ns</sup>	0.37 <sup>ns</sup>	0.12 <sup>ns</sup>
AMF×TM		0.00 <sup>**</sup>	0.00 <sup>**</sup>	0.02 <sup>*</sup>	0.01 <sup>*</sup>	0.00 <sup>**</sup>

The combination of the transplanting media consisting of manure, sawdust, and sand in a ratio of 1/3:1/3:2/3 along with the inoculation of 4 g AMF seedling<sup>-1</sup> (M2T1), resulted in the highest number of leaf midrib. This treatment differed significantly ( $p < 0.01$ ) from other treatment combinations at the ages of 18, 24, and 30 MAT (Table 2). These findings demonstrate that this particular treatment is the most effective in improving both plant height and the number of leaf midribs during the early stages of coconut growth.

**Table 2.** Treatment combination effects of transplanting media (TM) and doses of arbuscular mycorrhizal fungi (AMF) on the number of coconut leaf midribs at the ages of 6-30 MAT. Numbers followed by the same letters in the same column did not differ significantly at  $p < 0.05$  according to Duncan's multiple range test. T1: Manure:sawdust:sand 1/3:1/3:2/3; T2: manure:sawdust:sand 2/3:1/3:1/3; T3: manure:sawdust:sand 1/3:2/3:1/3; MAT: month after transplanting. \*, \*\*, <sup>ns</sup>Significant, very significant, nonsignificant, respectively.

AMF g seedlings <sup>-1</sup>	Transplanting media	6	12	18	24	30
2 (M1)	T1	3.00 <sup>b</sup>	6.33 <sup>b</sup>	6.66 <sup>d</sup>	8.66 <sup>cd</sup>	9.00 <sup>d</sup>
2 (M1)	T2	3.00 <sup>b</sup>	9.66 <sup>a</sup>	8.66 <sup>b-d</sup>	10.33 <sup>bc</sup>	12.00 <sup>a-c</sup>
2 (M1)	T3	3.30 <sup>b</sup>	7.00 <sup>ab</sup>	8.00 <sup>cd</sup>	10.00 <sup>bc</sup>	10.66 <sup>b-d</sup>
4 (M2)	T1	4.00 <sup>ab</sup>	10.00 <sup>a</sup>	11.66 <sup>a</sup>	12.66 <sup>a</sup>	13.33 <sup>a</sup>
4 (M2)	T2	5.00 <sup>a</sup>	7.00 <sup>ab</sup>	9.66 <sup>a-c</sup>	10.00 <sup>bc</sup>	11.33 <sup>a-d</sup>
4 (M2)	T3	3.60 <sup>b</sup>	9.66 <sup>a</sup>	10.33 <sup>a-c</sup>	10.66 <sup>b</sup>	11.66 <sup>a-c</sup>
6 (M3)	T1	4.00 <sup>ab</sup>	10.00 <sup>a</sup>	8.33 <sup>a-c</sup>	7.66 <sup>d</sup>	9.00 <sup>d</sup>
6 (M3)	T2	3.30 <sup>b</sup>	10.00 <sup>a</sup>	8.00 <sup>cd</sup>	9.66 <sup>bc</sup>	10.33 <sup>cd</sup>
6 (M3)	T3	5.00 <sup>a</sup>	7.00 <sup>ab</sup>	10.66 <sup>ab</sup>	11.00 <sup>ab</sup>	13.00 <sup>ab</sup>
Significance						
AMF		0.00 <sup>**</sup>	0.16 <sup>ns</sup>	0.00 <sup>**</sup>	0.00 <sup>**</sup>	0.04 <sup>*</sup>
TM		0.57 <sup>**</sup>	0.35 <sup>ns</sup>	0.30 <sup>ns</sup>	0.19 <sup>ns</sup>	0.11 <sup>ns</sup>
AMF×TM		0.02 <sup>*</sup>	0.00 <sup>**</sup>	0.04 <sup>*</sup>	0.00 <sup>**</sup>	0.00 <sup>**</sup>

### Stem circumference and leaf midrib length

There was an interaction between the application of AMF inoculum doses and transplanting media influencing the stem circumference and leaf midrib length at the age of 6-30 MAT (Tables 3 and 4). The transplanting media combination of manure, sawdust, and sand in a ratio of 1/3:1/3:2/3 along with the inoculation of 4 g AMF seedling<sup>-1</sup>, has resulted in the highest stem growth. This treatment had significant differences ( $p < 0.01$ ) compared to the other treatment combination at the ages of 12-30 MAT (Table 3). Therefore, this treatment can be considered the most effective to promote stem growth in coconut plants.

Similarly, the combination of transplanting media (PK:SG:P = 1/3:1/3:2/3) and inoculation of 4 g AMF seedling<sup>-1</sup> also resulted in the highest leaf midrib length. This treatment showed significant differences ( $p < 0.01$ ) compared to other treatment combinations at the ages of 24 and 30 MAT (Table 4). This indicates that this treatment can be considered the most effective for promoting the growth of the stem and the length of the leaf midrib length at the early growth stage of coconut plants.

### NRA and leaf chlorophyll content

The interaction between the transplanting media and the AMF inoculation dose has significantly ( $p < 0.01$ ) influenced the NRA, Chl a, Chl b, and Chl of coconut leaf at 24 MAT (Table 5). The mixture of transplanting media of manure, sawdust, and sand in a ratio of 1/3:1/3:2/3 with the inoculation of 4 g AMF seedling<sup>-1</sup> (M2T1), also led in the highest NRA. This treatment showed significant differences ( $p < 0.01$ ) compared to the other treatment combinations, except for the combination of higher doses of AMF inoculation with a media composition of 2/3 manure (M3T2).



The combination of the transplanting media of manure, sawdust, and sand in a ratio of 1/3:1/3:2/3, along with the inoculation of 4 g AMF seedling<sup>-1</sup> (M2T1), resulted in the highest Chl a and Chl levels, which were significantly different ( $p < 0.01$ ) from all other treatment combinations (Table 5). This treatment also produced the highest Chl b levels, significantly different from several other treatment combinations.

**Table 3.** Treatment combination effects of transplanting media (TM) and doses of arbuscular mycorrhizal fungi (AMF) on coconut stem circumference at the ages of 6-30 MAT. Numbers followed by the same letters in the same column did not differ significantly at  $p < 0.05$  according to Duncan's multiple range test. T1: Manure:sawdust:sand 1/3:1/3:2/3; T2: manure:sawdust:sand 2/3:1/3:1/3; T3: manure:sawdust:sand 1/3:2/3:1/3; MAT: month after transplanting. \*, \*\*, <sup>ns</sup>Significant, very significant, nonsignificant, respectively.

AMF g seedlings <sup>-1</sup>	Transplanting media	cm				
		6	12	18	24	30
2 (M1)	T1	23.517 <sup>b</sup>	31.350 <sup>c</sup>	30.117 <sup>bc</sup>	37.02 <sup>bc</sup>	47.45 <sup>c</sup>
2 (M1)	T2	24.167 <sup>ab</sup>	49.100 <sup>a-c</sup>	43.067 <sup>ab</sup>	62.67 <sup>ab</sup>	71.33 <sup>a-c</sup>
2 (M1)	T3	20.717 <sup>bc</sup>	40.483 <sup>a-c</sup>	42.300 <sup>ab</sup>	45.47 <sup>a-c</sup>	72.82 <sup>ab</sup>
4 (M2)	T1	17.683 <sup>c</sup>	61.667 <sup>a</sup>	49.367 <sup>a</sup>	63.37 <sup>ab</sup>	95.38 <sup>a</sup>
4 (M2)	T2	25.400 <sup>ab</sup>	39.667 <sup>bc</sup>	34.433 <sup>bc</sup>	42.38 <sup>a-c</sup>	54.05 <sup>bc</sup>
4 (M2)	T3	25.500 <sup>ab</sup>	40.700 <sup>a-c</sup>	38.033 <sup>a-c</sup>	45.40 <sup>a-c</sup>	49.07 <sup>bc</sup>
6 (M3)	T1	20.467 <sup>bc</sup>	47.767 <sup>a-c</sup>	37.100 <sup>a-c</sup>	51.40 <sup>a-c</sup>	56.73 <sup>bc</sup>
6 (M3)	T2	20.233 <sup>bc</sup>	32.800 <sup>c</sup>	24.783 <sup>c</sup>	26.43 <sup>c</sup>	46.80 <sup>c</sup>
6 (M3)	T3	29.067 <sup>a</sup>	54.033 <sup>ab</sup>	35.800 <sup>a-c</sup>	74.05 <sup>a</sup>	88.72 <sup>a</sup>
Significance						
AMF		0.93 <sup>ns</sup>	0.29 <sup>ns</sup>	0.08 <sup>ns</sup>	0.95 <sup>ns</sup>	0.91 <sup>ns</sup>
TM		0.01 <sup>*</sup>	0.35 <sup>ns</sup>	0.31 <sup>ns</sup>	0.37 <sup>ns</sup>	0.12 <sup>ns</sup>
AMF×TM		0.00 <sup>**</sup>	0.00 <sup>**</sup>	0.02 <sup>*</sup>	0.01 <sup>*</sup>	0.00 <sup>**</sup>

**Table 4.** Treatment combination effects of transplanting media (TM) and doses of arbuscular mycorrhizal fungi (AMF) on coconut leaf midrib length at the ages of 6-30 MAT. Numbers followed by the same letters in the same column did not differ significantly at  $p < 0.05$  according to Duncan's multiple range test. T1: Manure:sawdust:sand 1/3:1/3:2/3; T2: manure:sawdust:sand 2/3:1/3:1/3; T3: manure:sawdust:sand 1/3:2/3:1/3; MAT: month after transplanting. \*, \*\*, <sup>ns</sup>Significant, very significant, nonsignificant, respectively.

AMF g seedlings <sup>-1</sup>	Transplanting media	cm				
		6	12	18	24	30
2 (M1)	T1		93.050 <sup>a-c</sup>	109.933 <sup>a-c</sup>	115.717 <sup>c</sup>	117.28 <sup>c</sup>
2 (M1)	T2		93.067 <sup>a-c</sup>	104.833 <sup>ac</sup>	119.983 <sup>bc</sup>	126.67 <sup>c</sup>
2 (M1)	T3		82.167 <sup>c</sup>	94.333 <sup>c</sup>	124.417 <sup>bc</sup>	131.75 <sup>c</sup>
4 (M2)	T1		82.850 <sup>c</sup>	115.400 <sup>ab</sup>	151.800 <sup>a</sup>	171.92 <sup>a</sup>
4 (M2)	T2		105.550 <sup>ab</sup>	106.450 <sup>a-c</sup>	121.867 <sup>bc</sup>	128.43 <sup>c</sup>
4 (M2)	T3		97.500 <sup>a-c</sup>	111.833 <sup>ab</sup>	119.850 <sup>bc</sup>	131.37 <sup>c</sup>
6 (M3)	T1		84.217 <sup>c</sup>	101.867 <sup>bc</sup>	106.200 <sup>c</sup>	118.53 <sup>c</sup>
6 (M3)	T2		89.083 <sup>bc</sup>	111.067 <sup>a-c</sup>	116.900 <sup>bc</sup>	137.90 <sup>bc</sup>
6 (M3)	T3		109.383 <sup>a</sup>	119.517 <sup>a</sup>	135.233 <sup>ab</sup>	161.80 <sup>ab</sup>
Significance						
AMF		0.35 <sup>ns</sup>	0.40 <sup>ns</sup>	0.11 <sup>ns</sup>	0.06 <sup>ns</sup>	0.04 <sup>*</sup>
TM		0.05 <sup>ns</sup>	0.08 <sup>ns</sup>	0.92 <sup>ns</sup>	0.41 <sup>ns</sup>	0.35 <sup>ns</sup>
AMF×TM		0.00 <sup>**</sup>	0.01 <sup>*</sup>	0.04 <sup>*</sup>	0.00 <sup>**</sup>	0.00 <sup>**</sup>

**Table 5.** Treatment combination effects of transplanting media (TM) and doses of arbuscular mycorrhizal fungi (AMF) on NRA and leaf chlorophyll content at age 24 MAT. Numbers followed by the same letters in the same column did not differ significantly at  $p < 0.05$  according to Duncan's multiple range test. T1: Manure:sawdust:sand 1/3:1/3:2/3; T2: manure:sawdust:sand 2/3:1/3:1/3; T3: manure:sawdust:sand 1/3:2/3:1/3; MAT: month after transplanting. \*, \*\*, <sup>ns</sup>Significant, very significant, nonsignificant, respectively.

AMF g seedlings <sup>-1</sup>	Transplanting media	NRA $\mu\text{mol NO}_2 \text{ g}^{-1} \text{ h}^{-1}$	Chl <i>a</i>	Chl <i>b</i>	Chl
		$\text{mg g}^{-1}$			
2 (M1)	T1	1.40 <sup>bc</sup>	0.34 <sup>b-d</sup>	0.28 <sup>b-d</sup>	0.62 <sup>b-d</sup>
2 (M1)	T2	1.63 <sup>b</sup>	0.32 <sup>d</sup>	0.25 <sup>d</sup>	0.57 <sup>d</sup>
2 (M1)	T3	1.12 <sup>c</sup>	0.33 <sup>cd</sup>	0.26 <sup>cd</sup>	0.59 <sup>b-d</sup>
4 (M2)	T1	2.01 <sup>a</sup>	0.45 <sup>a</sup>	0.37 <sup>a</sup>	0.82 <sup>a</sup>
4 (M2)	T2	1.59 <sup>b</sup>	0.36 <sup>b-d</sup>	0.33 <sup>a-c</sup>	0.69 <sup>bc</sup>
4 (M2)	T3	1.27 <sup>c</sup>	0.36 <sup>b-d</sup>	0.33 <sup>a-c</sup>	0.69 <sup>bc</sup>
6 (M3)	T1	1.42 <sup>bc</sup>	0.32 <sup>cd</sup>	0.22 <sup>d</sup>	0.55 <sup>d</sup>
6 (M3)	T2	1.98 <sup>a</sup>	0.39 <sup>b</sup>	0.34 <sup>ab</sup>	0.70 <sup>bc</sup>
6 (M3)	T3	1.36 <sup>bc</sup>	0.37 <sup>cb</sup>	0.34 <sup>ab</sup>	0.71 <sup>b</sup>
<b>Significance</b>					
AMF		0.01*	0.00**	0.00**	0.00**
TM		0.00**	0.37 <sup>ns</sup>	0.54 <sup>ns</sup>	0.93 <sup>ns</sup>
AMF×TM		0.00**	0.00**	0.01*	0.00**

### Soil nutrient status

The interaction between the transplanting media and the application dose of AMF has significantly affected the soil pH, C-organic, N total, available K, and P<sub>2</sub>O<sub>5</sub> (Table 6). The treatment combination of the transplanting media of manure, sawdust, and sand in a ratio of 1/3:1/3:2/3, along with the inoculation of 4 g AMF seedling<sup>-1</sup> (M2T1), again resulted in the highest levels of N total, available K and P. Specifically for K availability, this treatment showed a significant increase ( $p < 0.01$ ) compared to other treatments (Table 6).

The results indicated that the combination of the transplanting media of manure, sawdust, and sand with a ratio of 1/3:1/3:2/3, along with the inoculation of 4 g AMF seedling<sup>-1</sup> (M2T1), has proven to be the most effective for increasing N total, available K and P levels. In addition, this combination of treatment also contributed to the achievement of optimal C-organic content and soil pH, resulting in improved agronomic traits such as plant height, stem circumference, leaf midribs number, and leaf midrib length, as well as enhanced plant physiology in terms of NRA and Chl.

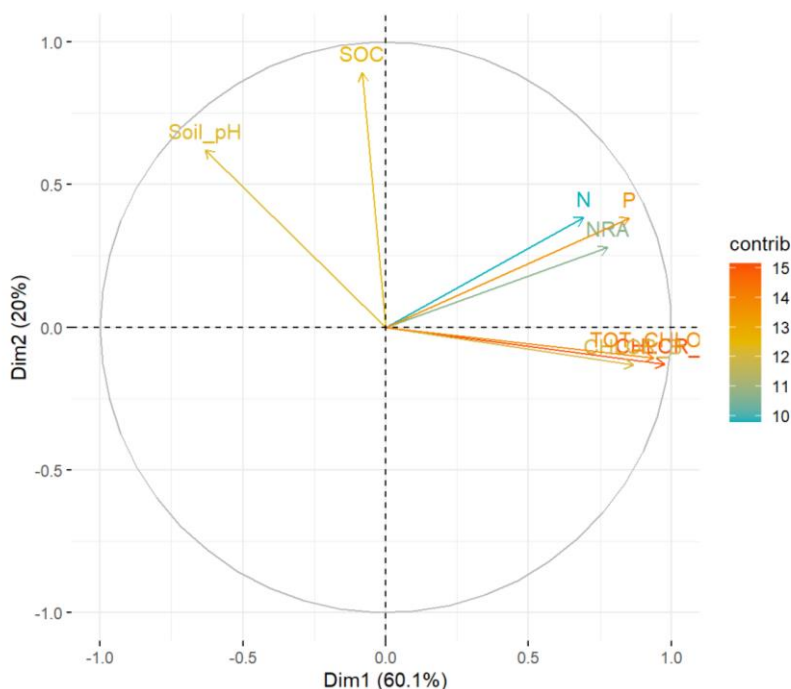
### Parameters contribution on early growth of coconut

The early growth of coconut was characterized by the greatest contribution (15%) of leaf chlorophyll, which was the most significant parameter among other parameters observed. The soil pH, soil organic C, availability of K and P showed moderate contributions (12% -13%) whereas total N and NRA showed low contributions (Figure 2).



**Table 6.** Combined treatment effects of transplanting media (TM) and doses of arbuscular mycorrhizal fungi (AMF) on soil nutrient status. Numbers followed by the same letters in the same column did not differ significantly at  $p < 0.05$  according to Duncan's multiple range test. T1: Manure:sawdust:sand 1/3:1/3:2/3; T2: manure:sawdust:sand 2/3:1/3:1/3; T3: manure:sawdust:sand 1/3:2/3:1/3; MAT: month after transplanting. \*, \*\*, <sup>ns</sup>Significant, very significant, nonsignificant, respectively.

AMF g seedlings <sup>-1</sup>	Transplanting media	pH	C-organic	N-Total	K <sub>2</sub> O %	P <sub>2</sub> O <sub>5</sub>
2 (M1)	T1	6.33 <sup>a</sup>	1.64 <sup>b</sup>	0.25 <sup>b-d</sup>	153.5 <sup>cb</sup>	176.5 <sup>c</sup>
2 (M1)	T2	6.22 <sup>a</sup>	2.03 <sup>a</sup>	0.31 <sup>a-c</sup>	157.0 <sup>cb</sup>	215.0 <sup>bc</sup>
2 (M1)	T3	5.85 <sup>ab</sup>	1.53 <sup>b</sup>	0.30 <sup>a-d</sup>	136.5 <sup>cb</sup>	321.5 <sup>a</sup>
4 (M2)	T1	5.28 <sup>b</sup>	1.71 <sup>b</sup>	0.44 <sup>a</sup>	233.0 <sup>a</sup>	333.5 <sup>a</sup>
4 (M2)	T2	6.28 <sup>a</sup>	1.80 <sup>ab</sup>	0.30 <sup>a-d</sup>	181.0 <sup>b</sup>	246.5 <sup>a-c</sup>
4 (M2)	T3	5.96 <sup>ab</sup>	1.77 <sup>ab</sup>	0.15 <sup>cd</sup>	139.5 <sup>cb</sup>	202.5 <sup>bc</sup>
6 (M3)	T1	5.76 <sup>ab</sup>	1.62 <sup>b</sup>	0.14 <sup>d</sup>	130.5 <sup>c</sup>	214.0 <sup>bc</sup>
6 (M3)	T2	5.25 <sup>b</sup>	1.53 <sup>b</sup>	0.36 <sup>ab</sup>	149.5 <sup>cb</sup>	278.0 <sup>ab</sup>
6 (M3)	T3	5.31 <sup>b</sup>	1.62 <sup>b</sup>	0.17 <sup>cd</sup>	158.0 <sup>bc</sup>	276.5 <sup>ab</sup>
Significance						
AMF		0.00 <sup>**</sup>	0.06 <sup>ns</sup>	0.17 <sup>ns</sup>	0.00 <sup>**</sup>	0.59 <sup>ns</sup>
TM		0.53 <sup>ns</sup>	0.11 <sup>ns</sup>	0.04 <sup>*</sup>	0.06 <sup>ns</sup>	0.53 <sup>ns</sup>
AMF×TM		0.02 <sup>*</sup>	0.03 <sup>*</sup>	0.00 <sup>**</sup>	0.00 <sup>**</sup>	0.00 <sup>**</sup>
CV, %		6.77	9.08	31.42	14.69	19.92



**Figure 2.** Contribution of observed parameters to the growth of *Cocos nucifera* 'Bido'. NRA: Nitrate reductase activity; Chlor\_A: chlorophyll *a*; Chlor\_B: chlorophyll *b*; Tot\_Chlor: total chlorophyll; Soil\_pH: soil pH H<sub>2</sub>O; SOC: soil organic C; N: total N; P: P availability; K: K availability; PH: plant height; SC: stem circumference; TM: total of midrib; LB: length of branch.

## DISCUSSION

The most effective treatment for increasing P levels was a combination of a mixture of transplanting media comprising manure:sawdust:sand in a ratio of 1/3:1/3:2/3 with the inoculation of 4 g AMF seedling<sup>-1</sup> (M2T1). The observed increase in P content could be attributed to the development of optimal AMF colonization facilitated by the specific transplanting media composition (John and Ray, 2023). These findings aligned with previous studies on coconut transplanting-AMF inoculated which stated that AMF inoculation increased colonization of coconut plants significantly ( $p < 0.01$ ) by 41.06% (Sulistiono et al., 2020). Additionally, the inoculation of AMF has increased the survival and growth of micro-propagated coconut plantlets (Gómez-Falcón et al., 2023).

Optimal AMF colonization is widely recognized for its ability to increase inorganic nutrients, especially P (Nirukshan et al., 2022). Several studies have indicated significant increases in P contents in the rhizosphere due to AMF effects, ranging from 54.39% to 76.13% (El-Sherbeny et al., 2022). The increased in P availability is attributed to the Pi transporter mechanism, which captures dissolved P in the soil, originated from AMF polyphosphate hydrolysis during symbiosis (Huo et al., 2022).

The incorporation of AMF in the transplanting media also plays a significant role in determining the optimal C organic. This increase in C organic was due to the production of glycoprotein (glomalin) by extrametrical mycelia (Singh et al., 2020). Glomalin binds soil micro aggregates (diameter < 259  $\mu\text{m}$ ), reduces the loss of nutrients and soil organic matter in the rhizosphere (Singh et al., 2020). The results were in line with the report by Jeewani et al. (2021), which highlights the AMF's role in increasing the return C organic (6.1 mg C kg<sup>-1</sup> d<sup>-1</sup>) by 74% through the mechanism known as rhizosphere priming effect.

This study found that the best combination of treatment resulted in the highest K<sub>2</sub>O levels, which differed significantly from all other treatment combinations. The results indicated that AMF inoculation could reduce the amount and volume of K leached from the soil. These findings are consistent with those reported by Hao et al. (2021) that the inoculation of AMF on suitable planting media increased the K absorption capacity of corn by 27.40%-441.7%. Soil K nutrient availability was regulated by the combination of AMF dosage and transplanting media (M2T1), resulting in an improvement of 88.9% compared to other treatments.

The treatment combination influenced the soil pH. These results suggested that soil pH levels influenced the development of AMF. At pH 5.28, the performance of AMF was optimal in terms of plants nutrient availability. This is consistent with the research of Barrow and Hartemink (2023), who emphasized the significant role of pH in releasing phosphate into the soil solution and absorption by plants. This study found that the highest P absorption occurred within the slightly acidic pH range (4-7).

The AMF colonization plays an essential role in this study, leading to superior agronomic and physiological properties that increased the availability of essential nutrients such as N, K, and P in the rhizosphere. This research confirms that AMF contributes to nutrient availability, plant nutrition, and soil fertility (Singh et al., 2020; Ebbisa, 2022). These findings are consistent with previous reports that indicate the positive effects of AMF inoculation in planting media on maize growth (Kazadi et al., 2022).

The superior agronomic traits observed in the best treatment combination (M2T1) were accompanied by notable improvements in physiological traits, namely NRA and leaf chlorophyll. These results were consistent with previous reports that AMF application increased the NRA by 74.6% under salt stress (Ma et al., 2022). This study found that the treatment led to a significant increase in NRA (79.5%). In addition, AMF in the biochar can be utilized as a source of N for coconut seedlings, due to reduced absorption of chemical N fertilizer (Nirukshan et al., 2022). This increase in N was due to a symbiotic system (extra radicle system), in the form of C protein pools that capture C from NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> from the soil (Ebbisa, 2022). The increase in N was also attributed to the improvements in coconut's root morphology traits, specifically the surface area affected by AMF (Sulistiono et al., 2020).

The observed increase in leaf NRA in the transplanting media resulted in the highest N total (Tables 5 and 6), aligned with the findings of Savolainen and Kytöviita (2022). Their report indicates that the presence of N sources in the planting medium is crucial in determining the AMF's activity in nutrient absorption, primarily N and P.

The current findings showed that the best treatment combination also resulted in a significant increase ( $p < 0.01$ ) in leaf chlorophyll content (Table 5), which is consistent with previous reports that have highlighted the role of AMF in enhancing the Chl in leaves (Popescu and Popescu, 2022). These results further support the observations of Sulistiono et al. (2020), who reported that AMF inoculation combined with NPK chemical fertilizer, did not lead to a significant increase in leaf chlorophyll during the early growth of coconut. Consequently, it is essential to optimize the application doses of AMF and to select the appropriate transplanting media to facilitate the interactions to significantly improve the leaf chlorophyll content.

Both Chl a and Chl b had a growth of 40.6% and 49.1% respectively, which was higher than that reported by Saboor et al. (2021). The total leaf chlorophyll content of maize under Zn stress fluctuated up to 15% due to the AMF effect, compared to the absence of AMF inoculation. The higher chlorophyll content in AMF-inoculated plants is known to increase the photosynthesis rate, resulting in higher production of photosynthate, plant growth, biomass, and crop production (Ebbisa, 2022). This increase in the chlorophyll content observed in this study also consistent with improvements in plant height, stem circumference, leaf midribs number, and leaf midrib length. Therefore, the total leaf chlorophyll content is a parameter with the largest contribution (15%) among other parameters observed during early coconut growth.

Application of AMF (commercial species) on appropriate media in this case was goat manure:sawdust:sand in a ratio of 1/3:1/3:2/3, complements the results of research Gómez-Falcón (2023) which states that the effectiveness of AMF is more optimal in mixed inoculations of the AMF genus. This result show that apart from AMF inoculation which contains several genera such as *Glomus* sp., *Funneliformis* sp., *Acaulospora* sp., *Gigaspora* sp., and *Scutellospora* sp., an optimal soil media composition (goat manure, sawdust, sand) is required. This planting medium is needed in alluvial soil which has characteristics, including loam to sandy loam, very sticky soil consistency when wet, and organic C which is classified as very low (Gayo et al., 2022).

A media composition of 2/3 sand will reduce the sticky soil consistency, and the clay structure will round so that the soil becomes looser, thereby increasing soil porosity. Thus, sandy soil will make it easier for AMF-infected coconut roots to grow. This is because AMF inoculation significantly increases the root surface area compared to the control in coconut (Sulistiono et al., 2020).

Goat manure plays a role in increasing the content of soil macro nutrients such as N, P, K, and C-Organic. The macro nutrient content of goat manure is N = 0.70%, P = 0.40%, K = 0.25%, while C-Organic = 31.00%. This is in line with the report by Marín-Martínez et al. (2021) states that goat manure increases organic N,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, and grape yield. Thus, the combination of a planting media consisting of manure: sawdust: sand in a ratio of 1/3:1/3:2/3 with inoculation of 4 g AMF seedling<sup>-1</sup> is the best combination for the early growth of coconuts in the field. This has been proven to improve agronomic properties, physiological properties of coconut plants, and soil nutrient status

## CONCLUSIONS

The combination of the transplanting media consisting of manure, sawdust and sand in a ratio of 1/3:1/3:2/3, along with the inoculation of arbuscular mycorrhizal fungi (AMF) at 4 g seedlings<sup>-1</sup>, resulted in the improved nutrient availability (N, P, K), physiological processes (nitrate reductase activity and leaf chlorophyll content) and enhanced the early growth of coconut, including plant height, stem circumference, leaf midribs number, and leaf midrib length. This technology has the potential to be utilized for the rejuvenation and development of sustainable coconut plantations.

### Author contribution

Conceptualization: W.S., B.B., H.B.A. Methodology: W.S., H.B.A., S.T., Z.A. Software: W.S., T.A. Validation: W.S., S.T., Z.A. Formal analysis: W.S., H.B.A., T.A. Investigation: G.G., M.S., S.S. Resources: W.S., M.S. Data curation: W.S., H.B.A., M.M. Writing-original draft: W.S. Writing-review & editing: W.S., B.B. Visualization: T.A. Supervision: G.G., M.S. Project administration: W.S. Funding acquisition: W.S., M.S. All co-authors reviewed the final version and approved the manuscript before submission.

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