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Morpho-Physiological Responses of Malabayabas (*Tristaniopsis decorticata* (Merr.) Peter G. Wilson & J.T. Waterh.) Seedlings Inoculated with Arbuscular Mycorrhizal Fungi

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ABSTRACT

Malabayabas (*Tristaniopsis decorticata* (Merr.) Peter G. Wilson & J.T. Waterh.) of the Myrtaceae family is a vulnerable endemic tree species in the Philippines. Poor development and low regeneration rates of seedlings in their natural habitats have led to a considerable population decline of the species. This study aimed to determine the morphological and physiological growth responses of *T. decorticata* seedlings in different soil media inoculated with arbuscular mycorrhizal fungi applied once at a rate of 5 g per seedling for the entire duration of the experiment. Morphological parameters were collected using conventional methods, while physiological parameters were measured using LI-6400 XT Portable Photosynthesis System. Results of the study revealed that the morphological growth of seedlings was generally better in Bantay soil regardless of treatments than in Lipa soil. Interestingly, the net photosynthesis, transpiration rate, and water-use efficiency were generally higher in Lipa unsterilized soil inoculated with arbuscular mycorrhizal fungi. However, this study can not yet confirm the percentage of Arbuscular Mycorrhizal Fungi (AMF) root colonization. Thus, further research must be conducted to determine the AMF root colonization and identify potential indigenous mycorrhizal fungi in both soil media.

1. Introduction

Malabayabas (*Tristaniopsis decorticata* (Merr.) Peter G. Wilson & J.T. Waterh.) is a large tree belonging to the family Myrtaceae. It is found primarily in natural forests at low and medium elevations. The species is endemic to the Philippines and reportedly found in Cagayan, Ilocos Norte, Bataan, Aurora, Quezon Province, Rizal, Camarines, Polillo, and Davao (Malabrigo et al. 2016; Pelser et al. 2011). The wood of *T. decorticata* is naturally as hard as that of the ironwood species, which makes it suitable for heavy constructions such as bridges, housing, and naval construction (ITTO 2021). Other wood uses include piles, posts, salt-water piling, tool handles, saw guide blocks, and paperweights (EDC 2021). However, illegal logging and shifting cultivation

have resulted in a significant decline in the species' population. Accordingly, there are approximately 839 threatened species in the Philippines, including *T. decorticata,* classified as vulnerable in both the Philippine Red List (DENR 2017) and the IUCN Red List of Threatened Species (IUCN 2020) and are among the priority species for conservation.

The Quezon Protected Landscape (QPL) in the Quezon Province, Region IV-A Calabarzon, is one of the known habitats of *T. decorticata*. The QPL is a protected area in Quezon Province, Philippines, encompassing the municipalities of Pagbilao, Padre Burgos, and Atimonan. Malabayabas Forest stands in the QPL are natural forest patches dominated by *T. decorticata*. The stand is a tourist attraction in the area because of the distinctive reddish color of *T. decorticata* tree trunks. However, despite its natural beauty and cultural significance, this attraction was threatened by the declining number of Malabayabas species because of poor natural regeneration. The seedlings were slow-growing and could not advance to the sapling stage due to increased mortality and low survival rates at the early stages. Therefore, biological interventions such as biofertilizer application are important tools in improving seedlings' survival and vigor, especially when planted outside their natural habitat.

Over the years, mycorrhizal research has gained much interest due to its importance in plant growth and development through root colonization. Arbuscular Mycorrhizal Fungi (AMF) can create symbiotic associations with over 80% of land plants (Berruti et al. 2016; Hildebrandt et al. 2002). AMF provides host plants with essential mineral nutrients from the soil and increases water absorption in exchange for photosynthetic products. Moreover, AMF colonizes the host plant's root system, and the fungal mycelium acquires nutrients from soil volumes that are generally inaccessible to plant roots. Fungal hyphae can penetrate smaller soil pores because they are much thinner than plant roots. In general, mycorrhizal association helps plants attain their optimal growth and development because of the beneficial nutrient acquisition activity (Allen 2011; Smith et al. 2010; Wang et al. 2017).

Traditionally, seedling performance has been measured through its morphological response. However, technological advances have made physiological measurements both rapid and possible. Furthermore, physiological measurements are the most reliable indicators for monitoring plant growth and development. The physiological processes are the important mechanisms by which genetic potential and environment interact to determine the quantity and quality of growth (Lee et al. 2012). The nutritional status of the soil and growth conditions highly influence these processes. According to Zhu et al. (2014), the growth and net photosynthesis of black locust seedlings with AMF inoculation were significantly higher than those of the uninoculated control. However, there is a dearth of studies on *T. decorticata* applied with AMF. Thus, the present study aimed to determine the morphological and physiological growth responses of the species planted in two different soil media sources with and without AMF inoculations.

2. Materials and Methods

2.1. Experimental Designs and Treatments

The factorial experiment was arranged in a completely randomized design with three replications (10 sample seedlings per replicate) under greenhouse conditions in the Department of Forest Biological Sciences (DFBS), University of the Philippines Los Baños (UPLB), from November 2019 to March 2021. The study site has a mean annual temperature and precipitation

of 27°C and 915 mm, respectively. May was considered to have the warmest temperature at 28.1°C, while January had the lowest average temperature at 24.8°C (Hernandez et al. 2023). Two factors were considered in the study, Factor A as two soil series (S1 = QPL Bantay soil and S2 = Mount Makiling Forest Reserve (MMFR) Lipa soil) and Factor B as four microbial treatments (MT1 = unsterilized, MT2 = sterilized soil, MT3 = unsterilized soil with AMF, and MT4 = sterilized soil without AMF). The treatment combinations were assigned as follows: S1MT1 = Bantay unsterilized soil (control); S1MT2 = Bantay sterilized soil; S1MT3 = Bantay unsterilized soil with AMF; S1MT4 = Bantay sterilized soil with AMF; S2MT1 = Lipa unsterilized soil; S2MT2 = Lipa sterilized soil; S2MT3 = Lipa unsterilized soil with AMF; and S2MT4 = Lipa sterilized soil with AMF.

2.2. Soil Media Collection, Preparation, and Sterilization

A bulk soil sample was collected from 0–30 cm depth. Bantay soil was collected from the Malabayabas stand, QPL (the natural habitat), and Lipa soil from Mount Makiling Forest Reserve-Permanent Field Laboratory Area 1 (MMFR-PFLA 1) at the UPLB. The soil was air-dried for one week, pulverized, and sieved in a 2 mm wire mesh. Subsamples were sterilized in a pressure cooker at 121°C for 20 minutes, and the rest were prepared for bagging the unsterilized treatments. The physicochemical analysis of the soil media was carried out by the Institute of Plant Breeding-Physiology laboratory in the College of Agriculture and Food Science in UPLB.

2.3. Source of Planting Materials and Transplanting

The three-month-old seedlings of *T. decorticata* were acquired from a small-scale nursery in QPL. These were directly brought to the greenhouse of DFBS and acclimatized for over a month. The acclimatized seedlings were transplanted into individual polybags measuring 4 inches × 4 inches × 7 inches and filled with 1 kg of soil media. Transplanted seedlings were randomly arranged above the bench surface on steel matting, 4 m long and 1 m wide. Initially, the seedlings' height and root collar diameter were obtained and further acclimatized for one month before the inoculation of AMF. The Ecosystem Research and Development Bureau developed the AMF inoculant, Hi-Q VAM 1, used in the National Greening Program in the Philippines. The inoculant was composed primarily of *Glomus* species. The inoculation of AMF was done by making a 4-inch-deep hole beneath and in contact with the roots applied once at a rate of 5 g for each seedling.

2.4. Morphological Measurements

This study assessed six morphological parameters to determine the growth performance of *T. decorticata,* including plant height increment, root collar diameter increment, percentage survival, leaf area, root length, and root-shoot ratio. Initial measurements for both plant height and root collar diameter were done one month after transplanting of seedlings. The plant height was measured from the root collar of the seedling up to its terminal bud and was recorded every three months for one year using a calibrated meter stick. Similarly, the root collar diameter was measured using a digital vernier caliper. The increment data for plant height and root collar diameter was calculated using the difference between the three-month and initial measurements (Aggangan et al. 2012).

Meanwhile, the percentage survival was quantified by counting the number of living seedlings after one year and expressing it as a percentage. If all the aboveground plant organs were desiccated, seedlings would be considered dead. The percentage survival (Mishra et al. 2015) was calculated using the Equation 1.

$$Survival\ (\%) = \frac{Number\ of\ Seedlings\ Alive}{Number\ of\ Seedlings\ Used} \times 100\% \quad (1)$$

The third expanded leaf from the seedlings' apex was used to compute the leaf area using the grid method (Dey et al. 2019). The leaves were traced on a graphing paper with a 0.5 cm × 0.5 cm grid, and the leaf area was computed using Equation 2.

$$Leaf\ area\ (cm^2) = Area\ of\ 1\ grid \times Number\ of\ grids \quad (2)$$

Root length was measured from the root collar down to the root tip. It was measured once at the end of the experiment after seedlings were uprooted. Fresh and dry weights were determined using a digital weighing scale and oven-dried at 80°C until constant weight. The roots were separated from the shoots. The root-shoot ratio (RSR) was calculated using the Equation 3.

$$RSR = \frac{Dry\ weight\ of\ roots}{Dry\ weight\ of\ shoots} \quad (3)$$

2.5. Physiological Measurements

Net photosynthesis, stomatal conductance, and transpiration rate were taken with a broad-leaf cuvette of Portable Photosynthesis System (LI-6400XT, LI-COR Biosciences, Inc., USA) equipped with a standard leaf chamber and CO_2 injection system adjusted to a constant CO_2 concentration of 400 µmol m^{-2} s^{-1}. The leaf was sealed, and CO_2 concentration was maintained at ambient levels. The average cuvette temperature and relative humidity were maintained at 32°C and 60%, respectively. Water use efficiency was calculated using the ratio of net photosynthesis and transpiration rate. Foliar nitrogen content analysis was conducted in the Agricultural Systems Institute's Central Analytical Services Laboratory of the College of Agriculture and Food Science, UPLB. Photosynthetic nitrogen-use efficiency was calculated using the ratio of net photosynthesis and foliar nitrogen. Total chlorophyll was determined using a Chlorophyll Meter (SPAD-502, Konica Minolta Sensing, Inc., Japan).

2.6. Data Analysis

The gathered data were encoded and organized in the Microsoft Excel Office 365 Program. Before statistical analysis, percentage survival data were converted using arcsine transformation. On the one hand, data on physiological parameters were converted by square root transformation to attain normality distribution for the analysis of variance and followed by a post hoc test using Tukey's Honest Significant difference (HSD). The analysis used the freeware Statistical Tool for Agricultural Research (STAR) version 2.0.1 (IRRI 2014).

3. Results and Discussion

3.1. Chemical Properties of Soil Media

Table 1 shows the chemical properties of the Bantay Clay Loam Soil from QPL and Lipa Soil from MMFR. Chemical analysis showed that the Bantay Clay Loam Soil from the QPL was

strongly acidic (pH = 5.23) with low OM (1.74%), very low N (0.087%), moderately high available P (13.98 ppm), and high K (0.77 meq/kg soil). On the other hand, Lipa Soil from the MMFR was slightly acidic (pH = 6.04) with moderately high OM (2.38%), low N (0.119%), high P (18.27 ppm), and adequate K (0.96 meq/kg soil). The Lipa soil generally has better chemical properties than the Bantay soil.

Table 1. Chemical properties of soil media

Property	Bantay Clay Loam Soil	Lipa Soil
pH	5.23	6.04
OM (%)	1.74	2.38
N (%)	0.087	0.119
Available P (Bray P2, ppm)	13.98	18.27
K meq/100g soil	0.77	0.96

3.2. Morphological Growth Responses

3.2.1. Plant height increment

Fig. 1 shows the plant height increment of seedlings after 3, 6, 9, and 12 months after transplanting (MAP). During the first 3 months, seedlings grown in Bantay unsterilized soil had the highest increase in plant height. These results were consistent after 6, 9, and 12 months, with an average increase of 5.77 cm, 17.78 cm, and 25.65 cm, respectively. Generally, seedlings grown in Bantay soil had longer shoots with large, fully expanded leaves than those grown in Lipa soil (**Fig. 2** and **Fig. 3**). This result may be attributed to the presence of ubiquitous microbiota present in the soil media, which can increase mineral absorption thereby promoting plant growth. Several studies have demonstrated that soil microbiota can significantly impact the production of arbuscular mycorrhizal fungi spores (Azcon-Aguilar et al. 1986; Meyer and Linderman 1986; Azcon et al. 1990). It was also previously reported by Scheublin et al. (2010) that groups of bacteria can attach to AMF hyphae, indicating their involvement in mycorrhizal interactions. Due to the limitations of the study, we cannot yet confirm the role of AMF on the increase of plant height because there is no available data on AMF root colonization. However, the soil physico-chemical analysis results reveal that the two soil types vary in terms of soil chemical properties. The Bantay soil is more acidic than the Lipa soil. Soil pH is an important indicator of the availability of nutrients. Lower pH may not always have negative implications for plants because some plants are well-adapted to acidic soil, for example, the malabayabas species that grows naturally in Bantay soil.

Accordingly, lower soil pH will lead to the availability of some mineral nutrients such as aluminum (Al), iron (Fe), and manganese (Mn), which may be available for plant uptake and growth (Londo et al. 2006). These elements, other than aluminum, are considered essential. However, low aluminum concentrations can cause beneficial effects on plants Al, including root growth stimulation and increased nutrient uptake and enzyme activity. On the one hand, Fe is an essential micronutrient required for metabolic processes such as DNA synthesis, respiration, and photosynthesis. It is also required for the synthesis of chlorophyll and the maintenance of chloroplast structure and function. The deficiency of this element will result in retarded growth and interveinal chlorosis, but too much iron is toxic to cells. Additionally, manganese influences iron availability (Kozlowski and Pallardy 1997). Manganese is also essential in photosynthesis,

especially the photosystem-II water oxidizing system. A sufficient amount of manganese is essential in the photolysis (light splitting) of water molecules and provides energy for photosynthesis. An inadequate supply of manganese will significantly hamper photosynthesis, decreasing soluble sugar concentration in different plant parts and declining dry matter production.

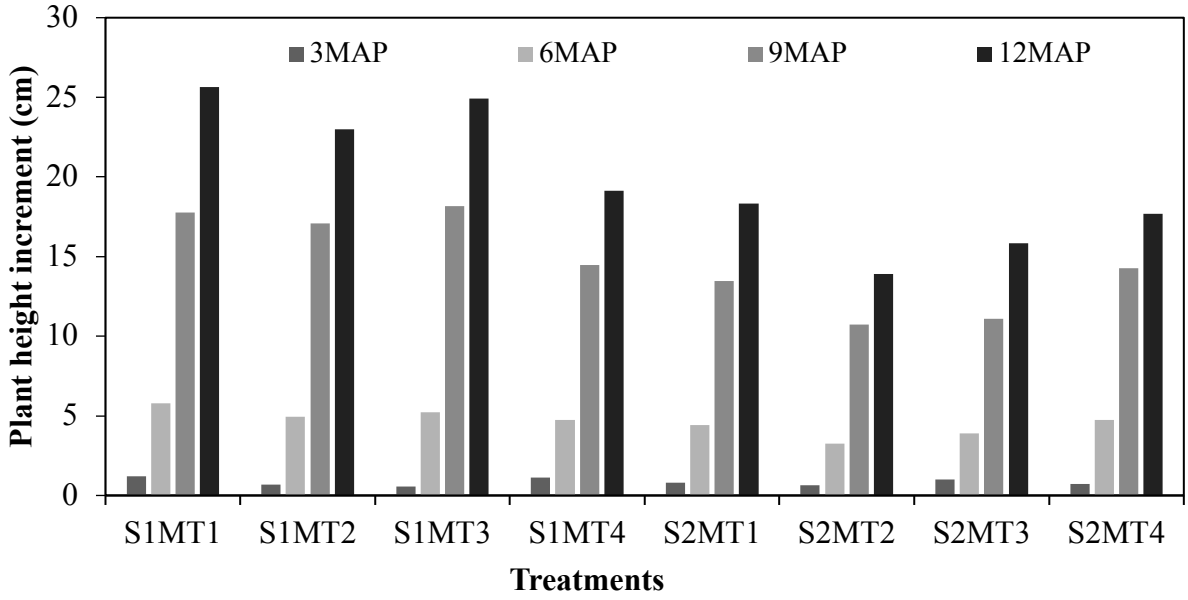

Fig. 1. Plant height increment of *T. decorticata* seedlings grown in two soil media inoculated with AMF (Notes: S1MT1 = Bantay unsterilized soil (control); S1MT2 = Bantay sterilized soil; S1MT3 = Bantay unsterilized soil + AMF; S1MT4 = Bantay sterilized soil + AMF; S2MT1 = Lipa unsterilized soil; S2MT2 = Lipa sterilized soil; S2MT3 = Lipa unsterilized soil + AMF; S2MT4 = Lipa sterilized soil + AMF at 12 months after transplanting).

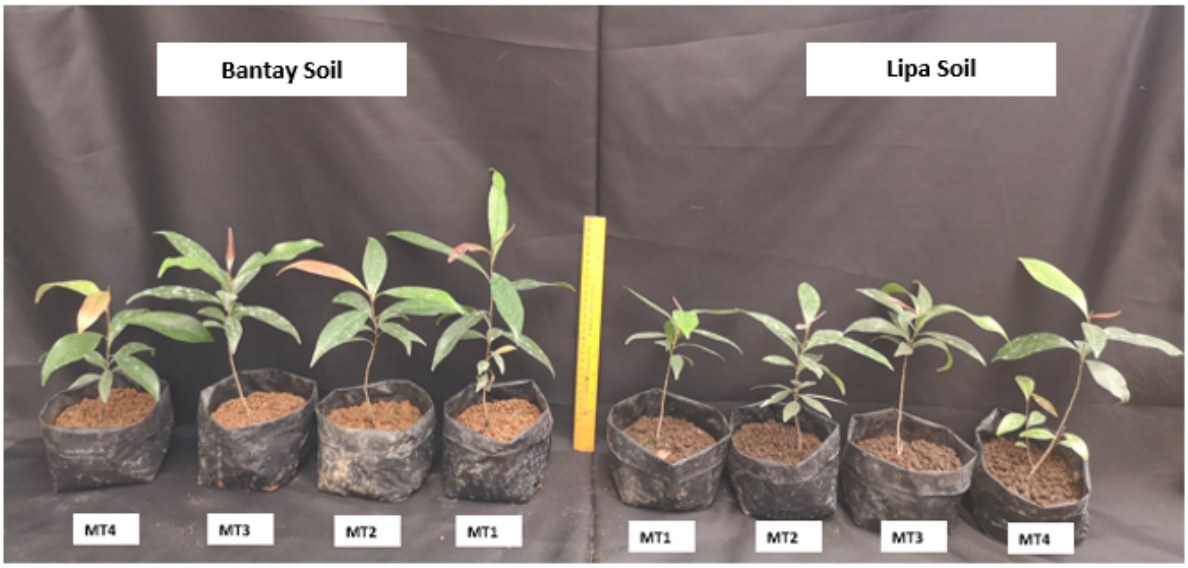

Fig. 2. Plant height increment of *T. decorticata* seedlings grown in two soil media inoculated with AMF after three months (Notes: S1MT1 = Bantay unsterilized soil (control); S1MT2 = Bantay sterilized soil; S1MT3 = Bantay unsterilized soil + AMF; S1MT4 = Bantay sterilized soil + AMF; S2MT1 = Lipa unsterilized soil; S2MT2 = Lipa sterilized soil; S2MT3 = Lipa unsterilized soil + AMF; S2MT4 = Lipa sterilized soil + AMF at 3 months after transplanting).

Fig. 3. Plant height increment of *T. decorticata* seedlings grown in two different soil media inoculated with AMF (Notes: S1MT1 = Bantay unsterilized soil (control); S1MT2 = Bantay sterilized soil; S1MT3 = Bantay unsterilized soil + AMF; S1MT4 = Bantay sterilized soil + AMF; S2MT1 = Lipa unsterilized soil; S2MT2 = Lipa sterilized soil; S2MT3 = Lipa unsterilized soil + AMF; S2MT4 = Lipa sterilized soil + AMF at 12 months after transplanting).

3.2.2. Root collar diameter increment

Table 2 shows the root collar diameter increment of *T. decorticata* seedlings grown in different soil media inoculated with AMF. Results revealed no significant difference in the root collar of seedlings across all treatments after the first three months. Subsequently, six months after transplanting, the mean difference became statistically significant ($p < 0.05$). Seedlings grown in Lipa unsterilized soil and Lipa sterilized soil + AMF were comparable to seedlings grown in Bantay soil. On the other hand, Lipa sterilized soil, and Lipa unsterilized soil + AMF have the lowest increase in terms of root collar diameter after 6 months. However, these results were inconsistent after 9 and 12 months of seedling growth, showing no significant difference at $p < 0.05$. This tendency implies that the growth of seedlings in terms of root collar diameter was not affected by the different types of soil and the inoculation of AMF. Diameter growth decreased as height growth increased, indicating that the appearance of inherited features for collar diameter growth and development was species-oriented. Aside from these factors, seedling crowding at the nursery bed may significantly impact root collar diameter growth. Root collar diameter development and growth appeared more responsive to environmental influences than height (Azad et al. 2014).

3.2.3. Percentage survival

Percentage survival was not significantly different across treatments (**Table 3**). However, seedlings grown in Lipa unsterilized soil, Lipa unsterilized soil + AMF, and Lipa sterilized soil +

AMF obtained a 100% survival of seedlings after 12 months. The application of AMF did not significantly affect the survival rate of seedlings, but notably, seedlings inoculated with AMF have higher survival rates. This result implies that arbuscular mycorrhizal fungi inoculation enhances the survival rate of seedlings. Accordingly, inoculation of mycorrhizal fungi contributes to an increased nutrient and water uptake from the soil by extending their extraradical hyphae (Allen 2007; Atkinson et al. 1994; Smith and Read 2008), and the host plants do not have to forage farther away into the soil by fine roots. In a study by Rousseau et al. (1994), external mycelium comprised up to 75% of the uptake potential of eight-week-old loblolly pine (*Pinus taeda*). This tendency supports the idea that improved nutrient and water uptake may contribute to higher plant survival rates. Additionally, AMF increases plant resistance against pathogenic microorganisms and protects the plant in exchange for photosynthetic products (Berruti et al. 2016; Wang et al. 2017).

Table 2. Root collar diameter increment of *T. decorticata* seedlings grown in two different soil media inoculated with AMF from 3 to 12 months after transplanting

Treatment	Diameter Increment (mm)			
	3 MAP	6 MAP	9 MAP	12 MAP
S1MT1 (control)	0.23	0.44[a]	1.23	1.81
S1MT2	0.06	0.46[a]	1.14	1.67
S1MT3	0.18	0.46[a]	1.22	1.94
S1MT4	0.12	0.38[a]	0.98	1.57
S2MT1	0.20	0.65[a]	1.38	1.94
S2MT2	0.13	0.41[c]	1.06	1.56
S2MT3	0.16	0.50[bc]	1.23	1.72
S2MT4	0.16	0.61[ab]	1.33	1.86
Significance	*ns*	*	*ns*	*ns*

Notes: Means in a column with a common letter are not significantly different based on HSD, ns- not significant, * - significant, ** - highly significant at a 5% significance level.

3.2.4. Leaf area

The leaf area of seedlings 12 months after transplanting was not significantly different in two different soil media and across treatments (**Table 3**). However, regardless of treatments, seedlings grown in Bantay soil have a larger leaf surface area than seedlings grown in Lipa soil. Moreover, the largest leaf area of seedlings was found in Bantay unsterilized soil + AMF (125. 28 cm^2), while the lowest was found on seedlings grown under Lipa sterilized soil + AMF (97.10 cm^2). These results demonstrate that AMF inoculation did not significantly affect seedlings' leaf area, opposing the previous work of Zhu et al. (2014). They reported that arbuscular mycorrhizal fungi enhanced the leaf area of black locust seedlings.

3.2.5. Root length

The root lengths of *T. decorticata* seedlings were not significantly different in two different soil media and across treatments (**Table 3**). However, *T. decorticata* seedlings grown in unsterilized Bantay soil had the most extended root length at 49.33 cm, while the shortest was observed on those grown under unsterilized Lipa soil at 33.67 cm. This result suggests that different soil media and inoculation with AMF did not influence the root length of *T. decorticata* seedlings. This tendency also implies that *T. decorticata* seedlings can thrive in Bantay and Lipa soils with and without inoculation of AMF.

3.2.6. Root/Shoot ratio

The root-shoot ratio of *T. decorticata* seedlings 12 months after transplanting was not significantly different in soil media sources and across treatments (**Table 3**). However, seedlings grown in Lipa unsterilized soil + AMF were numerically highest, having a value of 0.30 g. At the same time, the lowest root-shoot ratio was found on seedlings grown under Bantay unsterilized soil, having a value of 0.21 g.

Table 3. Percentage survival, leaf area, root length, and root-shoot ratio of *T. decorticata* seedlings grown in two different soil media inoculated with AMF after 12 months from transplanting

Treatment	Survival (%)	Leaf Area (cm^2)	Root Length (cm)	Root-Shoot Ratio
S1MT1 (control)	93.33	121.62	49.33	0.21
S1MT2	93.33	124.63	40.00	0.24
S1MT3	96.67	125.28	34.67	0.22
S1MT4	90.00	103.22	34.00	0.29
S2MT1	100.00	112.63	33.67	0.24
S2MT2	93.33	108.78	37.33	0.23
S2MT3	100.00	104.50	36.67	0.28
S2MT4	100.00	97.10	35.33	0.30
Significance	ns	ns	ns	ns

Notes: Means in a column with a common letter are not significantly different based on HSD, ns- not significant, * - significant, ** - highly significant at a 5% significance level.

3.3. Physiological Growth Responses

3.3.1. Net photosynthesis

Fig. 4 shows the net photosynthetic rates of *T. decorticata* seedlings grown in two different soil media inoculated with AMF after 12 months from transplanting. Results revealed that net photosynthesis of seedlings in all treatments has responded positively to increasing light intensity. Light intensity or quantity determines the photosynthesis rate (Chapman and Carter 1976; Taiz and Zeiger 2002). In this study, net photosynthetic rates are significantly different across treatments. Seedlings grown in Lipa unsterilized soil + AMF and Bantay unsterilized soil obtained the highest net photosynthetic rates with peak values of 5.14 µmol CO_2 m^{-2} s^{-1} and 4.37 µmol CO_2 m^{-2} s^{-1}, respectively. The lowest value of net photosynthesis was observed on seedlings grown in Lipa sterilized soil + AMF with 0.12 µmol CO_2 m^{-2} s^{-1}. These results demonstrate that the inoculation of AMF enhances the rate of photosynthesis on seedlings grown under Lipa unsterilized soil. AMF forms vesicles, arbuscules, hyphae, and spores and hyphae in the rhizosphere. Accordingly, AMF improves plant nutrition by increasing nutrient availability and translocation of various nutrients (Rouphael et al. 2015). A hyphal network within plant roots will be manifested due to the activity of AMF, which is primarily responsible for the increased uptake of water and nutrients from the soil (Bowles et al. 2016). Furthermore, AMF may influence atmospheric CO_2 fixation to host plants by increasing the "sink effect" and the movement of photo assimilates from aerial parts to roots. The result of the study corroborates with Zhu et al. (2014), who reported that the net photosynthesis of Black locust seedlings was significantly higher on seedlings inoculated with AMF. Similarly, Chen et al. (2017) also said that inoculation of multiple

arbuscular mycorrhizal fungi significantly improved net photosynthesis. Arbuscular mycorrhizal fungi have boosted plant growth, photosynthesis, nutrient acquisition, and tolerance to biotic and abiotic stresses (Cavanagro et al. 2015; Liu et al. 2014, 2015). Arbuscular mycorrhizal fungi need photosynthetically fixed carbon from the host to maintain their growth. The carbon sink strength stimulates the host plants to the arbuscular mycorrhizal fungi symbiosis, increasing the photosynthetic rate (Kaschuk et al. 2009).

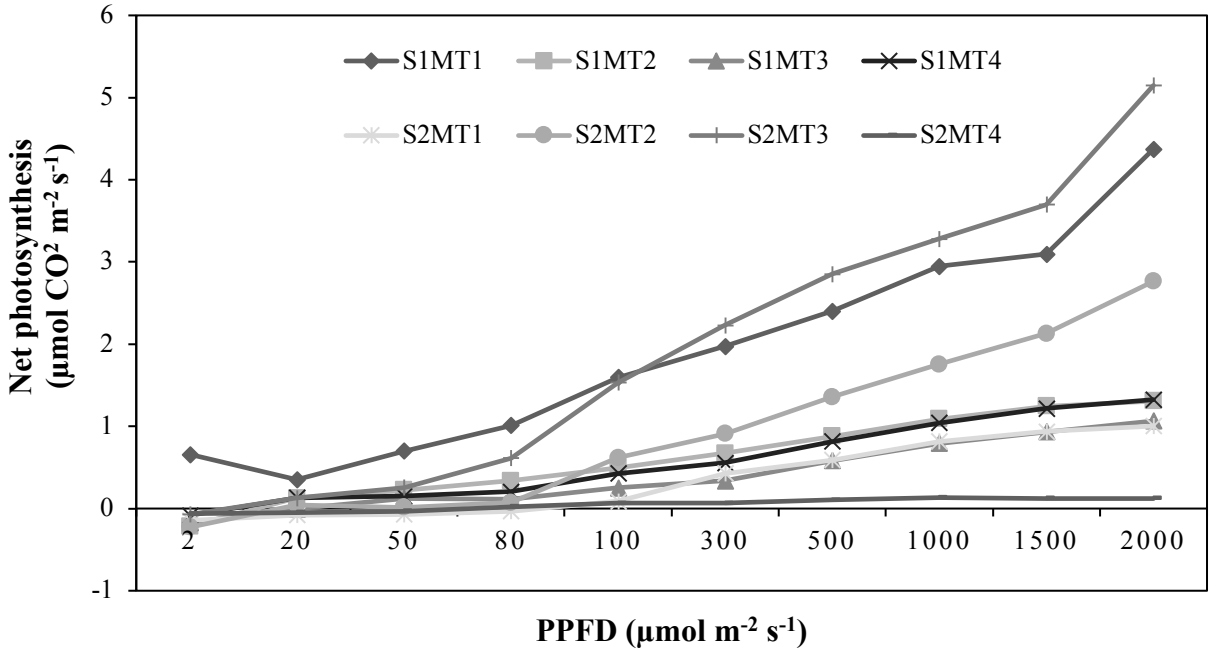

Fig. 4. Net photosynthetic rates of T. *decorticata* seedlings grown in two different soil media inoculated with AMF (Notes: S1MT1 = Bantay unsterilized soil (control); S1MT2 = Bantay sterilized soil; S1MT3 = Bantay unsterilized soil + AMF; S1MT4 = Bantay sterilized soil + AMF; S2MT1 = Lipa unsterilized soil; S2MT2 = Lipa sterilized soil; S2MT3 = Lipa unsterilized soil + AMF; S2MT4 = Lipa sterilized soil + AMF after 12 months from transplanting).

3.3.2. Transpiration rate

Table 4 shows the transpiration rates of *T. decorticata* seedlings grown in two different soil media inoculated with AMF. Results revealed that transpiration rates of seedlings were statistically different at $p < 0.05$. The highest transpiration rates were recorded on seedlings grown under Lipa unsterilized soil + AMF, followed by Bantay unsterilized soil having the values of 0.13 mmol H_2O m^{-2} s^{-1} and 0.12 mmol H_2O m^{-2} s^{-1}, respectively. However, these values were comparable to the rest of the treatments except for seedlings grown in Lipa sterilized soil + AMF with the lowest transpiration rate of 0.01 mmol H_2O m^{-2} s^{-1}. These results confirmed that using arbuscular mycorrhizal fungi enhances the transpiration rate of *T. decorticata* seedlings under Lipa unsterilized soil. This result corroborates the findings of Yang et al. (2014), who reported that arbuscular mycorrhizal fungi significantly enhanced the transpiration rate of black locust seedlings.

3.3.3. Stomatal conductance

Table 4 shows the stomatal conductance of *T. decorticata* seedlings grown in two different soil media inoculated with AMF. The stomatal conductance of seedlings was statistically different

at $p < 0.05$ across all treatments. The highest measurement was observed on seedlings grown under Lipa unsterilized soil + AMF at 0.0031 mol H_2O m^{-2} s^{-1}, followed by seedlings grown under Bantay unsterilized soil with 0.0026 mol H_2O m^{-2} s^{-1}. However, these values were comparable to the other treatments except seedlings grown under Lipa sterilized soil + AMF with the lowest stomatal conductance of 0.0003 mol H_2O m^{-2} s^{-1}. This result demonstrates that using arbuscular mycorrhizal fungi enhances the stomatal conductance of *T. decorticata* seedlings grown under Lipa soil. Net photosynthesis and stomatal conductance are positively correlated; thus, arbuscular mycorrhizal fungi enhance both parameters. By principle, an increase in stomatal conductance (gs), which regulates gas exchange (CO_2 and water), can allow plants under well-watered growth conditions to increase their CO_2 uptake and subsequently enhance photosynthesis (Kusumi et al. 2012).

3.3.4. Water use efficiency

The water use efficiency (WUE) of *T. decorticata* seedlings grown in different soil media inoculated with AMF after 12 months from transplanting is shown in **Table 4**. Results revealed that the interaction between treatments is significantly different at $p < 0.05$. *T. decorticata* seedlings grown under Bantay unsterilized soil (- control) were most efficient in water use at 29.05 µmol CO_2 $m^{-2}s^{-1}$ per mmol H_2O $m^{-2}s^{-1}$ followed by seedlings grown under Lipa soil unsterilized soil + AMF and Lipa sterilized soil at 27.74 and 26.32 05 µmol CO_2 $m^{-2}s^{-1}$ per mmol H_2O $m^{-2}s^{-1}$. Seedlings grown under Lipa sterilized soil + AMF at 8.61 per mmol H_2O m^{-2} s^{-1} were less efficient in water use. Water use efficiency is ultimately determined by stomatal behavior. When the stomata are wide open, the plant captures CO_2 at the highest rate but reduces WUE. Stomata control the leaf gas exchange in higher plants, providing pathways for CO_2 intake and regulating the efflux of water vapor to the atmosphere during transpiration (Ruiz-Lozano and Aroca 2010). Adequate stomatal regulation keeps the evaporation rate to a minimum while maintaining the rate of CO_2 assimilation, which improves plant WUE. Yang et al. (2014) also reported that WUE on black locust seedlings was enhanced significantly with AMF-inoculated seedlings compared to uninoculated seedlings.

3.3.5. Photosynthetic nitrogen use efficiency

Nitrogen is an essential component for photosynthesis in plants. Generally, higher nitrogen contents are associated with higher rates of maximum photosynthesis. The reason for this strong relationship is a large amount of leaf organic nitrogen (up to 75%) present in the chloroplasts, most of it in the photosynthetic machinery (Evans and Seemann 1989). **Fig. 5** shows the photosynthetic nitrogen use efficiency (PNUE) of *T. decorticata* seedlings grown in two different soil media inoculated with AMF after 12 months from transplanting. Results revealed that treatment interactions were significantly different at $p < 0.01$. *T. decorticata* seedlings grown in Lipa unsterilized soil inoculated with AMF were 32% higher (5.1686 µmol $g^{-1}s^{-1}$) than control. It was followed by Bantay unsterilized soil (- control) with 3.4824 µmol $g^{-1}s^{-1}$. The lowest PNUE was recorded from seedlings grown under Lipa sterilized soil + AMF at 0.1662 µmol $g^{-1}s^{-1}$. This result implies that the application of AMF significantly improved the PNUE of *T. decorticata* seedlings. AMF inoculation can significantly increase the concentration of various macro- and micronutrients, resulting in increased photosynthate production and, thus, increased biomass accumulation (Chen et al. 2017; Mitra et al. 2019). Furthermore, AMF inoculation improves P and N contents by improving water and intercellular CO_2, P, and N (Jixiang et al. 2017).

Table 4. Transpiration rate, stomatal conductance, and WUT of *T. decorticata* seedlings grown in two different soil media inoculated with AMF after 12 months of transplanting

Treatment	Transpiration Rate (E, mmol H_2O m^{-2} s^{-1})	Stomatal Conductance (gs, mol H_2O m^{-2} s^{-1})	Water Use Efficiency (µmol CO_2 m^{-2} s^{-1} per mmol H_2O m^{-2} s^{-1})
S1MT1	0.1171[a]	0.0026[a]	29.0483[a]
S1MT2	0.0718[a]	0.0016[a]	13.3126[b]
S1MT3	0.0782[a]	0.0017[a]	11.8491[b]
S1MT4	0.0744[a]	0.0017[a]	14.2031[ab]
S2MT1	0.073[b]	0.0016[bc]	12.4811[ab]
S2MT2	0.0951[ab]	0.0021[ab]	26.3156[a]
S2MT3	0.1354[a]	0.0031[a]	27.7434[a]
S2MT4	0.0145[c]	0.0003[c]	8.6099[b]
Significance	*	*	*

Notes: Means in a column with a common letter are not significantly different based on HSD, ns- not significant, * - significant, ** - highly significant at a 5% significance level.

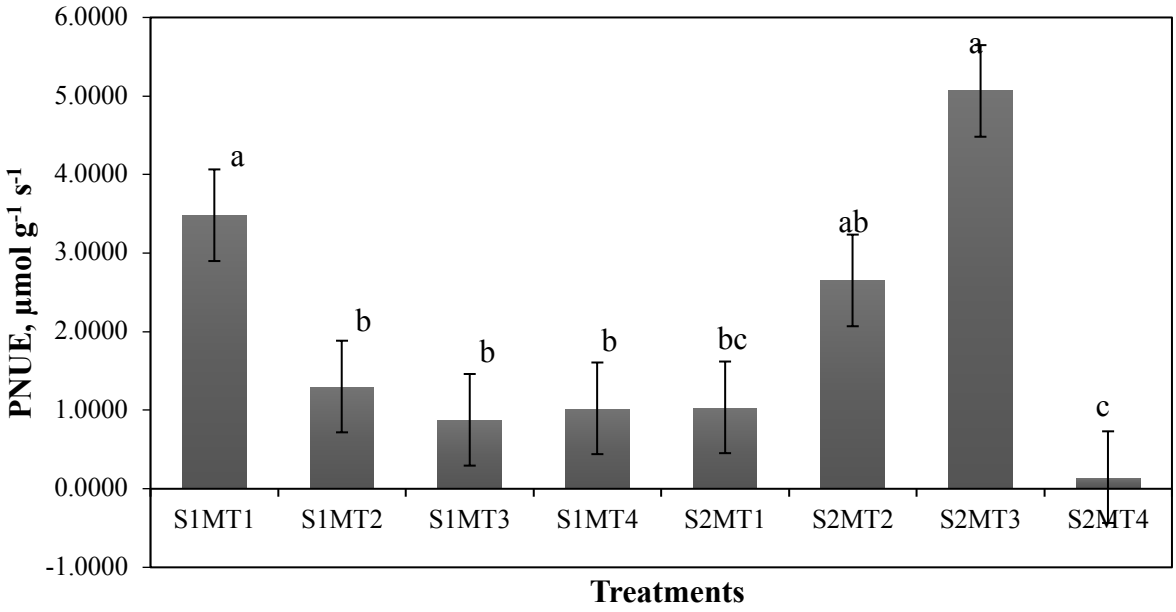

Fig. 5. Photosynthetic nitrogen use efficiency (PNUE) of *T. decorticata* seedlings grown in two different soil media inoculated with AMF (Notes: S1MT1 = Bantay unsterilized soil (control); S1MT2 = Bantay sterilized soil; S1MT3 = Bantay unsterilized soil + AMF; S1MT4 = Bantay sterilized soil + AMF; S2MT1 = Lipa unsterilized soil; S2MT2 = Lipa sterilized soil; S2MT3 = Lipa unsterilized soil + AMF; S2MT4 = Lipa sterilized soil + AMF after 12 months from transplanting).

4. Conclusions

Based on the findings of this study, the growth performance of *T. decorticata* seedlings was favorable in their natural habitat, even without biofertilizer application. However, this study affirmed that the species could grow significantly in soil other than from its natural habitat when aided with bio-inoculants such as AMF, which was demonstrated by the significantly improved physiological performance of seedlings grown in the unsterilized Lipa soil media from the MMFR,

which is not a typical natural habitat of the species. The study results render a warranty for the planting of the species outside of its natural habitat provided that an intervention such as inoculation of AMF is provided. Therefore, inoculation of AMF is a potential biotechnology tool for the ex-situ conservation of *T. decorticata* in Mount Makiling Forest Reserve. However, due to the limitations of the study, It is recommended that further studies be conducted to investigate the rate of AMF root colonization and the identification of potential indigenous mycorrhizal fungi present in both QPL and MMFR soil media.

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