Arbuscular mycorrhizal fungi improve biomass, photosynthesis, and water use efficiency of Opuntia ficus-indica (L.) Miller under different water levels (Postprint)

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Abstract

Abstract

Opuntia ficus-indica (L.) Miller is a CAM (Crassulacean Acid Metabolism) plant with remarkable drought adaptation capabilities, employing strategies such as nocturnal atmospheric CO_2 fixation, substantial water storage in stem segments, and reduced root growth. Plants grown under water stress conditions, characterized by thicker stems and fewer fine root hairs, form strong symbiotic relationships with arbuscular mycorrhizal fungi (AMF) to cope with drought. While water stress limits plant growth and biomass production, AMF symbiosis can ameliorate these limitations by enhancing physiological performance. This study investigated the effects of AMF inoculation and varying soil moisture levels on biomass, photosynthesis, and water use efficiency in both spiny and spineless O. ficus-indica varieties. The experiment was conducted in a greenhouse using a complete factorial design with seven replicates, examining O. ficus-indica type (spiny or spineless), AMF presence (with or without), and four soil water availability (SWA) treatments: 0%-25% SWA (T1), 25%-50% SWA (T2), 50%-75% SWA (T3), and 75%-100% SWA (T4). Drought stress reduced biomass and stem segment growth; however, AMF colonization significantly increased biomass production and substantially influenced the physiological performance of O. ficus-indica. The presence of AMF significantly enhanced biomass in both plant types by improving growth, photosynthetic water use efficiency, and photosynthetic capacity. Spines on stem segment surfaces significantly reduced photosynthetic rate and water use efficiency. Net photosynthesis, photosynthetic water use efficiency, transpiration rate, and stomatal conductance all decreased significantly with intensifying drought stress. Under drought conditions, mother stem segments without AMF inoculation failed to establish daughter segments, whereas AMF-inoculated mother segments successfully produced complete daughter segments. AMF root colonization increased significantly as SWA decreased. AMF improved biomass production, enhanced drought tolerance, and optimized photosynthetic and water use efficiency performance in O. ficus-indica. The drought adaptation potential of O. ficus-indica is governed by morphophysiological performance associated with AMF symbiosis.

Full Text

Preamble

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Arbuscular mycorrhizal fungi improve biomass, photosynthesis, and water use efficiency of *Opuntia ficus-indica* (L.) Miller under different water levels

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Abstract: *Opuntia ficus-indica* (L.) Miller is a CAM (crassulacean acid metabolism) plant with extraordinary capacity to adapt to drought stress through its ability to fix atmospheric CO_2 at nighttime, store significant amounts of water in cladodes, and reduce root growth. Plants growing in moisture-stressed conditions with thick, less fine root hairs form strong symbioses with arbuscular mycorrhizal fungi (AMF) to adapt to drought stress. Water stress can limit plant growth and biomass production, which can be ameliorated by AMF association through improved physiological performance. The objective of this study was to investigate the effects of AMF inoculation and variable soil water levels on the biomass, photosynthesis, and water use efficiency of spiny and spineless *O. ficus-indica.* The experiment was conducted in a greenhouse using a full factorial design with *O. ficus-indica* type (spiny or spineless), AMF (presence or absence), and four soil water availability (SWA) treatments with seven replications.

The water treatments consisted of 0%-25% SWA (T1), 25%-50% SWA (T2), 50%-75% SWA (T3), and 75%-100% SWA (T4). Drought stress reduced biomass and cladode growth, while AMF colonization significantly increased biomass production and altered the physiological performance of *O. ficus-indica*. AMF presence significantly increased biomass of both *O. ficus-indica* plant types through improved growth, photosynthetic water use efficiency, and photosynthesis. The presence of spines on cladode surfaces significantly reduced photosynthetic rate and photosynthetic water use efficiency. Net photosynthesis, photosynthetic water use efficiency, transpiration, and stomatal conductance rate significantly decreased with increased drought stress. Under drought stress, some planted mother cladodes without AMF failed to establish daughter cladodes, whereas AMF-inoculated mother cladodes fully established daughter cladodes. AMF root colonization significantly increased with decreasing SWA. AMF caused increased biomass production, enhanced drought stress tolerance, and improved photosynthesis and water use efficiency performance of *O. ficus-indica*.

The potential of *O. ficus-indica* to adapt to drought stress is controlled by morpho-physiological performance related to AMF association.

Keywords: biomass; cactus pear; cladode growth; photosynthesis; water stress; water use efficiency

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1 Introduction

Drought stress affects plant performance (Taghizadeh et al., 2023) by influencing physiological and metabolic processes (Mohammadi et al., 2017; Hu et al., 2020), which can cause functional and interaction alterations of soil microorganisms (Wang et al., 2022; Qianqian et al., 2023). In arid and semi-arid areas, interactions between plants and soil microorganisms play important roles in inducing plant resistance to drought stress and maintaining plant community performance (Birhane et al., 2012; Hu et al., 2020; Taghizadeh et al., 2023). Among soil microorganisms, rhizobacteria and fungi are key actors for maintaining host plant productivity under drought stress (Wang et al., 2022; Taghizadeh et al., 2023). For instance, bacteria like Actinobacteria produce substances (Stevenson and Hallsworth, 2014), and Ascomycota fungi maintain plant productivity by decomposing complex organic matter (Chen et al., 2019). Arbuscular mycorrhizal fungi (AMF) also play significant roles in inducing plant resistance to drought stress and maintaining plant productivity (Hu et al., 2020; Stevens et al., 2020).

Plants benefit from water uptake by AMF hyphae, while AMF acquire carbohydrates from plants (Aiqun et al., 2017), facilitating their mutualistic association (Garcia et al., 2017). AMF can acquire up to 20% of the total carbon fixed by their host plants (Parniske, 2008). Under water-limited conditions, rhizosphere soil water availability is inadequate and terrestrial plant roots cannot easily spread and capture water from the bulk zone (Cristina et al., 2017). Naturally, roots become more active and develop stronger associations with AMF to enhance water uptake through AMF hyphae (Alho et al., 2015; Cristina et al., 2017).

Previous studies have examined AMF inoculation effects on inducing drought resistance and maintaining plant community productivity. For instance, Birhane et al. (2012) studied artificial AMF inoculation effects on inducing drought stress resistance and maintaining *Boswellia papyrifera* (Del.) Hochst. seedling productivity, finding significant effects on plant resistance to drought stress and productivity maintenance. Pereira et al. (2021) investigated AMF inoculation effects on drought tolerance and survival of *Cenostigma microphyllum* Tul. seedlings, finding that AMF-inoculated plants easily rehabilitated from rehydration under water stress. Hu et al. (2020) found that AMF-colonized maize plants better induced drought stress tolerance and improved productivity compared to non-mycorrhizal plants. These investigations indicate that AMF inoculation improves drought resistance and productivity performance of host plants. However, these studies mainly focused on C_4 and C_3 plants. The impacts of AMF inoculation on different plant species, particularly crassulacean acid metabolism (CAM) plants, are relatively rare, with only a few studies assessing native AMF colonization on some CAM plants growing in different land use types. For instance, Pimienta-Barrios et al. (2001) found that native AMF colonization of *Opuntia robusta* J.C. Wendl. improved adaptations for prolonged drought conditions. Birhane et al. (2017, 2020) observed that *O. ficus-indica* growing in arid and semi-arid areas of the Tigray region, Ethiopia was colonized by AMF. Moreover, these studies focused on the degree of native AMF colonization in dry and rainy seasons. However, the role of artificial AMF inoculation on drought stress adaptation of *O. ficus-indica* has not been investigated.

O. ficus-indica belongs to the genus *Opuntia*, sub-family Opuntiodeae, and family Cactaceae (Snyman, 2005; Astello-Garcia et al., 2015). It is indigenous to Central Mexico and the Caribbean region (Salem-Fnayou et al., 2014). *O. ficus-indica* is characterized by spiny or spineless types (Astello-Garcia et al., 2015). It is a CAM plant (Scalisi et al., 2015) widely cultivated in arid and semi-arid areas (Ochoa and Barbera, 2017; Berhe et al., 2022) and is considered drought resistant, tolerant, and an escaping plant (Liguori et al., 2013; Scalisi et al., 2015). Previous studies on drought stress adaptation in this plant have mainly focused on its ability to fix atmospheric CO_2 at nighttime (Ranjan et al., 2016), store significant amounts of water in tissue (Nobel, 2010; Scalisi et al., 2015), and transfer water from water-storage tissue (parenchyma) to photosynthetic tissue (chlorenchyma) (Andrade et al., 2009). Morphological traits such as the presence of spines, areoles, and cladode surface area can be related to drought stress tolerance of *O. ficus-indica* (Pena-Valdivia et al., 2007;

Ranjan et al., 2016). These investigations showed that drought stress adaptation of the plant was related to the nature and phenomena of the cladode. However, drought stress adaptation related to the root is rare. Snyman (2004, 2005) investigated the relationship between drought stress and root dynamics of *O. ficus-indica*, finding that root biomass, thickness, length, and the number of fine roots decreased significantly with increased drought stress. However, these studies did not assess the relationship between AMF root colonization and drought stress adaptation of *O. ficus-indica*. Under drought stress conditions, AMF root colonization of plants can relate to root dynamics (Brundrett et al., 1996; Birhane et al., 2012). *O. robusta*, for example, has higher root thickness, length, and fine roots than *O. ficus-indica* (Snyman, 2005), and AMF colonization increases under water-limited conditions (Pimienta-Barrios et al., 2001). These investigations may indicate that AMF could be more beneficial under drought stress because plants with short, thick, and fewer fine root hairs benefit better, and AMF can replace fine root hairs and serve in the absorption and transport of minerals and water from soil micro-pores (Brundrett et al., 1996). AMF improves morphological performance, mainly biomass production of host plants, by facilitating increased growth, root collar diameter, root length, number of leaves, and leaf area (Birhane et al., 2012; Andrino et al., 2020; Pereira et al., 2021). It also helps improve physiological performance by increasing photosynthesis rate and water use efficiency (Birhane et al., 2012; Andrino et al., 2020; Pereira et al., 2021). Increases in morpho-physiological performance are ensured by combinations of nutritional, morphological, and physiological (Birhane et al., 2012), nutritional and non-nutritional (Auge et al., 2016), and physiological and metabolic mechanisms (Hu et al., 2020). These microbes are also significantly important in increasing photosynthetic water use efficiency through decreased transpiration rates (Birhane et al., 2012; Pereira et al., 2021). AMF colonization also enhances morpho-physiological performance of plants through improved soil physical and chemical properties (Hailemariam et al., 2017). However, AMF abundance (spore density) in soil can decrease with increased drought stress and soil phosphorus availability (Stevens et al., 2020). Its function is also affected by soil disturbance (Trejo et al., 2016).

O. ficus-indica growing in the Tigray region, Ethiopia is traditionally cultivated without irrigation and inorganic fertilizers (Belay et al., 2011). The plant is also dominated by the spiny type (Ochoa and Barbera, 2017). In arid and semi-arid areas, frequent drought occurrence and dominance of the spiny type are major constraints on plant performance as they negatively affect growth and biomass production of *O. ficus-indica* (Snyman, 2005; Belay et al., 2011; Scalisi et al., 2015), photosynthesis rate (Pimienta-Barrios et al., 2005; Liguori et al., 2013), and water use efficiency (Snyman, 2005). Thus, improving plant accessions and increasing plant soil water availability can improve the morpho-physiological performance of *O. ficus-indica* cladodes (Snyman, 2005; Scalisi et al., 2015). However, the interactive effect of plant soil water availability and *O. ficus-indica* type on the morpho-physiological performance of cladodes in response to AMF inoculation is unclear. In this study, we focused on the role of AMF symbiosis on the performance of spiny and spineless *O. ficus-indica* under various soil water availability (SWA) treatments. We also reported how AMF symbiosis affected the productivity performance of *O. ficus-indica*. We hypothesized that: (1) spiny *O. ficus-indica* cladodes show lower morpho-physiological performance than spineless; (2) mycorrhizal *O. ficus-indica* cladodes show higher morpho-physiological performance than cladodes without AMF; (3) morpho-physiological performance of *O. ficus-indica* cladodes increases with increase in soil water availability; (4) mycorrhizal spineless *O. ficus-indica* cladode types show higher morpho-physiological performance than mycorrhizal spiny cladodes under less water levels; and (5) AMF improve the performance of both spineless and spiny *O. ficus-indica* cladodes with decrease in plant soil water availability.

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2 Materials and methods

The experiment was conducted at the greenhouse of Mekelle Research Center in Mekelle City, Tigray, Ethiopia (13°29 N, 39°28 E; 2000 m a.s.l.) from September 2019 to March 2021. The greenhouse had mean day/night temperatures of 26°C/22°C and mean daily average relative humidity of 51%.

2.1 Mother cladodes and soil preparation

One-year-old spiny and spineless mother cladodes of green *O. ficus-indica* were obtained from Mekelle Research Center. We selected mother cladodes based on their morphological traits following procedures by Snyman (2005) and Belay et al. (2011). Cladodes used for the study were of similar weight, size, and thickness, and number of areoles per pad differed only in the presence or absence of spines (Table 1).

The cladodes were green, healthy, and fresh. The spiny cladodes were 35.58 ($±0.21)*cmlong*, 19.53(±0.16)*cmwide*, *and*1.09(±0.54)*cmthick*, *respectively*(*Table*1).*Thenumberofareolesand*
areoles.

After collection, cladodes were air-dried at room temperature under shelter and shade for four weeks (Snyman, 2004). The samples were preserved with a mean daily relative humidity of 51% and temperatures of 25°C–30°C during the day and 15°C–18°C at night. This promoted healing of the cut area of the planting materials for successful root development (Snyman, 2005).

The soil mix used for the study was composed of three parts soil excavated from the rhizosphere of *O. ficus-indica* plantations in the field and two parts pure river sand. Mixed soil prepared for all pots was of uniform character. The clay, silt, and sand contents of the soil were 12.8%, 7.1%, and 80.1%, respectively. The soil mix had a bulk density of 1.52 g/cm^3, pH of 7.77, and electrical conductivity of 0.11 dS/m. Organic carbon, total nitrogen, available potassium, and available phosphorous contents of the soil were 1.04%, 0.7%, 0.5 mg/g, and 1.103 mg/g, respectively.

2.2 AMF extraction, cultivation, and application

Soil samples were excavated using a handheld hoe and collected from the rhizosphere of *O. ficus-indica* growing areas following Birhane et al. (2017). For spore extraction, sample soils were first air-dried and passed through a 0.75-mm sieve to remove unnecessary materials, and were weighed using a sensitive balance to obtain a 25 g sub-sample (Brundrett et al., 1996). The weighed sample was soaked in 100 mL tap water for at least 30 min and shaken for 30 min, then decanted through a series of sieves arranged in ascending order with the smallest sieve size (50 µm) at the bottom, followed by 100, 300, and 750 µm largest sieve size at the top.

Spores on the three different sieve sizes were poured into their respective jars. Jars were well fixed and then centrifuged at 2000 r/min for 5 min with water and poured into the smallest sieve size, and the pellet was discarded. Sieves were washed for the second time and poured into a jar filled with 50% sucrose, centrifuged for 1 min at 2000 r/min, poured onto the finest sieve (50 µm), and carefully washed with water to remove the sucrose. The pellet was discarded. Finally, spores were washed and poured onto pre-wetted filter paper in a funnel and placed in an inverted petri dish to store the filter papers with spores. Spores were counted using a dissecting microscope with $×$400 magnification. Then the sterile soil was mixed with *Glomus*, *Acaulospora*, and *Scutellospora* AMF genera types, with an average spore density of 196.6 spores/100 g dry soil.

Pots were filled with 10.5 kg of soil each and AMF pot cultures were grown in a greenhouse using *Sorghum bicolor* (Linn.) Moench plants following the method of Birhane et al. (2012). The viability of the sorghum seeds was determined before planting. Twelve sorghum seeds were planted in each pot and grown for 60 d. Soil and root samples were collected from each pot culture for further determination of AMF root colonization and spore density. Spore density and AMF root colonization were determined using a compound microscope with $×$400 magnification.

The average spore density and AMF root colonization 60 d after planting *S. bicolor* were 198.8 spores/100 g soil and 99.21%, respectively, and these values were used to determine the weight of AMF inoculum added to the pot. The number of spores added to the center of each pot was close to 400 spores (198.8 spore$×$201.0 g/100 g soil). Fungal inoculums were composed of a mixture of soil, spores, and root fragments, and 201.0 g of inoculum was added to the center of each pot of the mother cladode. To mimic the optimum rhizosphere ecosystem and increase AMF performance, we added 300 mL microbial wash created through the extraneous extraction solution (without spores) from fungi inoculums to the center of each pot. Adding 400 spores and 300 mL microbial wash is recommended for improved plant performance and positive protection of insects (Frew et al., 2017).

2.3 Planting cladodes

Mother cladodes of *O. ficus-indica* were planted in soil-filled cylindrical plastic containers with top and bottom diameters of 32 and 27 cm, respectively, and 30 cm in height. The cladodes were planted upright with one-quarter of their height covered with soil and were grown for 18 months. Each pot was filled with 18.50 kg of autoclaved and dried mix soil. Experimental plants were grown individually in the filled autoclaved growing media. The pots had 5 drainage holes of 6.5 mm diameter at the bottom. The pots were placed on their covers to prevent nutrient and water loss through the holes (Snyman, 2004).

2.4 Experimental design and treatment

The treatments consisted of three factors: AMF (present or absent), *O. ficus-indica* type (spiny or spineless), and four water treatments (0%-25% SWA (T1), 25%-50% SWA (T2), 50%-75% SWA (T3), and 75%-100% SWA (T4)) (Snyman, 2004). Each pot contained one cladode, and each mother cladode was replicated seven times across treatments, making a total of 112 mother cladodes (2 *O. ficus-indica* type × 2 AMF × 4 SWA × 7 replications) in 112 pots. In this experiment, an additional 12 experimental pots filled with the same quantity of autoclaved mix soil (18.50 kg each) were considered as controls and used to determine the percent of SWA.

The weight values were determined as the permanent wilting point (PWP) of the soil pots. The controlled soil pots were saturated with water and reweighed after 48 h, and the saturated soil weight of the pots was 20.64 kg, which was used to determine pot water capacity (PWC). The amount of water added to every plant was determined following Snyman (2004).

Plant soil water available = Pot water capacity -Permanent wilting point
Volumetric soil water = Surface area (pot) × Height (water depth)

Accordingly, SWA values at PWP and PWC were 0.073 and 0.285 mm water/mm soil pot depth, respectively. The total SWA was 0.212 mm water/mm soil pot depth or 63.600 mm water/pot. This value was used to determine and monitor SWA for exact water treatment.

2.5 Mycorrhizal colonization

Root samples were collected from treated cladodes and preserved in plastic jars filled with 50% ethanol (Birhane et al., 2012). Collected roots were cut into 1 cm pieces and bleached using 10% potassium hydroxide in a heat-resistant jar. The roots were autoclaved at 121°C for 15 min (Brundrett et al., 1996). The roots were immersed in 10% hydrogen peroxide for about 15 min for further bleaching and clearing, and acidified with 2% HCl for about 1 h at room temperature. They were stained in trypan blue (0.05% in 5:1:1 lactic acid:glycerol:distilled water ratio) overnight. Stained roots were washed, immersed in 50% glycerol for 1-2 h, de-stained, and preserved until further processing. Afterwards, stained roots were mounted lengthwise on slides in replicates of nine from every individual treated cladode. Proportional root colonization by AMF was estimated using the magnified intersection method with a hairline graticule inserted into an eyepiece that acted as the lines of intersection at 0, 30, 60, and 90 inches following Giovannetti and Mosse (1980). AMF structures (arbuscules, vesicles, and hyphae) were identified using a compound microscope at $×$400 magnification.

2.6 Measurement of morpho-physiological cladode traits

Morpho-physiological trait measurements were conducted at the end of the experiment. Morphological traits measured in this experiment were cladode biomass, number of daughter cladodes, number of spines, and areoles. The total number of 1st and 2nd emerging and up-growing daughter cladodes per mother cladode, number of spines per areole, and number of areoles per cladode were manually counted and recorded. Cladode height and breadth were measured using a ruler. Cladode thickness was measured using a digital caliper. Fresh plant biomass per pot was measured using a digital balance. Cladode area was calculated using the method of Tiznado-Hernández et al. (2010).

Cladode area = Height × Breadth

The height of cladodes was recorded twice per month and continuously measured for the first four months. It was used to evaluate the rate of growth (Scalisi et al., 2015).

Net photosynthesis rate (Pn), transpiration rate (E), and stomatal conductance (C) were measured as physiological traits of the daughter cladodes. These traits were measured using a portable CI-340 Handheld (CID Bio-Science, Inc., Camas, USA) photosynthesis system. Photosynthetic water use efficiency (PWUE) was determined following Birhane et al. (2012). The traits were also measured after 18 months of growth under the treatments. The traits were taken six times over a 2400 h period with 400 h intervals on 4 March, 2021.

2.7 Statistical analysis

Data for the number of 1st emerged cladodes, biomass, height, breadth, and thickness of daughter cladodes, number of spines per areole, areoles per cladode, number of up-growing daughter cladodes, Pn, E, C, and PWUE were subjected to analyses of variance (ANOVA) using SPSS 2004 software. Variations of AMF in *O. ficus-indica* daughter cladode traits were tested using three-way ANOVA. Fractional root colonization of AMF in plant roots was tested using two-way ANOVA. After checking for normality of data distribution, we performed Gabriel post hoc test for unequal sample size and least significant difference (LSD) for main effect comparison. Standard errors were used to show estimates of variability. LSD (α=0.05) was used to compare means. P-values were used to show significance levels.

3 Results

3.1 Morphological traits of daughter cladodes

After nurturing the mother cladodes in the greenhouse for 18 months, we measured and analyzed the main effects of *O. ficus-indica* type, AMF, water treatment, and their interaction on the morphological traits of the daughter cladodes (Fig. 1 [Figure 1: see original paper]; Table 2). Numbers of established and up-growing daughter cladodes were not significantly affected by *O. ficus-indica* type and AMF inoculation. Fresh biomass was significantly affected by treatments. The presence of spines significantly reduced the height, breadth, and surface area of the cladodes, but improved the number of areoles per cladode (Table 2). AMF inoculation significantly increased the height, breadth, thickness, and surface areas of the cladodes. Water treatments were a significant source of variation for numbers of established, up-growing, height, breadth, thickness, and surface area of cladodes, and these traits significantly decreased with increasing water stress (Table 2). Significantly lower numbers of areoles were recorded in highly water-stressed cladodes. Significant interactions were observed for *O. ficus-indica* type×AMF and *O. ficus-indica* type×SWA for up-growing, cladode biomass, height, breadth, number of areoles, and total spines per cladode. *O. ficus-indica* type×AMF×SWA interaction significantly affected up-growing, cladode biomass, height, breadth, thickness, number of areoles, spines per areole, and total spines per cladode.

3.2 Cladode growth

Spineless daughter cladodes showed significantly higher growth rates at 30 and 45 d of growing periods (Table 2). AMF was not a significant source of variation. Cladode growth rates were significantly affected by water treatments, which increased growth with increasing SWA (Table 2). Cladode growth rates were significantly affected by the interactions of *O. ficus-indica* type×AMF, *O. ficus-indica* type×SWA, AMF×SWA, and *O. ficus-indica* type×AMF×SWA. Cladodes exhibited the highest growth rates at 60 d of growing period, followed by 75 and 45 d of growing periods. Growth rates of the cladodes had similar rates at 75 d of growing period (Fig. 1 [Figure 1: see original paper]).

3.3 AMF root colonization

AMF root colonization was observed only in the roots of *O. ficus-indica* inoculated with AMF. AMF root colonization was significantly affected by SWA, but the presence of spines was not a significant source of variation (Table 3). Mycorrhizal colonization significantly varied by the interaction effect of *O. ficus-indica* type×SWA. The highest average fractional colonization was recorded for hyphal colonization, followed by arbuscular and vesicular colonization. Hyphal, arbuscular, and vesicular colonization significantly decreased with increasing levels of SWA (Table 3).

3.4 Physiological traits of daughter cladodes

Photosynthesis rate and water use efficiency were significantly affected by *O. ficus-indica* type, AMF, and SWA (Table 2). Transpiration rate was not significantly affected by *O. ficus-indica* type and AMF, but SWA was a significant source of variation. AMF and SWA were significant sources of variation in stomatal conductance. The presence of spines in the cladodes significantly reduced the rate of photosynthesis and photosynthetic water use efficiency throughout day and night times (Table 2). Stomatal conductance was significantly reduced during daytime. At nighttime, AMF-inoculated cladodes showed significantly higher photosynthesis rates than plants without AMF. Water use efficiency was significantly higher for AMF-inoculated cladodes than for controls during both day and night times. Photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency decreased with increasing water stress during both day and night times (Table 2). Significantly lower physiological traits were recorded at 0%–25% SWA level. Except for water use efficiency, all traits were not significantly affected by the interactions (Table 2).

4 Discussion

The hypothesis that spiny cladodes show lower morpho-physiological performance than spineless is supported in this study. The presence of spines significantly influenced morphological traits, mainly fresh biomass and growth rates at 30 and 45 d of growing periods (Fig. 1 [Figure 1: see original paper]; Table 2). The negative spine effect is due to reduced height, breadth, and surface area of the cladodes, which were evident by the significantly lower height, breadth, and area of spiny cladodes compared to spineless. According to Pea-Valdivia et al. (2008), spines in *O. ficus-indica* significantly influenced the breadth and area of the cladodes. In contrast, Adli et al. (2016) reported that wide and large areas of the cladodes did not significantly vary between spiny and spineless accessions. The presence of spines also significantly affected the photosynthetic rate and photosynthetic water use efficiency of the cladodes during both day and night times (Table 2). The decrease in Pn and PWUE in spiny cladodes was due to the presence of spines. The spines in spiny cladodes were distributed throughout the surface area of the cladodes. Spines dispersed on the surface area of the cladodes can interrupt solar energy that functions to reduce surface temperature and water loss of the cladodes (Ranjan et al., 2016). Due to different Pn and PWUE of the cladodes of the two accessions, spineless *O. ficus-indica* had higher fresh biomass than spiny ones. Biomass of the cladodes increased with an increase in Pn (Scalisi et al., 2015) and water use efficiency (Snyman, 2013).

The hypothesis that cladodes with AMF show higher biomass performance than cladodes without AMF is supported in this study. Several previous studies have reported that AMF inoculation improves biomass production of host plants by facilitating increased growth, root collar diameter, root length, number of leaves, and leaf area (Birhane et al., 2012; Hailemariam et al., 2017; Andrino et al., 2020). These microbes are also significantly important in increasing biomass production through decreased transpiration rates (Birhane et al., 2012). In contrast, Pereira et al. (2021) observed that biomass production of plants growing at high soil water availability did not significantly vary between AMF-inoculated and non-inoculated plants. Our findings suggest the presence of AMF significantly improved the height, breadth, thickness, and area of daughter cladodes, which in turn improved the biomass of *O. ficus-indica* (Table 2; Fig. 2 [Figure 2: see original paper]). Biomass of cladodes increased with an increase in thickness (Scalisi et al., 2015). Daughter cladode growth rates were not improved by AMF alone but were clearly increased when AMF was associated with *O. ficus-indica* type and SWA. These results are consistent with the findings of Hailemariam et al. (2017) who investigated the effects of different SWA levels and AMF inoculation on growth of *Faidherbia albida* (Del.) A. Chev. Specifically, the result of this study showed that at lower SWA levels, higher growth was observed for AMF-inoculated *F. albida* than non-mycorrhizal plants. Growth of cladodes could not be affected by a single environmental factor, but also by many other factors (Scalisi et al., 2015). At low soil water availability, the root of *O. robusta* associated with AMF positively influences the establishment of new cladodes (Pimienta-Barrios et al., 2001). Similarly, Pereira et al. (2021) observed that at low plant soil water availability, AMF inoculation significantly affects the growth and survival of *Cenostigma microphyllum* (Mart. ex G. Don) E. Gagnon and G. P. Lewis. Developing new daughter cladodes could improve the growth of mother cladodes (Scalisi et al., 2015). Cladodes with AMF improved Pn and PWUE (Table 2; Fig. 3 [Figure 3: see original paper]). This was similarly reported by Birhane et al. (2012) with *Boswellia papyrifera* (Del.) Hochst. According to Birhane et al. (2012), *B. papyrifera* seedlings with AMF significantly influenced Pn and PWUE. Andrino et al. (2020) observed that AMF inoculation increased the leaf area of *Solanum lycopersicum* L. species, which positively influenced Pn.

We found that the morpho-physiological cladodes were more beneficial at lower SWA levels (Table 2; Fig. 3 [Figure 3: see original paper]). Increasing SWA level can improve root biomass production of *O. ficus-indica* (Snyman, 2004, 2005). It also improves the cladode relative water content, cladode thickness, and rhizosphere soil moisture content of *O. ficus-indica* (Scalisi et al., 2015). Thus, improving the biomass production of mycorrhizal *O. ficus-indica* plant was ensured by the combinations of morpho-physiological performance change.

During nighttime, mycorrhizal cladodes showed significantly higher Pn than control cladodes (Table 2). PWUE was significantly higher for mycorrhizal cladodes than non-mycorrhizal cladodes during both day and night times. During daytime, PWUE of *O. ficus-indica* plant was observed negative with 0%–25% SWA level. PWUE was related to Pn of the plant because Pn of *O. ficus-indica* plant can be negative during extreme drought (Nobel and De la Barrera, 1999, 2004).

ANOVA result shows that all physiological traits (Pn, E, C, and PWUE) were significantly related and varied with time difference (Table 4). Higher physiological traits were observed during nighttime. This was related to the high water-stress levels of SWA. During daytime, the transpiration rate decreases with the increase in surface temperature of the cladodes (Nobel and De la Barrera, 2004). With the water loss of the cladodes, Pn and PWUE also decreased by improving the degree of stomatal closure during drought stress (Nobel and De la Barrera, 1999, 2004; Liguori et al., 2013).

5 Conclusions

The potential of *O. ficus-indica* to adapt to drought stress is not only related to its morpho-physiological performance but also its association with AMF. AMF caused an increase in biomass production, increased tolerance to drought stress, and improved photosynthesis and water use efficiency performance of *O. ficus-indica*. Under drought stress, some planted mother cladodes without AMF failed to establish daughter cladodes, whereas AMF-inoculated mother cladodes fully established daughter cladodes. High degrees of AMF root colonization were recorded under low plant soil water availabilities. The level of water supply affects the impact of AMF root colonization performance on *O. ficus-indica*. AMF has interacted with decreasing levels of SWA. Thus, mycorrhizal *O. ficus-indica* plants with low levels of SWA increased their benefits. AMF associated with *O. ficus-indica* type and SWA increase growth and improve biomass, Pn, and PWUE. The presence of spines on the surface area of *O. ficus-indica* cladodes can cause decreased growth, biomass, height, breadth, and surface area by reducing Pn and PWUE. These results have direct consequences for the management of AMF technology. Drought stress and dominance of spiny *O. ficus-indica* plants in the areas should be considered to improve the early growth and biomass of *O. ficus-indica*, and allow the rapid establishment of *O. ficus-indica* daughter cladodes.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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