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# Arbuscular Mycorrhizal Inoculation Enhances Drought Tolerance and Physiological Performance in Avocado (*Persea americana*).

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**Abstract**

Drought severely limits avocado production, necessitating sustainable resilience strategies. This study investigated the effects of arbuscular mycorrhizal fungi (AMF) inoculation (*Gigaspora* and *Rhizophagus* spp.) on the drought tolerance of two avocado races ('Mexicana' and 'Guatemalan') under controlled shade house conditions. A factorial experiment with four moisture-withholding intervals (daily, 7, 14, and 21 days) and AMF treatments were conducted. AMF inoculation significantly enhanced seedling morpho-physiology under all stress levels. Inoculated plants exhibited a 12% increase in plant height and a 10% increase in internode length compared to non-inoculated controls. Leaf area, fresh weight, and dry weight improved by 32.5–43.9%, 24.2–29.9%, and 20–26.6%, respectively, under drought. Root system development was profoundly enhanced, with root number and taproot length increasing by 26–38.5%, and root biomass surging by 46.2% (fresh) and 38.8% (dry) under the severest 21-day stress. Physiologically, AMF increased stomatal conductance by 23–41% and total chlorophyll content by up to 24.4%, supporting higher photosynthetic rates. The 'Mexicana' race consistently outperformed 'Guatemalan,' showing superior leaf area, biomass, and water use efficiency under stress. AMF colonization rates were highest (67.9%) under daily watering and declined (39.7%) with severe drought. We conclude that AMF symbiosis significantly mitigates drought impairment in avocado seedlings, with 'Mexicana' demonstrating greater inherent tolerance. This synergy offers a viable strategy for enhancing resilience in water-limited avocado cultivation.

**Keywords:** Mycorrhizal symbiosis; race variation; seedling physiology; water-stress mitigation

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## 1. Introduction

Avocado (*Persea americana* Mill.) is a nutrient-rich fruit crop of the Lauraceae family that flourishes in tropical and subtropical climates (Lyu et al., 2023; Zuazo et al., 2021). Unlike many fruits, it contains low sugar but is abundant in essential vitamins, minerals, and healthy lipids (Stephen & Radhakrishnan, 2022). In Ethiopia, it is increasingly cultivated by smallholder farmers, particularly in regions such as Sidama and Hararge (Legesse et al. 2024). However, recurrent drought intensified by climate change has become a major constraint to avocado productivity. Water deficit reduces soil moisture availability, hampers nutrient uptake, and disrupts photosynthetic performance (Torres-Quezada et al., 2025). In avocado specifically, drought stress leads to reduced stomatal conductance and diminished photosynthetic efficiency (Lin et al., 2022).

Plants often rely on root-associated microbiomes to enhance drought tolerance through escape, avoidance, or physiological adaptation mechanisms (Munir et al., 2022). Arbuscular mycorrhizal fungi (AMF), which form symbioses with more than 80% of terrestrial plants, improve water and nutrient acquisition, enhance antioxidant activity, and mitigate oxidative damage under drought stress (Diagne et al., 2020). They also contribute to soil carbon cycling (Sheteiwy et al., 2021) and increase the availability of phosphorus and micronutrients (Khaliq et al., 2022). While AMF has shown promise in improving avocado seedling vigor and drought resilience, comparative studies across different avocado races and their physiological and biochemical responses to AMF under progressive drought remain limited. Given avocado's shallow root system and strong mycorrhizal dependency, addressing these gaps is essential. Therefore, this study aims to evaluate shoot morpho-physiological responses to AMF under moisture stress; Assess root growth and development in AMF-inoculated seedlings under drought; Determine key biochemical responses influenced by AMF during moisture stress.

## 2. Materials and Methods

### *2.1. Study Site, Plant Materials, and Experimental Design*

The experiment was conducted in a controlled shade-house environment at Hawassa University, Ethiopia (7°4′ N, 38°31′ E; 1669 m a.s.l.) between February and August 2022. The site receives 900–1100 mm of annual rainfall, with temperatures ranging from 12°C to 27°C. Two avocado races Mexicana and Guatemalan were selected due to their uniform germination behavior and suitability as rootstocks (Rendón-Anaya et al., 2019). Seed materials were sourced from Yirgalem Agro-Processing Industry.

A factorial completely randomized design (CRD) was used with three replications. Treatments included four moistures-stress regimes (daily watering; irrigation withheld for 7, 14, and 21 days), two AMF treatments (inoculated with *Gigaspora* and *Rhizophagus* spp. vs. non-inoculated), and two avocado races, resulting in 16 treatment combinations. Each treatment was replicated in four pots, yielding 48 experimental units and 192 seedlings in total.

### *2.2. Methodology*

Five-liter pots (22 cm diameter; 20 cm height) were filled with a 3:2:1 mixture of topsoil, compost, and sand. Two seeds were planted per pot. AMF inoculum, propagated on *Sorghum bicolor*, exhibited >80% colonization and >750 spores per 100 g soil after 45 days. Seedlings were irrigated daily for two weeks and thereafter every two days. At eight weeks, 150 g of AMF inoculum was applied to each seedling. Moisture-stress treatments commenced when plants reached the pencil-size stage (~15 cm height) (Esgrina & Tan, 2025). Pre-planting soil characterization identified a sandy loam texture (62% sand, 14% clay, 24% silt), slightly alkaline pH (7.8), CEC of 32.4 meq/100 g, organic carbon (1.17%), total nitrogen (0.1009%), and available phosphorus (11 ppm).

*2.3. Weather Monitoring*

Microclimate conditions inside the shade house were recorded on 24 randomly selected days using Testo 174 data loggers. Temperature varied from 17.20°C (early morning) to 34.97°C (midday), averaging 22.4°C. Relative humidity ranged from 38.27% to 74.35%, with a mean of 62.4%. Vapor pressure deficit (VPD) fluctuated between 0.51 kPa and 3.46 kPa, averaging 1.33 kPa.

*2.4. Data Collection*

Morphological measurements included plant height, leaf number, and leaf area measured using a LI-3000A leaf area meter (LiCor, Lincoln, Nebraska, USA) on three randomly selected plants per experimental unit. Leaf fresh and dry weights were determined after oven-drying samples at 75°C for 48 hours to constant weight. Internode length was measured along the main stem.

Root morphological traits including total root length, diameter, number, and biomass were assessed from three seedlings per treatment. Root anatomical characteristics, including xylem structure, were examined following fixation protocols described by Hiti-Bandaralage et al. (2022). AMF colonization percentage was quantified using the method of Kaur & Singh (2024).

Physiological measurements of gas-exchange parameters using a LI-6400 portable photosynthesis system, chlorophyll concentration via spectrophotometry (Ramazan et al. 2021), stomatal anatomical features, and relative leaf water content following Pérez-Harguindeguy et al. (2013). Biochemical analyses focused on proline quantification following the method of (Bita & Gerats, 2013). Biomass assessment involved determining fresh and dry weights of whole plants five months after planting.

*2.5. Data analysis*

All parameters were analyzed using PROC GLM for ANOVA via SAS software version 9.2 (Moodie and Johnson 2022). Means were separated using the LSD test at a 5% significance level. Pearson correlation analysis was conducted to assess variable relationships.

## 3. Results and Discussion

*3.1. Morphological and growth parameters*

Statistical analysis revealed significant effects of arbuscular mycorrhizae (AM) inoculation and moisture intervals on plant height and internode length ($P$<0.001) but not race ($P$>0.05) (Table 1). AM-inoculated, daily watered avocado seedlings achieved maximum height (27.50 cm) and internode length (0.79 cm), outperforming non-inoculated, water-stressed plants (24.18 cm, 0.71 cm), with 12% and 10% increases, respectively, consistent with Solórzano-Acosta et al. (2024). Growth improvement may result from AMF-induced phytohormones (Santoyo et al., 2021), influencing cell morphogenesis (Jing et al., 2022). Daily watering yielded better growth (29.88 cm, 0.88 cm) than 21-day drought (22.95 cm, 0.62 cm), aligning with Sun et al. (2023).

**Table 1.** Effect of arbuscular mycorrhizae (AMF) and moisture withholding on plant height and internode length

| AMF | Plant height | Internode length |
|---|---|---|
| Inoculated | 27.504[a] | 0.79[a] |

| AMF | Plant height | Internode length |
|---|---|---|
| Non-inoculated | 24.18[b] | 0.71[b] |
| LSD (1%) | 1.05 | 0.005 |
| | | |
| Daily | 29.88[a] | 0.88[a] |
| 7 days | 25.6 [b] | 0.78[b] |
| 14 days | 24.9[b] | 0.72[b] |
| 21 days | 22.95[c] | 0.62[c] |
| LSD (5%) | 1.495 | 0.0781 |
| CV (%) | 6.94 | 12.44 |

Means with different letters in a column are statistically significant at p<0.05.

### *3.1.1. Leaf number, area, fresh and dry weight of avocado leaves*

ANOVA revealed that AMF and moisture withholding significantly affected avocado seedlings' leaf number, area, fresh weight, and dry weight, with their interaction also significant. However, race interactions with moisture and AMF, and the three-way interaction, were non-significant (Table 2). AMF-inoculated, daily watered seedlings had the highest leaf number, while non-inoculated, 21-day water-stressed seedlings had the lowest. AMF increased leaf number by 10.6–14.8% across stress levels (Table 2), aligning with Sun et al. (2024). AMF also enhanced leaf area (32.5–43.9%), fresh weight (24.2–29.9%), and dry weight (20–26.6%) under stress, consistent with Chandrasekaran, (2022). Mexicana outperformed Guatemalan in leaf area (282.88 cm² higher under 14-day stress), supporting Chután et al. (2023) and Ruiz-Chután et al. (2023).

**Table 2.** Interaction effect of arbuscular mycorrhiza (AMF)and moisture stress on leaf traits in avocado seedlings

| AMF | Moisture withholding | Leaf number | Leaf area (cm²) | Leaf fresh weight (gm) | Leaf dry weight(gm) |
|---|---|---|---|---|---|
| Inoculated | Daily | 21.20[a] | 1268.6[a] | 25.08[a] | 6.7[a] |
| | 7-days | 16.85[c] | 899.3[b] | 18.12[c] | 4.5[c] |
| | 14-days | 15.20[d] | 730.7[c] | 13.2[d] | 3.6[d] |
| non-inoculated | Daily | 18.10[b] | 652.0[c] | 18.9[b] | 5.4[b] |
| | 7-days | 14.35[f] | 503.9[d] | 12.7[d] | 3.3[d] |
| | 14-days | 13.58[f] | 493.1[d] | 9.4[f] | 2.7[e] |
| | 21-days | 11.23[g] | 316.9[e] | 7.8g | 2.4[f] |
| LSD (5%) | | 0.74 | 55.063 | 0.36 | 0.13 |
| CV (%) | | 4.07 | 13.7 | 4.3 | 5.6 |

Means with different letters in a column differ significantly at ***P*** < 0.05 (LSD test). CV = Coefficient of Variation.

### *3.1.2. Root number, tap root length, and diameter*

Root number and length were significantly affected by races, AMF, and moisture withholding (*P*<0.01) Table 3. The Guatemalan race had the highest root number under daily watering, while stressed, non-inoculated seedlings showed the lowest. AMF increased root number and taproot length by 38.5%, 36.5%, and 37.6% in Mexicana and 32.3%, 26%, and 28.8% in Guatemalan at 21, 14, and 7 days of water withholding, respectively, aligning with Solís-Ramos et al. (2023) and Almanza et al. (2025) Root diameter was also significantly influenced (p<0.01), with the maximum (8.03 mm)

in AMF-inoculated Mexicana under daily watering and the minimum (1.80 mm) in non-inoculated Guatemalan seedlings after 21 days, consistent with Lopes (et al., 2021)
and Solís-Ramos et al. (2023).

**Table 3.** Interaction effects of AMF, moisture withholding and avocado races on root number, root length and average root diameter

| **Race** | **Moisture** | **Root number** | | **Taproot length (cm)** | | **Average root diameter (mm)** | |
|---|---|---|---|---|---|---|---|
| | Withholding | inoculated | Non inoculated | inoculated | non-inoculated | inoculated | non-inoculated |
| Mexican | | 70.76$^{a}$ | 49.66$^{f}$ | 58.16$^{a}$ | 42.33$^{f}$ | 8.03$^{a}$ | 5.76$^{c}$ |
| | 7-days | 59.33$^{b}$ | 39.33$^{h}$ | 56.16$^{b}$ | 40.23$^{g}$ | 5.33$^{de}$ | 3.50$^{h}$ |
| | 14-days | 52.66$^{de}$ | 35.00$^{ij}$ | 52.83$^{c}$ | 34.23i | 4.73$^{f}$ | 3.23$^{i}$ |
| | 21-days | 51.00$^{ef}$ | 31.33$^{k}$ | 48.66$^{d}$ | 26.33$^{ij}$ | 3.10$^{i}$ | 2.2$^{k}$ |
| Guatemalan | daily | 71.33a | 54.33$^{cd}$ | 47.00$^{e}$ | 38.50$^{h}$ | 6.36$^{b}$ | 5.53$^{cd}$ |
| | 7-days | 55.33$^{b}$ | 37.00$^{i}$ | 57.66$^{b}$ | 40.23g | 5.26$^{e}$ | 3.80$^{g}$ |
| | 14-days | 47.33$^{g}$ | 33.66$^{ij}$ | 53.66$^{c}$ | 25.36$^{j}$ | 4.78$^{f}$ | 2.53$^{i}$ |
| | 21-days | 46.00$^{g}$ | 31.33$^{k}$ | 43.66$^{f}$ | 37.00$^{h}$ | 3.60$^{gh}$ | 1.80$^{l}$ |
| **LSD (1%)** | | **2.26** | | **1.63** | | **0.25** | |
| **CV (%)** | | **2.83** | | **2.22** | | **3.52** | |

Means with different letters in a column are statistically significant at P < 0.01. CV = coefficient of variance.

*3.1.3. Root fresh and dry weight*

Root fresh and dry weights were significantly influenced (P<0.001) by the interaction of AMF × moisture and race × moisture, but not by the three-way interaction ($P$>0.05). Daily watered, AMF-inoculated avocado seedlings had the highest root numbers, while water-stressed, non-inoculated ones had the lowest. Inoculated plants showed 10–45% higher root weights across water treatments (Table 4), with 46.2% (fresh) and 38.77% (dry) increases under 21-day stress. Soti et al. (2021) observed reduced root biomass under prolonged stress, mitigated by AMF.

**Table 4.** Interaction effect of arbuscular mycorrhiza (AMF)and moisture withholding interval on root fresh weight and root dry weight

| **AMF** | **Moisture withholding** | **Root fresh weight (gm)** | **Root dry weight (gm)** |
|---|---|---|---|
| Inoculated | Daily | 23.63$^{a}$ | 4.78$^{a}$ |
| | 7$^{th}$ day | 18.73$^{c}$ | 3.56$^{c}$ |
| | 14$^{th}$ day | 18.08$^{c}$ | 3.25$^{d}$ |
| | 21$^{st}$ day | 14.28$^{d}$ | 2.40$^{f}$ |
| Non-inoculated | Daily | 20.98$^{b}$ | 4.26$^{b}$ |
| | 7$^{th}$ day | 14.16$^{d}$ | 2.60$^{e}$ |
| | 14$^{th}$ day | 12.65$^{e}$ | 2.20$^{g}$ |
| | 21$^{st}$ day | 7.68$^{f}$ | 1.45$^{h}$ |
| **LSD (5%)** | | **1.02** | **0.27** |
| **CV (%)** | | **3.77** | **5.34** |

Means with different letters in a column are statistically significant at P < 0.05. CV = coefficient of variance.

*3.1.4. Root xylem vessel, number and width*

Xylem number and width were significantly influenced ($p$<0.01, Figs. 1 and 2) by race and moisture interactions. Mexicana seedlings under 21-day water withholding had the highest xylem number,

while daily watered Guatemalan seedlings had the lowest. These findings align with Andrés-Andrés-Hernández et al. (2023)
who reported drought-adapted pine species develop xylem with larger lumens and narrower walls. Similarly, Hakim et al. (2021) found AM-inoculated fruit crops rootstock produced more metaxylem with larger diameters. Drought increased xylem number but reduced width, likely due to additional protoxylem formation (Cardoso et al., 2019). Oxidative stress may drive xylem differentiation via cytokinin and jasmonic acid Jang and Choi (2018), while narrower xylem reduces embolism risk, as seen in soybean.

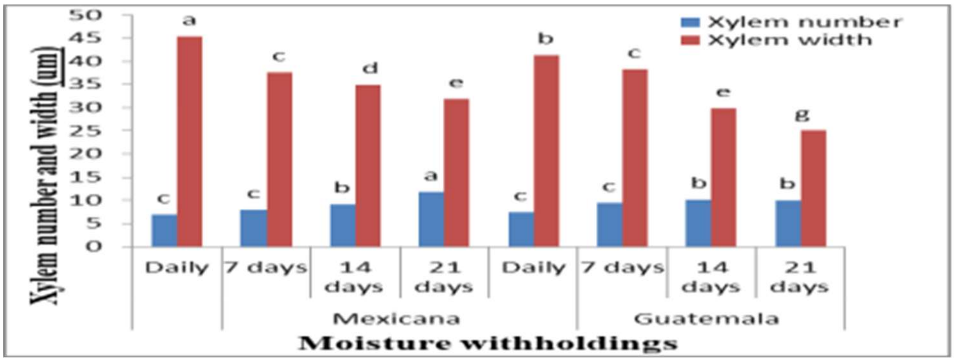
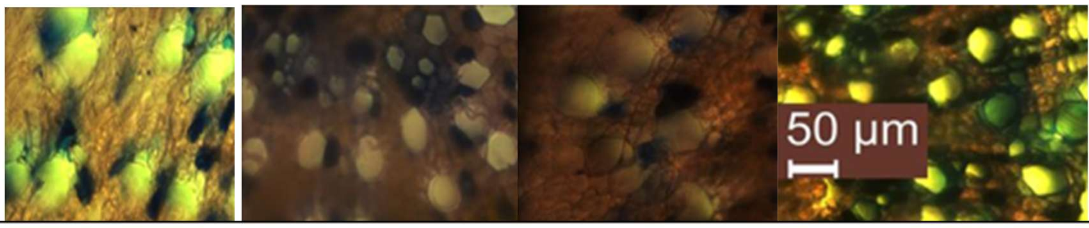



**Fig. 1:** Mean value of xylem number and width of 2 avocado races as influenced by soil moisture withholding

a B C D




a=Daily mexicana,B=21 days mexicana,C= Daily Guatemalan and D=21 days Guatemalan,

**Fig. 2:** Pictorial representations of xylem numbers affected by moisture stress

*3.2. Arbuscular mycorrhizal fungi colonization rate*

AMF colonization was significantly influenced ($p<0.01$) by AMF presence, moisture levels, and their interaction. However, race interactions (with AMF, moisture, or both) were non-significant ($p<0.05$). The highest colonization (67.88%) occurred under daily watering, while the lowest (39.65%) was after 21 days of withholding water (Table 5). These results align with Sabagh et al., (2022), who noted reduced colonization in drought-stressed damask rose. Similarly, Khaliq et al. (2022) reported decreased colonization in *Artemisia* under drought and competition.

**Table 5:** Interaction effect of moisture stress and arbuscular mycorrhizae (AMF) on root mycorrhizae colonization rate

| Moisture withholding levels | Colonization rate AMF-inoculated |
|---|---|
| Daily | 67.88$^{a}$ |
| 7 days | 60.93$^{b}$ |

| | |
|---|---|
| 14 days | 55.63[c] |
| 21 days | 39.65[d] |
| **LSD (1%)** | **0.85** |
| **CV (%)** | **2.58** |

Means with different letters in a column are statistically significant at P < 0.01, CV = coefficient of variance.

### *3.3. Physiological Parameters*

#### *3.3.1. Photosynthesis and Transpiration Rates*

Photosynthesis was significantly influenced ($p$<0.01) by race, AMF inoculation, and drought intervals, with interactions. The highest rate occurred in AMF-inoculated Guatemalan plants under daily watering, followed by Mexicana. The lowest rates were in non-inoculated Guatemalan plants under 21-day drought (Fig. 3). Mexicana maintained better photosynthesis under stress than Guatemalan. AMF enhances photosynthesis and chlorophyll content. Transpiration was also significantly affected p$P$<0.001) by race, AMF, and drought. The highest rate was in AMF-inoculated Mexicana under daily watering; the lowest in non-inoculated Guatemalan plants under drought (Fig. 3). AMF-inoculated plants had double the root hydraulic activity of non-inoculated ones. Msimbira and Smith (2020) found AMF regulates ABA, balancing transpiration and water uptake. AMF symbiosis enhances water and or nutrient uptake, increasing transpiration Keesstra et al. (2016), who showed AMF improves drought tolerance via antioxidant activation and osmotic regulation.

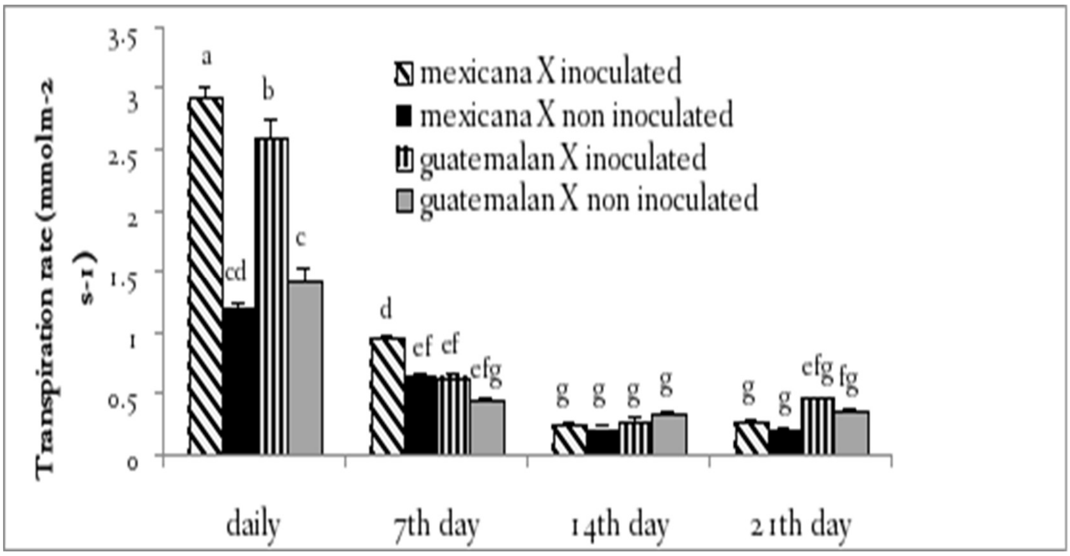



**Fig. 3:** Photosynthetic rate (A) and Transpiration rate (E) of avocado seedlings under AMF inoculation and moisture stress treatments.

#### *3.3.2. Water use efficiency (WUE)*

It was significantly influenced ($p$<0.05) by avocado races, arbuscular mycorrhiza (AMF), and their interactions. AMF-inoculated Mexicana races under 14-day drought showed the highest WUE, while non-inoculated Guatemalan races under 21-day drought had the lowest. AMF enhanced WUE via improved water uptake, stomatal regulation, and osmotic adjustment, maintaining turgor and photosynthesis under stress (Albasri et al. 2024). Albasri et al. (2024) observed higher intrinsic WUE under water stress.

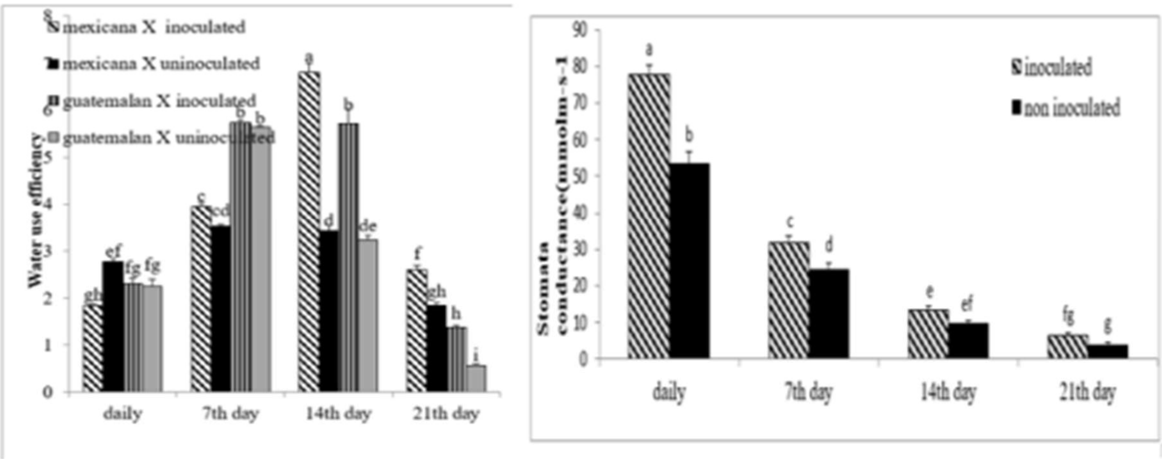



**Fig. 4:** The stomata number per leaf area and the size of stomata scored higher values in arbuscular mycorrhizae inoculated cassava plants than non-inoculated.

*3.3.3. Stomatal conductance, number, and width*

Stomatal conductance was significantly affected ($P$<0.05) by the interaction between arbuscular mycorrhizae (AMF) and moisture withholding (Fig. 4). Daily watered, AMF-inoculated seedlings showed the highest conductance, while stressed, non-inoculated plants had the lowest. AMF increased conductance by 31.2%, 23.15%, 26.25%, and 41.07% daily, 7-, 14- and 21-day withholding, respectively. AMF enhances water uptake, improving efficiency and conductance (Kumar et al. (2023), and reduces ABA levels, promoting stomatal opening. These findings align with studies showing AMF sustains higher conductance and water potential under drought (Albasri et al. 2024). Analysis variance showed that stomata number was significant (p<0.01)

affected by main factors arbuscular mycorrhizae (AMF) and moisture withholding. The inoculation of arbuscular mycorrhizae (AMF) increased stomata number by 8.3% compared to AMF non-inoculated avocado seedlings. Similarly, a high stomata number was counted on daily water-treated seedlings, while as stomata number decreased as the moisture stress level increased (Table 6). Modification of stomatal density in response to drought varies between plant species and is contingent on the severity of water deficit.

**Table 6.** Effect of AMF inoculation and moisture withholding on stomatal number

| **AMF** | **Stomata number** |
|---|---|
| Inoculated | 17.00[a] |
| non-inoculated | 15.58[b] |
| LSD (5%) | 0.79 |
| CV (%) | 8.27 |
| **Water withholding** | **Stomata number** |
| daily | 19.91[a] |
| 7 days | 17.91[b] |
| 14 day | 14.25[c] |
| 21 days | 13.08[d] |
| LSD (5%) | 1.12 |
| CV (%) | 8.27 |

*Means with different letters in a column are statistically significant at P < 0.05. CV = coefficient of variance*

Stomatal width was significantly influenced (*P*<0. 1) by races, AMF, and moisture interactions. The Guatemalan race had the largest stomata under daily watering with AMF, while non-inoculated, 21-day stressed plants had the smallest (Table 7). AMF mitigated drought-induced size reduction. Similar results were observed in olives (Hamzehzadeh et al. (2024

**Table 7.** Interaction effect of arbuscular mycorrhiza (AMF), moisture withholding and avocado races on stomata width at seedling stage

| Race | Moisture withholding interval | Inoculated | Non inoculated |
|---|---|---|---|
| Mexicana | Daily | 10.96[b] | 10.30[c] |
| | 7 days | 9.66[de] | 9.10[fg] |
| | 14 days | 7.56[i] | 8.33[h] |
| | 21days | 7.33[ij] | 5.96[k] |
| Guatemalan | Daily | 11.53[a] | 10.33[c] |
| | 7 days | 10.03[cd] | 9.30[ef] |
| | 14 days | 8.90[g] | 8.16[h] |
| | 21 days | 7.0[j] | 5.16[l] |
| **LSD (1%)** | **0.397** | | |
| **CV (%)** | **2.73** | | |

Means with different letters in a column are statistically significant at P<0.01. CV=coefficient of variance.

### 3.3.4. Chlorophyll and Leaf Relative Water Content

Leaf chlorophyll content (Chl a, Chl-b, and total) was significantly influenced (*p*<0.1) by avocado race AMF–moisture interactions. Highest levels occurred in AMF-treated Mexicana and Guatemalan under daily watering; lowest in drought-stressed Guatemalan. AMF increased total chlorophyll by 7.2–8.8% (daily watering) and 10.5–24.4% (21-day withholding) for Mexicana and Guatemalan, respectively (Table 8), aligning with Diagne et al. 2020). Leaf relative water content was affected (*P*<0.01) by race–moisture interactions, with Mexicana exceeding Guatemalan by 7.23%, 3.23%, and 8.78% under daily, 14-, and 21-day withholding (Table 8).

**Table 8.** AM fungi, avocado variety, and drought effects on seedling chlorophyll a, b, and total content.

| Races | Inoculations | Moisture | chl a | chl b | TC |
|---|---|---|---|---|---|
| | inoculated | daily | 23.42[a] | 7.68[a] | 31[a] |
| | Non-inoculated | daily | 21.44[b] | 7.23[b] | 28.72[b] |
| | Inoculated | 7 days | 19.55[d] | 7.12[b] | 26.67[b] |
| | Non-inoculated | 7 days | 18.35[e] | 6.2[c] | 24.55[e] |
| Mexicana | Inoculated | 14 days | 18.11[ef] | 5.09[e] | 23.93[g] |
| | Non-inoculated | 14 days | 17.37[g] | 4.29[fg] | 21.67[i] |
| | Inoculated | 21 days | 16.07[h] | 4.15[g] | 20.22[j] |
| | Non-inoculated | 21 days | 15.01[i] | 3.07[i] | 18.08[l] |
| | Inoculated | daily | 23.2[a] | 7.6a | 30.8[a] |
| | Non-inoculated | daily | 21.03[c] | 7.07[b] | 28.13[c] |
| | Inoculated | 7 days | 19.65[d] | 7.1[b] | 26.75[d] |
| Guatemalan | Non-inoculated | 7 days | 18.11[f] | 6.16[c] | 24.26[f] |
| | Inoculated | 14 days | 18.24[ef] | 5.69[d] | 23.93[g] |
| | Non-inoculated | 14 days | 17.43[g] | 4.42[f] | 21.83[k] |
| | Inoculated | 21 days | 16.14[h] | 3.58[h] | 19.72[k] |
| | Non-inoculated | 21 days | 13.24[j] | 1.76[j] | 15[m] |

| | | | |
|---|---|---|---|
| **LSD (1%)** | **0.24** | **0.23** | **0.2** |
| **CV (%)** | **1.8** | **2.54** | **1.54** |

Means with different letters in a column are statistically significant at P<0.01. TC=Total Chlorophyll, CV=coefficient of variance.

### 3.4. Proline Content

Proline content was significantly affected ($P$<0.05), Table 9, by race, arbuscular mycorrhiza (AMF), and moisture stress interactions. Highest levels occurred in AMF-inoculated Mexicana after 21-day drought, while lowest were in non-inoculated Guatemalan under daily watering. Inoculated seedlings showed 12.6% (Mexicana) and 9.2% (Guatemalan) higher proline under drought. Proline aids drought tolerance, recovery, and dry mass gains Bonomelli et al. (2018).

**Table 9.** Proline content (μmol $g^{-1}$ FW) in avocado seedlings as influenced by race, AMF, and moisture stress.

| Moisture withholding | AMF | Mexicana | Guatemalan |
|---|---|---|---|
| Daily | inoculated | $0.5^{g}$ | $0.46^{h}$ |
| Daily | non-inoculated | $0.44^{h}$ | $0.37^{i}$ |
| 7 days | inoculated | $0.64^{e}$ | $0.59^{f}$ |
| 7 days | non-inoculated | $0.58^{f}$ | $0.65^{e}$ |
| 14 days | inoculated | $0.8^{b}$ | $0.81^{b}$ |
| 14 days | non-inoculated | $0.71^{d}$ | $0.74^{c}$ |
| 21 days | inoculated | $0.86^{a}$ | $0.76^{c}$ |
| 21 days | non inoculated | $0.75^{c}$ | $0.69^{d}$ |
| **LSD (5%)** | | **0.031** | |
| **CV (%)** | | **2.89** | |

Means with different letters in a column are statistically significant at P<0.05. CV=coefficient of variance.

### *3.5. Fresh and Dry Biomass Weight*

Biomass weights were significantly influenced ($p$<0.01) by race, moisture withholding, their interaction, arbuscular mycorrhizae (AM), and withholding intervals. No effects were found for race × AM or the three-way interaction. The Mexicana race under daily watering had the highest biomass (56.40 g fresh, 13 g dry), while the Guatemalan race showed the lowest after 21 days without water (Table 10). These findings agree with Kumar et al. (2023), who reported biomass variations among drought-tolerant and sensitive varieties. Morphological traits like biomass significantly affect drought performance.

### *3.6. Associations Between Xylem Traits, Biomass, and Avocado Races*

Correlation analysis revealed strong positive correlations shown in Tables 10 and 11. Root xylem vessel number and width were positively correlated with physiological and biomass traits. Stomatal number and width also showed strong positive correlations (p≤0.001) with physiological parameters. However, WUE had no significant correlation (p<0.05) with physiological or biomass traits (Table 11). All morphological and physiological parameters displayed significant strong positive correlations (p≤0.001).

**Table 10:** Pearson Correlation coefficient (r) among xylem traits, physiological parameters, and biomass of avocado races under AMF and moisture stress

| | XN | XW | BFW | BDW | Chl (a+b) | A | E | WUE | Gs |
|---|---|---|---|---|---|---|---|---|---|
| **XN** | 1 | | | | | | | | |
| **XW** | 0.95*** | 1 | | | | | | | |
| **BFW** | 0.89*** | 0.98*** | 1 | | | | | | |
| **BDW** | 0.89* | 0.98*** | 0.99*** | 1 | | | | | |
| **Chl (a+b)** | 0.94* | 0.99*** | 0.98*** | 0.98*** | 1 | | | | |
| **A** | 0.73*** | 0.89* | 0.94*** | 0.94*** | 0.89*** | 1 | | | |
| **E** | 0.63*** | 0.79* | 0.86*** | 0.87*** | 0.80*** | 0.94*** | 1 | | |
| **WUE** | 0.88*** | 0.88*** | 0.85*** | 0.84*** | 0.88*** | 0.73*** | 0.51*** | 1 | |
| **Gs** | 0.65*** | 0.84*** | 0.90*** | 0.91** | 0.84*** | 0.97** | 0.97*** | 0.60** | 1 |

Where, XN=Xylem number, XW=Xylem width, BFW= Biomass fresh weight, BDW= biomass dry weight, Chl (a+b) = Chlorophyll (a+b), A= Photosynthetic rate, E=Transpiration rate, WUE=Water use efficiency, Gs=Stomata conductance. *Significant at p≤0.05, **Significant at p≤0.01, and ***Significant at p≤0.001.

**Table 11.** Pearson Correlation coefficient (r) among stomatal traits, physiological parameters, and biomass of avocado races under AMF and moisture stress

| | SN | SW | chla | chlb | Chl(a+b) | LRWC | A | E | WUE | Gs |
|---|---|---|---|---|---|---|---|---|---|---|
| **SN** | 1 | | | | | | | | | |
| **SW** | 0.843** | 1 | | | | | | | | |
| **chla** | 0.83*** | 0.95*** | 1 | | | | | | | |
| **chlb** | 0.82*** | 0.9*** | 0.98*** | 1 | | | | | | |
| **Chl** | 0.85*** | 0.97*** | 0.98*** | 0.97*** | 1 | | | | | |
| **LRWC** | 0.59*** | 0.59*** | 0.73*** | 0.63*** | 0.70*** | 1 | | | | |
| **A** | 0.84*** | 0.90*** | 0.97*** | 0.89*** | 0.93*** | 0.69*** | 1 | | | |
| **E** | 0.72* | 0.73* | 0.83*** | 0.69*** | 0.78* | 0.75*** | 0.87*** | 1 | | |
| **WUE** | 0.031NS | 0.206NS | 0.0989NS | 0.2648NS | 0.1682NS | 0.1379NS | 0.0344NS | 0.352NS | 1 | |
| **gs** | 0.86*** | 0.84*** | 0.9242*** | 0.8188*** | 0.89*** | 0.78*** | 0.92*** | 0.94*** | 0.19NS | 1 |

*Where, SN= stomata number, SW= stomata width, Chla=Chlorophyll-a, Chl-b=Chlorophyll-b, Chl= Chlorophyll (a+b), LRWC = Leaf Relative Water Content, A=Photosynthetic rate, E=Transpiration rate, WUE=Water use efficiency, Gs=Stomata conductance; *Significant at p≤0.05, **Significant at p≤0.01, and ***Significant at p≤0.001; NS=Not Significant*

## 5. Conclusions

Inoculation with arbuscular mycorrhizal fungi (AMF) clearly enhances the resilience of avocado seedlings to moisture stress. Across all drought levels, AMF-treated plants exhibited superior performance in growth parameters (including plant height and internode length), leaf area expansion, root development (length, number, and diameter), and physiological processes such as photosynthesis, chlorophyll concentration, and water-use efficiency when compared to non-inoculated plants. Under severe moisture stress (21-day watering interval), root biomass increased by 38–46% in AMF-inoculated seedlings. Among the avocado races tested, the Mexicana race consistently demonstrated greater drought tolerance than the Guatemalan raceparticularly under severe stress. Mexicana seedlings maintained higher growth rates, larger leaf area, greater biomass accumulation, and stronger physiological performance, with the largest improvements occurring when AMF inoculation was applied. AMF inoculation proved especially important for improving drought resilience in the more vulnerable Guatemalan race. Overall, the combination of Mexicana's inherent drought tolerance and the symbiotic benefit of AMF offer a promising strategy for improving avocado production in water-limited regions of Ethiopia. However, since this research was conducted under greenhouse conditions, it should be repeated under field conditions to validate and confirm its applicability in real production environments

**Author Contributions: Contribution of authors**

The research team designed the study, with Hiwot Kelbo conducting data collection and analysis under the supervision of Meseret Tesema Terfa, PhD. Hiwot Kelbo prepared the initial manuscript draft, which was then reviewed and edited by Daniel Manore. Laboratory resources were made available under the oversight of Beyene Dobo, Ph.D.

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