GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF ARBUSCULAR MYCORRHIZA INOCULATED AVOCADO (*Persea americana* M.) RACESUNDER MOISTURE STRESS CONDITIONS

MSc. THESIS

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HAWASSA UNIVERSITY COLLEGE OF AGRICULTURE

HAWASSA, ETHIOPIA

MAY, 2023

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A THESIS SUBMITTED TO THE SCHOOL OF PLANT AND
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ADVISORS’APPROVAL SHEET

(Submission Sheet – 1)

This is to certify that the thesis ’’Growth, physiological and biochemical responses of arbuscular mycorrhiza inoculated avocado races under moisture stress conditions’’ submitted in partial fulfilment of the requirements for the degree of **Masters of Sciences** with specialization in **Horticulture** Graduate Program of the School of **Plant and Horticultural Sciences**, College of Agriculture, and has been carried out by **Hiwot Kelbo**, under my supervision and no part of the thesis has been submitted for any other degree or diploma. The assistance and help received during the course of this investigation have been well acknowledged. Therefore I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

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We, the undersigned, members of the Board of Examiners of the final open defense by **Hiwot kelbo** have read and evaluated her thesis entitled **“Growth, Physiological and Biochemical responses of arbuscular mycorrhiza inoculated avocado (***Persea americana*M.**) Races under moisture stress conditions”** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement for the degree **Master of Science** in Plant Sciences with specialization in **Horticulture**.

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I seriously declare that this MSc thesis is an original report of my work and that has not been submitted to any other institutions anywhere for the award of any academic degree, diploma or certificate. I confirm that appropriate credit has been given within this thesis where reference has been made to the work of others. This thesis is submitted in partial fulfilment of the requirements for MSc. degree at Hawassa University and deposited in Hawassa University library and is made available to users under the rules of the library.

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DEDICATION

I dedicate this thesis manuscript to my Brother Melese Kelbo who I lost when I was graduated my bachelor degree as well as my beloved Father Kelbo Hado and Mother Amarech Hirigo for nursing me with affection and love.

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ABBREVIATION

ANOVA	Analysis of Variance
AMF	Arbuscular mycorrhizae fungi
BMDW	Biomass Dry Weight
BMFW	Biomass Fresh Weight
CEC	Cation Exchange Capacity
CV	Coefficient of Variance
FAO	Food and Agriculture Organization
Gs	Stomata Conductance
LRWC	Leaf Relative Water Content
LSD	List Significant Difference
RC	Root Colonization
SAS	Statistical Analysis Software
WUE	Water Use Efficiency

Growth, Physiological and Biochemical Responses of Arbuscular Mycorrhiza Inoculated Avocado Races under Moisture Stress Conditions

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ABSTRACT

Avocado is one of the most economically important and widely grown fruit crops in tropical and subtropical regions. It is mainly grown in the southern, South Western, and Eastern parts of Ethiopia, with high economic returns. Despite its importance, production is hampered by abiotic factors like moisture limitation in the nursery and later in the orchard. This requires alternative and effective strategies and tools to overcome the challenge, such as inoculating with arbuscular mycorrhiza fungi as a soil amendment. This study was designed to investigate the effect of AMF inoculation on the morpho-physiological and biochemical responses of two avocado (Persea americana M.) races under different moisture levels. A factorial combination of two inoculation levels (inoculated and non-inoculated), two avocado races (Mexicana and Guatemalan), and four water withholding intervals (daily watering, 7-days, 14-days, and 21-days watering intervals) were arranged in a CRD design with three replications. Results of the study revealed that growth parameters of avocado seedlings such as internode length and seedling height were influenced by the main effects of arbuscular mycorrhizae and moisture while, leaf number, leaf area were influenced by main and two way interaction effect of arbuscular mycorrhizae and moisture while xylem number and xylem width affected by interaction effect of race and moisture. However, root length, root number, and average root diameter were influenced by the main, two-way, and three-way interaction effects of race, arbuscular mycorrhizae, and moisture levels. The highest mean values of leaf area, root number, root length, xylem number, xylem width, and biomass fresh and dry weight were observed from Mexicana treated with arbuscular mycorrhizae under daily and moisture stress treatments. Whereas the minimum value was recorded from Guatemalan race grown under non-inoculated and 21 days water withholding treatment. Moreover, photosynthesis rate, transpiration, water use efficiency, chlorophyll content, proline content, biomass fresh and dry weight were significantly influenced by the main, two ways and three-way interaction effect of avocado races, arbuscular mycorrhizae and moisture levels. All growth, physiological and biomass parameters were significantly decreased under drought stressed conditions. However, compared to non-inoculated seedlings, the inoculation of arbuscular mycorrhizae considerably increased all those growth, physiological, biochemical, and biomass parameters even under drought stress. Among the tested races, "Mexicana" showed significantly well performance under drought stress conditions than Guatemalan cultivar. In conclusion, the inoculation of arbuscular mycorrhizae considerably improved drought stress tolerance in both races compared to non-inoculated treatments. However, further research is needed under field condition before generalized conclusions can be drawn.

Keywords*:* Arbuscular Mycorrhizae, Avocado Race, Drought Stress, Morpho-Physiological

1. INTRODUCTION

Avocado (*Persea americana*M.) is a member of the Lauraceae family which is cultivated in tropical and sub-tropical regions from 40° N and 40° S (Bergh, 2018). Avocado is unique among fruit trees in that it is neither sweet nor acidic but the nature with remarkably high nutritional density. It contains eleven vitamins, fourteen minerals, and a high percentage of oil (up to 30%) with no cholesterol (Ouma *et al*., 2019). It is economically important fruit crop in Ethiopia mainly in the Southern, South Western and Eastern part of the country, mainly produced by small scale farmers (Abebe *et al*., 2006; Biazin *et al*., 2018). In Ethiopia major avocado producing areas are Sidama, Gedio and Wolayita areas in the South, Jimma and Mizan areas in southwestern and Hararge area in eastern region of the country (Biazin *et al*., 2018).

Despite its importance, production is hampered by the wide spread incidences of biotic and abiotic stresses which is aggravated in recent years due to climate change. Abiotic factors like moisture and nutrient limitation stresses are considered as one of the most serious stresses that limit plant growth and reduce crop production in numerous regions worldwide (Fahad *et al.,* 2017). Drought stress as in moisture deficit, is estimated to affect nearly one-third of soils, thus, for supporting normal plant development (Mar *et al*., 2016). Drought occurs in almost all climatic regions, and it induces crop yield loss in a wide range of plants, while also increasing global tree mortality (Guillermo *et al*., 2021). Many factors including a lack of rainfall capacity, irregular rainfall distribution, drought intensity and duration, and progression rate of stress are responsible for water scarcity (Seleiman *et al.*, 2021). Drought stress often causes lower soil water potential, inducing cell dehydration, ultimately resulting in inhibiting cell expansion and division, leaf size, stem elongation, root proliferation, disturbed stomatal oscillations, plant water, nutrient uptake, and water use efficiency (Khatun *et al*., 2021). It is also known to alter a variety of biochemical and physiological processes ranging from photosynthesis to protein synthesis and solute accumulation (Hasanuzzaman *et al.*, 2013). Similarly, in avocado, water-stressed plants showed lower water potential and stomatal limitations of photosynthesis compared to control plants (Martínez-Ferri *et al.*, 2019).

Certainly, plants also develop sophisticated and complex mechanisms in morphological, physiological, and biochemical characteristics, dividing into escape, avoiding, and drought tolerance, to cope with drought stress (Khoyerdi *et al*., 2016).

Plants respond to stress in many ways, while manipulation of the root microbiome is one of the significant mechanism to deal with such stresses. Plant recruitment of a drought-specific microbiome could be an evolved trait, where generations of repeated drought events have led to evolution of stable and beneficial plant-microbe interactions that improve the reproductive fitness of both host and microbe (Naylor and Coleman-Derr, 2018; Chaudhry *et al.*, 2021). Plants have evolved complex morphological and metabolic responses to drought stress, many of which have been hypothesized or demonstrated to play a role in shaping root associated microbial communities (Naylor *et al.*, 2017; Xu and Coleman-Derr, 2019). Plants alter metabolism in accordance with available carbon pools, synthesize osmolytes to reduce osmotic stress, and activate stress pathways, such as antioxidant defense. These shifts in plant metabolism and exudation are mirrored by changes in plant morphological responses such as root physiological and morphological changes and adjustments (Ma *et al.*, 2020).

One of these symbiosis is with arbuscular mycorrhizal (AMF) fungi (Behrooz, *et al*., 2019). AMF is the most widespread soil microorganisms that form a symbiotic relationship with more than 80% of plants (Ma *et al.*, 2022). It forms vesicles, arbuscules, and hyphae in roots, and also spores and hyphae in the rhizosphere. Formation of hyphal network by the AMF with plant roots significantly enhances the access of roots to a large soil surface area, causing improvement in plant growth (Begum *et al*., 2019). Arbuscular mycorrhizal fungi facilitate host plants to grow vigorously under stressful conditions by mediating a series of complex communication events between the plant and the fungus leading to enhanced photosynthetic rate and other gas exchange-related traits (Begum *et al*., 2019) as well as increased water uptake. Numerous reports describe improved resistance to a variety of stresses including drought, salinity, herbivory, temperature, metals, and diseases due to fungal symbiosis (Begum *et al.*, 2019 ; Hoque *et al.*, 2022). Fungal hyphae can expedite the decomposition process of soil organic matter (Frey, 2019; Liu *et al.*, 2021). Furthermore, mycorrhizal fungi may affect atmospheric CO_2 fixation by host plants, by increasing “sink effect” and movement of photo-assimilates from the aerial parts to the roots (Begum *et al*., 2019; Busso and Busso, 2022).

Such a symbiotic association is believed also to regulate a variety of physio-biochemical processes in plants such as increased osmotic adjustment (Begum *et al.*, 2019), stomatal regulation by controlling ABA metabolism (Ouledali *et al.*, 2019b), enhanced accumulation of proline (Correia *et al.*, 2022 ; Behrooz, Vahdati, and Leslie, 2019), or increased glutathione level (Sharma *et al.*, 2017). The symbiosis between AM and plants is important for drought adaption and increased drought resistance in plants (Cheng *et al*., 2021). The regulation of plant drought resistance by AMF is a very complex process, involving a variety of metabolites and metabolic pathways (Tang *et al.*, 2022). This relationship involves enhancing survival (Begum *et al.*, 2019), enhance water absorption and transport (Ren *et al.*, 2019), change the root morphology, affect gas exchange and water use efficiency (Zhang *et al*., 2019), regulate plant endogenous hormone level (Ren *et al.*, 2019; Zhang *et al*., 2019; Jia-Dong *et al*., 2019), increased metabolites accumulation such as proline and promote the removal of reactive oxygen species (Amiri *et al*., 2015).

Furthermore, AMF promote growth of host plants by enhancing minerals availability and acquisition, (Begum *et al*., 2019). Mycorrhizal fungi can absorb, accumulate and transport a large quantity of phosphates within their hyphae and release it to cells of the root tissues. It has been shown that mycorrhizal plants can absorb and accumulate several times more phosphate from the soil solution than non-mycorrhizal plants (Manga *et al.*, 2022). These plants also accumulate P, K, Ca, Cu, Fe and Mn in the leaf in higher concentration than non-mycorrhizal plants (Kilpeläinen *et al.*, 2019). The increase in uptake of nutrients by mycorrhizal plants attributed to solubilization of the elements by increased root surface resulted by hyphal strands in soil regions inaccessible by the root hairs.

The production of avocado is highly dependent on the availability of water during seedling raising and irrigation during orchard transplant growth which is strongly dependent on water management. One particular feature of the avocado tree that is relevant for irrigation is its effective rooting zone, which is often limited to depths between 0.3 and 0.6 m, and a diameter of 2.0 m around the tree (Irrigation *et al*., 2020) . In several occasions it has been shown that adding mycorrhizal fungi improve a young plant´s subsequent resilience to drought by improving the roots' abilities to absorb water and nutrients (Manga *et al.*, 2022; Begum *et al.*, 2019). On planting out, such trees should have developed a more extensive root system which helps it to establish quickly in the field. This may be particularly important in conditions where drought conditions occur. Avocado has hairless mangrove-like roots that are highly dependent on mycorrhizal symbiosis (Alvarado *et al*., 2013).

Several studies reported positive impact of inoculation avocado seedlings and micro propagated plants with mycorrhiza. These observations showed increased growth and development of avocado seedlings (Menge *et al.*, 1980; Rivera *et al*., 2011), survival and development of micropropagated avocado plants during the *ex vitro* acclimatization (Vidal *et al*., 1992) and the plants are more tolerant to drought than the non mycorrhizal plants (Pozo and Azcón-Aguilar, 2007). Application of AMF (Gianinazzi and Vosátka, 2004) can reduce the negative effects of pesticide treatments and increase sustainability of the agricultural production systems (Alvarado *et al.*, 2013). In addition, inoculation with AMF alleviate seedling transplant shock and enhance hundred per cent field establishment of grapevine seedling(Krishna *et al*., 2015).

Hence, the benefits of inoculation with arbuscular mycorrhizae fungi can be seen in improved seedling survival, growth in shorter time, reduction of nursery time, saving in fertilizer costs, and increased production and product quality while also improving water use efficiency (Diagne *et al.,* 2020). Nonetheless the use of AMF to improve water stress in avocado during seedling raising and subsequent transplanting is less studied and explored despite its significant important in improving water use efficiency. Due to the importance of this association and the benefits that these fungi provide, it is valuable to evaluate how best to use mycorrhizal inoculants at nurseries to foster the rapid growth of seedlings, before they are planted in the field. Therefore this study was conducted to investigate the effect of arbuscular mycorrhizae fungi inoculation on growth, physiology and biochemical response of avocado seedlings under moisture stress condition. The scope and hypothesis of this study is mainly focused on evaluation of the effects of AMF on physiological and morphological response of avocado seedlings as a means to overcome moisture stress.

1.1 Objective of the study

1.1.1. General objective

- To investigate growth, biochemical and physiological responses of arbuscular mycorrhiza inoculated avocado races under moisture stress conditions

1.1.2 Specific Objectives

- To evaluate the shoot morpho-physiological responses of avocado seedlings to arbuscular mycorrhizae inoculation under moisture stress conditions
- To identify the root growth and development of two avocado race seedlings to arbuscular mycorrhizae inoculation under moisture stress conditions
- To evaluate the biochemical response of two avocado race seedlings to arbuscular mycorrhizae inoculation under moisture stress conditions

2. LITERATURE REVIEWED

2.1 Botanical description of Avocado

Avocado(*Persea americana*.) is polymorphic tree crop belongs to genus Persea family Lauraceae that originated from a broad geographical area stretching from the eastern and central highlands of Mexico through Guatemala to the Pacific coast of Central America (Janice *et al.*, 2018). The avocado plant is erect and grows up to ≥ 9 to 18 m high with bole diameter ranging from 30 to 60 cm. The leave could be of several shapes including lanceolate, elliptic, oval, ovate or obovate (Janice *et al*., 2018) and may be alternate, dark-green, glossy on the upper surface and whitish on the underside. According to Janice *et al*. (2018) leaf lengths ranging from 7.5 - 40 cm log and found the fruit to be pear-shaped, often necked, and oval or nearly round and 7.5-33 cm long in length and may reach 15 cm wide. The fruit skin is known to be yellow-green, deep-green, reddish-purple or very dark purple to almost black the fruit may be speckled with tiny yellow dots, may be smooth or pebbled, glossy or dull, thin or leathery and up to 6 mm skin thickness, pliable or granular and brittle *(*Araújo *et al*., 2018). Generally, the flesh of avocado is entirely pale to rich-yellow in colour but in some fruits, there is a thin layer of soft, bright-green flesh immediately beneath the skin. The avocado fruit has a single seed enclosed in two brown, thin, papery seed coats often adhering to the flesh cavity and may be oblate, round, conical or ovoid in shape, hard and heavy, ivory in colour and 5-6.4 cm long (Bergh, 2018).

2.2 Agro ecological requirements of Avocado

The main climatic requirements of the avocado tree are related to the temperature and rain fall, and the varieties behave differently according to their race (Anguiano *et al.*, 2007) pointed out that depending on the race of origin of the avocado, this can settle down from the level of the sea until the 3,000 m of altitude, although in the practical orchards to more than 2,400 m they are considered outside of the appropriate area for a profitable production. The Mexican race prospers in altitudes from 500 to 3,000 m; the Guatemalan of 1,000 to 2,000 m and the Antillean of 0 to 1,000 m. Thermal requirement (Whiley and Winston, 1987) point out that the avocado cv. Hass, for fruit mooring requires temperatures in a range that goes from 12-17 ºC to 28-33 °C. According to Bita and Gerats, (2013) that higher temperature than 42 ºC is unfavourable for the cultivation. Salazar-García and Lovatt,(2000) indicated that the biggest mooring of avocado fruits happened with temperatures between 20 and 25 °C, on the other hand he pointed out that temperatures above 28 °C cause the abscission of individual flowers. (Menzel and Paxton, 1985) pointed out as thermal thresholds of the avocado 10°C and 35 °C. Anguiano *et al.* (2007) mentions that in the cultivation of the avocado higher temperatures greater than 32 °C have negative effects due to a detriment on the fertilization and the grade of sterility of the pollen. (Cantuarias Aviles, 1995) referred that the respiration and the hydrological potential in the leaves of citric and avocado species was reduced when the temperature in the roots was low. The hydrological potential in the leaves was specially affected when the temperature of the air went down 5 °C.

Soil requirements a fine sandy loam with good drainage is necessary for long life and good health of the tree. As for soil requirements (Arpaia and Hofshi, 2004) mentions that the upper layer of the earth where the plants grow is very complex, because of the great variability of their physical and chemical components that possesses. The avocado requires a very good drainage in the soil to be able to live and to take place, since it is one from the most sensitive species to the radical asphyxia. Avocado doesn't need very deep soils since it possesses superficial roots, producing abundant crops in floors from 30 to 40 cm deep, provided it has an underground an excellent drainage, or that it is located on hillsides. Nevertheless, a deep soil and of textures stockings, they are decisive conditions in the quantity of water that it can retain (Anguiano *et al*., 2007).

2.3 Importance of Avocado

Avocado is unique among fruit trees in that it is neither sweet nor acidic but of bland nature with remarkably high nutritional density. It contains eleven vitamins, fourteen minerals, and a high percentage of oil (up to 30%) with no cholesterol (Ouma *et al*., 2019). Avocado is by itself a complete food containing all nine essential amino acids although not in a balanced proportion (Garedew and Tsegaye, 2011a). Avocado is a high calorie fruit with high nutritional value and is considered a major tropical fruit, since it is rich in protein and contains fat soluble vitamins lacking in other fruits, including Vitamins A and B, and median levels of vitamins D and E (Duarte *et al.*, 2016). It contains different oil levels in the pulp, thus it is widely used in pharmaceutical and cosmetic industries, and for obtaining commercial oils similar to olive oil, because of their similar fatty acid composition (Ko *et al.*, 2018). In addition, this fruit has been recognized for its health benefits, especially due to the compounds present in the lipid fraction, such as omega fatty acids, phytosterols, tocopherols, and squalene (Duarte *et al*., 2016). The fruit stands out on potassium levels (339mg 100g-1) when compared to other fruits, which regulates muscle activity and protects the body from cardiovascular diseases (Gaidhani *et al*., 2014). It also represents a source of glutathione, a powerful antioxidant that acts on potentially carcinogenic compounds (Abdelkader *et al*., 2022). In addition to the important major compounds, avocado contains substantial amounts of bioactive compounds such as phytosterols, especially in the lipid fraction, and the main representative is the β-sitosterol (Dossantos *et al*., 2014). Diets rich in phytosterols can lead to the reduction of the total cholesterol and

LDL cholesterol (Dedić *et al*., 2022). Avocado oil is easily absorbed by the skin, with high absorption power of perfumes, which is of great value to the cosmetics industry. In addition, it easily forms an emulsion, ideal for the manufacture of fine soaps (Gaidhani *et al*., 2014). In comparison to other vegetable oils, avocado oil is characterized by having high levels of monounsaturated fatty acids (oleic and palmitoleic acids), low polyunsaturated fatty acids (linoleic acid), and relatively high levels of saturated fatty acid (palmitic and stearic acids). This fatty acid composition is influenced by the cultivars, maturity stage, anatomical region of the fruit, and geographic location for plant growth (Araújo *et al*., 2018).

2.4 Avocado production status in the world and Ethiopia

The avocado (*Persea americana* M.) is a native of Central America and the West Indies. Counts of the fruit date back to the early 1500s when the Spanish conquistadors over ran the Aztec and Inca empires and found the avocado being extensively cultivated (Dirou, 2003). It was introduced into Florida, California and Hawaii in the early 1800s and is now found worldwide where growing conditions are suitable (Faris, 2016). The avocado was initially introduced to Ethiopia in 1938 by private orchardists to Hirna of Eastern Ethiopia and Wondogenet area in the south and then the production gradually spread to different areas and agro- ecologies in the country where the crop was adapted (Shumeta, 2010). Avocado production in the world has been increasing considerably (García *et al*., 2021); countries such as Mexico, Dominican Republic, Peru, Indonesia, Colombia, United States, Chile and Brazil are the largest producers (Selim *et al*., 2018) and (FAO, 2022). In the field of exports, Mexico leads the list with 46% of the total, while in imports, the United States, the Netherlands and France are the most demanding in the avocado market with 64% of total imports (García *et al*., 2021). Avocado's global production has now reached more than 3.8 million metric tons (Faris, 2016).

In Ethiopia avocados are second in total volume of fruit crop production, next to banana (Garedew and Tsegaye, 2011). Ethiopia stands the 10th leading producer and 6th most important consumer in the world (Faris, 2016). Annual avocado production in Ethiopia is 25633.16 tons. The crop is now produced by 1,149,074.00 farmers country wide who collectively farm more than 8938.24 ha of land (Jalata, 2021).

Avocado consumption has been very low in many parts of the world outside the American continent. Recently the consumption has rapidly increased globally especially Europe and avocado is now an important fruit in international trade (Juma *et al*., 2019). The increased avocado consumption is due to the rising awareness of the people for eating healthy foods, the increased avocado popularity on social media and the improved accessibility of ready-to-eat delicious avocados (Lourentzatos, 2021).

2.5 Production trends and constraints in Ethiopia

During the last century, avocado has been widely distributed to more than 50 countries around the world, which mainly includes sub-tropical and tropical areas such as sub-Saharan Africa. Ethiopia is one of the top five avocado producers in sub-Saharan Africa. Avocado was first introduced to Ethiopia around 1938 by private orchardists in Hirna (Eastern highlands of Ethiopia) and Wondo-genet (Southern Highlands of Ethiopia). Gradually, it has been distributed to different agro ecologic conditions where the crop could be adapted (Shumeta, 2010) . Despite its long history since introduction and the diverse agro-ecologic conditions of Ethiopia, its distribution is still limited to few areas of the country (Aschalew *et al*., 2021). Currently the main avocado producing areas include

Sidama and Wolayita areas in the South, Jimma and Mizan areas in south western and Hararge area in eastern region of the country (Área and Natural, 2022).

In Ethiopia, avocado trees are mainly grown as an integral component of coffee (*Coffee Arabica* L.) and enset (*Ensetventricosum*) agro forestry systems and to a lesser extent in combination with other crops like maize (Biazin *et al*., 2018). As coffee requires shade trees and known for its shade-tolerance, smallholder producers consider avocado as one of the important shade trees while also benefiting a lot from the sale of the avocado fruits (Berhanu, 2013) . It is also grown as individual trees around the court yards where it is used also as shades for people and livestock. Annual avocado production in Ethiopia is 25633.16 tons. The crop is now produced by 1,149,074.00 farmers countrywide who collectively farm more than 8938.24 ha of land (Awulachew, 2022).

Despite the suitable agro-ecological conditions to grow avocado and economic importance of the crop there are constrains on avocado production in Ethiopia. According to Faris (2016), even though avocado has economically and socially play a significant role its production is confronted by a number of constraints; this are Degeneration of fruits, Disease problem and absence of agronomic practices. According to Humble and Reneby (2014) report in Ethiopia No value adding activities of avocado take place at the farmer, broker or wholesaler level in the supply chains and the products are sold unprocessed. The value of the fruits increases when the products move closer to markets with high demand.

2.6 Effects of moisture stress on plant growth and development

Water-deficit stress can be defined as a situation in which plant water potential and turgor are reduced enough to interface with normal functions (Cheng *et al*., 2016). Water stress may arise from two conditions, either due to excess of water or water deficit. The more common water stress encountered is water-deficit stress, known as drought stress.

According to Shao *et al.* (2008), water stress is characterized by reduction of water content, turgor, total water potential, wilting, closure of stomata, and decrease in cell enlargement and growth. Seleiman *et al.* (2021) reported that there is strong corelation between plant growth and water stress. Drought stress reduces plant-growth inhibition of various physiological and biochemical processes, such as photosynthesis, respiration translocation, ion uptake, carbohydrates, nutrient metabolism, and hormones (Wahab *et al.*, 2022).

2.6.1 Effect of moisture stress on plant growth and Morphology

Water is one of the most important environmental factors regulating plant growth and development. Drought is a major stress that disrupts metabolic processes and constraints plant growth and development and thus limiting the crop productivity (Hayet *et al.*, 2021). Plant growth is mainly accomplished by cell division, enlargement, and differentiation. Moisture stress impairs mitosis and cell elongation which results in poor growth *(Fahad et al.*, 2017) . Moisture stress influences germination of seeds, leaf area, leaf expansion and root development of plant (Singh *et al.*, 2021) . According to Fahad *et al.* (2017), Number of leaves and the size of individual leaf are reduced under the drought conditions because the expansion of the leaf normally depends upon the turgor pressure and the supply of assimilates. Different morphological parameters (dry biomass, leaf number, root to shoot ratio) and the relative water content (RWC) were reduced in severe moisture stress treatment 25% field capacity (Hayet *et al.*, 2021). The root systems are essential to plant growth, which controls the growth of the shoots and the yield (Chen *et al.*, 2020). Root development, viability, functionality and plant growth are all significantly influenced by water stress (Kaysar *et al.*, 2023). Decrease in root length root relative growth rate and root dry weight was observed in different plants under moisture stress condition (Shao *et al.*, 2008)

2.6.2 Moisture stress effect on Physiological responses

Water stress, indeed, reduces plant growth and development by decreasing respiration, growth, photosynthesis, assimilate partitioning, moisture, and nutrient relationships (Tribulato *et al*., 2019). Water shortage typically increase stomata closure in plants, hence reducing gas exchange and decreasing photosynthetic in some species water unavailability determines smaller stomata formation ,able to have faster dynamic characteristics (Hussain *et al*., 2018). The water stress resulted in significant decreases in chlorophyll content and the leaf relative water content (Matricaria, 2011). Relative leaf water content (RLWC) is the most appropriate measure of the water status in plants (Hayet *et al.,* 2021). Moreover, decline in RLWC is generally correlated with changes in plant nutrition, carbon dioxide balance and water relations (Troughton, 1969). Moisture stress progressively decreases CO2 assimilation rates due to reduced stomatal conductance (Kabbadj *et al.*, 2017). The reduction in transpiration and co_2 content together improve the instantaneous water use efficiency (Vesala *et al*., 2017)

2.6.3 Effect of moisture stress on biochemical compounds

Plants accumulate different types of organic and inorganic solutes in the cytosol to lower osmotic potential there by maintaining cell turgor (Anjum *et al*., 2011). In plants, proline accumulation has been reported to occur after moisture stress (Liang *et al*., 2013).

Proline is greatly important for plants to cope with moisture stress; the correlation between the acquisition of stress tolerance and proline accumulation has been verified (Nanjo *et al*., 2003). Proline as an osmotic regulating substance, proline is preferentially stored in plant vacuoles. When the cell is subjected to osmotic stress, Proline is transported to the cytoplasm, and the osmotic potential is reduced by increasing the concentration of the cytoplasm so that the cell can still absorb extracellular water under the condition of low osmotic potential; thus, maintain the cell protoplasm and the external environment of osmotic balance (Yang *et al.*, 2021). Proline has a strong ability to hydrate, so it can also play a protective role in cell structure. In the event of plant injury, Proline interacts with proteins to form a hydrophobic skeleton to stabilize and protect biological macromolecules and cell membrane structures. Pro is also a variety of free radical scavengers (Yang *et al*., 2021)

Chlorophyll content tends to decrease under drought stress and the ratio of chlorophyll "a", "b", and carotenoid was changed, thus, in turn, causes changes in photosynthetic function (Pompelli *et al*., 2022). The reason for the decrease of chlorophyll content in leaves may be the degradation of chlorophyll directly caused by drought. Drought stress could significantly reduce the contents of chlorophyll a, chlorophyll b, and total chlorophyll in chickpea during vegetative growth and anthesis (Mafakheri *et al*., 2010).

2.6.4. Effects of moisture stress on Biomass

The performance and biomass production potential of trees depend on the maintenance of a higher physiological status and economical utilization of resources in agroforestry tree species (Recous *et al*., 2008). Prolonged water stress reduced the biomass of fibrous roots in Avocado races(Shao *et al*., 2008). A decrease in total dry matter may be due to the considerable decrease in plant growth, photosynthesis and canopy structure, as indicated by leaf senescence during water stress in *Abelmoschus esculentum*, (e.g., water or nutrient availability) may also affect the distribution of biomass. Drought stress decreases mean plant biomass, whereas it increases both the relative variation in plant biomass and the concentration of mass within a small fraction of the population (Shao *et al*., 2008). This is supported by earlier studies, conducted at single field sites or in pots. Drought stress decreased the plant biomass in Cyamopsis tetragonoloba and spring wheat (Taheri *et al*., 2011). Similar results were observed in earlier studies in wheat, Asteriscus maritimus, and Albizzia seedling.

The reduction in total biomass was reported in groundnut races under water stress due to the reduction in the pod mass rather than in the vegetative mass(Shao *et al.*, 2008). Morphological parameters like fresh and dry weights have a profound effect in water-limited conditions. There was a one-third reduction in fresh and dry weights of the *Zizphus rotundifolia* plant under drought conditions (Younis *et al*., 2017) .Progressive drought resulted in a significant reduction in early allocation of dry matter and decreased fresh and dry weight in all plant parts in *Populus davidiana* (Yin *et al*., 2005).

Under water-deficit stress, the biomass production was decreased in *Populus cathayana* and drought severely affected all growth parameters. Plant productivity under drought stress is strongly related to the processes of dry matter partitioning and temporal biomass distribution (Rauf and Sadaqat, 2007).

2.7. Effects of water stress on avocado growth

Avocado (*Persea americana* Mill.) is a subtropical fruit-tree highly sensitive to lack and excess of water in the root (Zuazo *et al*., 2021). In the current context of global climatic changes, the variation in water availability caused by the increasing occurrence of extreme climatic events, such as droughts and floods, might negatively affect avocado production, due to the high susceptibility of this species to the lack of water and oxygen in the soil (Cantuarias-Avilés *et al*., 2019). Zhu *et al.* (2020) also repoted that avocado has a shallow root system that is very sensitive to water deficit and soil water logging events, which may result in wilting or abscission of leaves and fruits and might irreversibly undermine final fruit quality.

Low soil moisture during days of high evapotranspiration can severely reduce avocado fruit yield and quality (Celedón *et al.*, 2012a), whereas water logging, caused by excessive irrigation or poor soil drainage can cause root hypoxia and provide suitable conditions for *Phytophthora cinnamomi* infection (Celedón *et al*., 2012b). Several studies have quantified the effects of water deficit on avocado trees, both in controlled environments and I the field (Cantuarias-Avilés *et al*., 2019). Net CO_2 assimilation rate (AN), transpiration rate (E), and stomatal conductance (gs) of avocado root stock showed a marked and significant decrease in both mild and sever moisture stress levels (Guillermo *et al*., 2021).

2.8. Use of Bio-fertilizers for enhancing productivity

Bio fertilizer utilization becomes urgent to substitute or mix it up to a single chemical fertilizer application. According to Kumar *et al*. (2022) Single uses of chemical fertilization continuously caused to several negative impacts of the soil and water environment pollution and weaken the sustainable agriculture structure. Moreover, bio fertilizer was recommended to use as due to growth prompting microorganism produce plant nutrient and replaced the chemical fertilizers function (Daniel *et al*., 2022). Organic substance in soil would be mineralized by microbes through enzymatic processes turn into plant nutritive value as well as plant growth factor (Kästner *et al*., 2021). Bio stimulant fertilizers, the role of Effective Microorganisms (EM1) increasing tree yield is widely reported by Bzdyk *et al*., (2018). There are ascending studies that mentioned EM improves soil fertility and rebalance plant nutrient ratios causing increase in growth, yield and fruit quality of several plants on

Avocado and Mango seedlings (Shama *et al.*, 2017*)*, (Song *et al*., 2022) ; (Abd El-Hameid and Elshazly, 2019)**.**

2.8.1 Use of arbuscular mycorrhizal fungi to increase growth and development in Avocado and other fruit trees

Fruit crops, like most horticultural plants, commonly develop arbuscular mycorrhizae relationships and ex-hibit a high degree of dependence on this symbiosis for normal development (Vidal *et al.*, 1992). Fruit crops are one of the vascular plants that are associated with AMF (Khade and Rodrigues, 2006). Depending upon the P and other nutrients level in the soil fruit crops are known to harbor diverse group of AMF that covers up to 50% of the plant roots mycorrhization (Belay *et al.*, 2014; Gaidashova *et al.*, 2012). Several reports showed that mycorrhizal dependency is different among tropical fruit tree species (Jaizme-Vega and Azcón, 1995; Menge *et al.*, 1978). The authors reported that different fruit crops as such that avocado (30.7%), banana (105%) and papaya (75.8%) showed different mycorrhization percentage after inoculated with four different AM fungi species (Jaizme-Vega and Azcón, 1995). In arbuscular mycorrhizae fungi, the plants symbiotically associate with glom eromycete fungi mainly developed to improve the uptake of nutrients and water by plants (Malhi *et al*., 2021). Mycorrhizal symbiosis is one of the initial symbiotic relationships on earth. It helps in the growth and development of the plant by supplementing the plant growth with an appropriate supply of mineral, and in return, the fungi draw food from the plant roots (Begum *et al*., 2019). In Ethiopia, various reports revealed the presence of varying species composition and community structure of AMF depending upon the type of plants in acacia woodland (Zerihun *et al*., 2013) and in coffee agroforestry system (Muleta *et al.*, 2007). Belay *et al*. (2014) has shown the presence of AMF diversity in species composition and community structure in different tropical fruit crops in Ethiopia.

2.8.2 Use of arbuscular mycorrhiza to overcome water stress in Avocado and other horticulture crops

Mycorrhizal symbiosis is a reciprocally beneficial association between soil fungi with plant roots. Arbuscular mycorrhizal fungi start a symbiotic union with roots of 80% land crops (Khaliq *et al*., 2022). AM fungi have a positive impact on the plants' stress tolerance that prevails in desert ecosystems is capable of enhancing resistance to drought stress and 0tolerance, improving plants' ability to absorb water and nutrients (Madouh and Quoreshi, 2023). Lots of eco physiological studies indicate that arbuscular mycorrhizal (AM) symbiosis is a crucial component in helping plant life to cope with water demands (Malhi *et al*., 2021). The fungi control the root water uptake by plants and enables plants to maintain bigger organ hydration and turgor, which will sustain general cell natural activity, mainly linked to the photosynthetic machinery (Rahman *et al*., 2021). Mycorrhizal fungi furthermore affect the hydraulic conductivity and gas exchange within the root and foliage (Chandrasekaran *et al.*, 2019). The alleviating effect of AM symbiosis in response to drought generally is determined by the positive consequences of AM fungi on the uptake and transport of water along with improved nutrient absorption, especially of accessible soil phosphorus (P) along with other immobile minerals (Etesami *et al.*, 2021). It results in the hydration of developing tissues, sustainable physiology and a clear promotion of growth.

Avocado trees are shallow rooted under water limit condition it is most likely that this tree depends on mycorrhiza to accomplish its nourishment. The use of AMF during the nursery stage of avocado has been successful in enhancing water and nutrient uptake (particularly P), and reduce the post-transplant stress (Osorio Vega *et al*., 2012).

3. MATERIALS AND METHOD

3.1. Description of study area

The experiment was carried out under shade house condition during February, 2022 – august, 2022 at Hawassa University, College of Agriculture campus. The site is located at about 275 km south of Addis Ababa with 7°4′ N latitude and 38°31' E longitudes, at an altitude of 1669 m.a.s.l. The area receives annual rainfall ranging from 900 – 1100mm and the average daily minimum and maximum temperature is 12^{o}c and 27^{o}c respectively.

3.2 Plant materials of Avocado races

Seeds of two avocado races Mexicana and Guatemalan were used in this experiment and the planting materials were collected from Yirgalem Agro processing industry by sorting out of seeds in size, shape and weight to maintain uniformity. The races are among the three botanical races mainly used as rootstocks. Guatemalan and Mexican seedlings were used as root stocks because Seeds are available, germinated uniformly, produce relatively thick shoots and suitable for the agro ecology. The description is indicated in Table 1.

Table 1.The distinct features of Guatemalan and Mexican races

	Races	
Characteristics	**Guatemalan**	**Mexican**
Origin	Tropical highlands	Tropical highlands
Foliage	No odour	Anise-scented
Blooming season	March to April	January to February
Maturity season	September to January	June to October
Fruit set to maturity	10 to 15 months	6 to 8 months
Fruit size	0.2 to 2.3 kg	0.5 kg
Skin texture	Woody-rough	Papery-smooth
Fruit oil content	Moderate to High	High
General rating	Moderate to High	High

Source: (Crane and Balerdi, 2008)

3.2.1. Experimental Design and treatments

The experiment was conducted in a factorial arrangement using completely randomized design (CRD) with three replications. The treatments of the experiment included four moisture stress levels which were employed as irrigation withholding (daily, 7-days, 14-days and 21-days), two levels of inoculation with arbuscular mycorrhizae (mixed inoculum of Gigaspora and Rihzophus spp) as inoculated and non-inoculated, two races of avocado (Mexican and Guatemalan races). Thus, the experiment had 16 treatments combinations replicated three times bringing into total of 48 treatments. To increase number of plants for sampling, there were four pots (plants) per replications which made the total number of plants (pots) 192 pots (plants) for the experiment.

Table 2: Treatment combination

Treatments	**Race**	**Water withholding**	**AM inoculation**
1	Mexican	Daily-watering	Inoculated
2	Mexican	7-days	Inoculated
3	Mexican	14-days	Inoculated
4	Mexican	21-days	Inoculated
5	Mexican	Daily	not inoculated
6	Mexican	7-days	not inoculated
7	Mexican	14-days	not inoculated
8	Mexican	21-days	not inoculated
9	Guatemalan	Daily	inoculated
10	Guatemalan	7-days	Inoculated
11	Guatemalan	14-days	Inoculated
12	Guatemalan	21-days	inoculated
13	Guatemalan	Daily	not inoculated
14	Guatemalan	7-days	not inoculated
15	Guatemalan	14-days	not inoculated
16	Guatemalan	21-days	not inoculated

3.3 Experimental Procedure

For the experiment, pots with five litre capacity with dimension of 22 cm diameter and 20 cm length was filled with propagation media composed of top soil, compost and sand with 3:2:1 ratio. The top soil up to 20 cm depth was collected from Hawassa University, College of Agriculture. Then after, two seeds per pot of the avocado races were planted and kept for germination. AMF inoculants of Gigaspora and Rihzophus spp were prepared using Sorghum bicolor as a host plant in Hawassa University, Biology department laboratory. The effectiveness of AMF inoculums was determined by inoculating Sorghum bicolor with an AMF mixture and allowing it to grow for 45 days. The test host plants (Sorghum bicolor) were watered every day for two weeks, and then every two days until the test ended. After 45 days, the host plants' growth parameters, such as length, stem diameter, leaf area index, and so on, were compared to the control. Finally, the rate of Sorghum AMF root colonization and spore density were determined. The mixture of the two AMF species was found to be effective in increasing all growth parameters with high rate colonization (>80%) and spore density (>750spores/100g dry soil). This inoculum was used in the current study.

Mixed AMF crude inoculums of Gigaspora and Rihzophus spp containing spores, sporcarps and roots were applied in to pots of germinating avocado seeds by digging 10 cm hole around each seedling at the age of 8 weeks after planting. The AMF inoculation was 150g of mycorrhiza inoculum per seedling. The water stress (irrigation withholding) treatment was commenced when the germinated seedlings reach the pencil size or 15cm ten weeks after planting and fifteen days after AMF inoculation to establish the inoculants and maximize the survival.

3.4. Experimental Soil media Sampling Analysis and climatic data

3.4.1. Experimental Soil media and Sampling Analysis

The composited soil media samples were dried and grounded to pass through 0.2 mm sieve before laboratory analysis, and the samples were analysed for parameters relevant to the study at Hawassa university soil analysis laboratory class (sand, silt and clay), soil pH, total N, available P, CEC and organic carbon were determined as per the following laboratory procedure. The soil pH values were determined in soil water suspension 1:2.5 using glass electrode pH meters Jackson (1967). Determination of particle size distribution (texture) was carried out hydrometrically following the methodology of Day (1965) Soil organic carbon (OC) was determined using Jackson (1958) and soil total nitrogen by the Kjeldahl as described by Dewis and Freitas (1970), and cation exchange capacity (CEC) determined using the Kjeldahl method (Ranist *et al.*, 1999). Available phosphorus was determined using the sodium bicarbonate solution extraction method, and the quantity was measured using a spectrophotometer as defined by way of Olsen *et al.,* (1954). The physical and chemical properties of the soil before planting are presented in table (3).

Table 3: Physical and chemical properties of the experimental soil media before-planting

Physical and chemical properties	**values**
PH	7.8
CEC (MEQ/100gsoil)	32.4
Sand (%)	62
Clay (%)	14
Silt (%)	24
Textural class	Sandy loam
Organic carbon (%)	1.17
Organic matter (%)	2.01
Total nitrogen %	0.1009
Available phosphorus (ppm)	11
EC(μm)	4.75

3.4.2. Climatic data during growth (screen house climate data)

The maximum and minimum temperature and relative humidity during the experimental period from February to August, 2022 were measured on randomly selected 24 days using mini data loggers (Testo 174, Version 5.0.2564.18771, and Lenzkirch, Germany). To avoid direct sunlight and moisture the data logger was covered from the top with flat carton and hanged closer to the plant canopy. The vapor pressure deficit of the shade house was calculated based on the temperature and relative humidity recorded using VPD-Auto grow software (https://cals.arizona.edu/ceac). The climate data were recorded every hour for 24 days and the average value of 24 days measurements is represented.

Table 4.Relative humidity, temperature and vapour pressure deficit (VPD) of the shade house data recorded from the experimental plot during the experimental period

Hours	**Temperature ^{O}C**	**Humidity (%)**	**VPD (kPa)**
10:36:00 AM	32.70	41.97	2.87
11:36:00 AM	32.97	41.54	2.94
12:36:00 PM	34.97	38.27	3.46
1:36:00 PM	29.43	47.41	2.16
2:36:00 PM	25.77	60.75	1.30
3:36:00 PM	21.90	67.92	0.84
4:36:00 PM	20.53	67.12	0.79
5:36:00 PM	20.03	69.69	0.71
6:36:00 PM	19.57	68.33	0.72
7:36:00 PM	19.57	69.46	0.70
8:36:00 PM	19.60	71.80	0.64
9:36:00 PM	19.37	72.04	0.63
10:36:00 PM	18.97	72.51	0.60
11:36:00 PM	18.33	71.64	0.60
12:36:00 AM	18.07	73.21	0.56
1:36:00 AM	18.07	73.48	0.55
2:36:00 AM	17.33	74.35	0.51
3:36:00 AM	17.53	72.36	0.55
4:36:00 AM	17.20	73.13	0.53
5:36:00 AM	17.20	73.93	0.51
6:36:00 AM	19.73	68.40	0.73
7:36:00 AM	21.83	64.14	0.94
8:36:00 AM	24.40	57.71	1.29
9:36:00 AM	28.17	51.01	1.87

3.5 DATA COLLECTION

3.5.1 Growth and Morphological Data

Seedling height (cm): The average height of randomly selected 3 seedlings from each experimental unit was measured from the ground level to the tip of the main stem at the end of seedling transplant stage.

Leaf Number (count): The number of leaves from 3 plants per treatment was counted and average was recorded at end of seedling transplant.

Leaf Area (cm_2): The leaf area was measured using an area meter model Ll3000A belt driven leaf area meter (LiCor Lincoln, Nebraska, USA) three month after the start of the irrigation withholding treatments from three selected plants per treatment.

Leaf fresh weight (g): It was measured using sensitive balance from all leaves of three selected plants per experimental unit at end of seedling transplant stage.

Leaf dry weight (g): It was measured by electronic sensitive balance after the samples had been oven-dried (48 hr. at 75℃) until constant weight was maintained.

Internode Length (cm): Average internode length was measured from the main stem of randomly selected plants at seedling transplant stage.

Root morphology:

Root length (cm)**:** Tap root length was measured from randomly selected three seedlings of each experimental unit after the roots were carefully separated from the soil and washed, at the seedling transplant stage by using ruler (cm).

Average root diameter (mm plant-^{1}): Root diameter was measured from randomly selected three seedlings after uprooting and proper washing of the roots by clean water, by using a digital calliper at seedling transplant stage.

Root number (count): Root number was counted from randomly selected three plants of each experimental unit at the seedling transplant stage after root was carefully separated from the pot and washed.

Root length (cm): Average tap root length was measured from randomly selected three plants at the end of seedling stage after root was carefully separated from the pot and washed.

Root fresh/dry weight (g): Root weight was determined by dry weight, the washed and cleaned roots was measured and dried in an oven at 105° C for about 10 to 20 h depending on the amount of roots.

Root anatomy parameters

Xylem number and Xylem width: The root sample was uprooted and washed properly during the transplant stage of seedlings. Then sample were taken from tap root up to 0.50 cm and fixed the plant cell by using fixatives which was prepared from 50ml ethanol, 5ml acetic acid, 10ml 37% formaldehyde, 35ml of H_2O and left over night in 4^0c by following method of Soukup and Tylová (2019) with little modification. The samples were embedded in paraffin wax using molds and stored at 4^0c up to the date of trimming. Before trimming deparaffinization was done through hydration using xylene, ethanol different concentration (100, 96, and 80) distilled water, tap water. Trimming was done by using paraffin microtome at 20 micrometre. The hot tap water at 60^0c was used during trimming for detaching the small sample from paraffin. Finally the samples were collected and put on slides and left for 24hr for drying. Then, the samples were stained by following staining procedures of cell histology. A 0.25g of toluene blue and eosin powder was dissolved by 20ml of distilled water in different container for staining purpose. The trimmed cell sections were then visualized by using the Automated Upright Leica Microscope DM5000B with a 100x magnification lens fixed with a digital Leica DFC425/DFC425C image processing camera by following the method of Soukup and Tylová (2019).

AMF Colonization: To determine the arbuscular mycorrhizal colonization, root segments 1 cm-long, were cleared by 10% KOH solutions at 95 °C for 1.5 h, bleached by 10% H_2O_2 solutions for 15 min, acidified with 0.2 mol l^{-1} HCl for 1 h, and stained with 0.05% (w/v trypan blue in lactophenol for 3 min (Phillips and Hayman, 1970). Mycorrhizal colonization was quantified using the magnified intersections method with a microscope (200 to × 400) as described by (Mcgonigle *et al.*, 1990).

Colonization rate = area of AMF mm^2 / area of root mm^2 * 100

3.5.2 Physiological data

Leaf gas exchange parameters: Leaf gas-exchange rates were measured using a Li 6400 portable photosynthesis system (LICOR Inc., Lincoln, NE, USA) from three seedlings of each experiment unit. Photosynthesis (A), Stomata conductance (gs) and transpiration rate (E) were measured at 3 months after the start of treatment on fully expanded intact leaves. Measurement was done between 10:00 AM and 12:00 PM setting instrumental modifications like: leaf surface area was 6.54 cm2 ambient carbon dioxide concentration 386 μmol mol-1,leaf chamber mass flow rate was250 μmol s-1, atmospheric pressure 840 bar and photosynthetic active radiation (PAR) was manually fixed to 1200 μmol m-2 s-1.

Water use efficiency was determined as the ratio between net CO_2 assimilation rate (A) and transpiration rate (E).

Determination of chlorophyll concentration: Leaf chlorophyll concentration was measured at 3 months after the start of the treatment on fully expanded young leaves of three plants from middle in each treatment. Leaves were placed in a bag sealed with aluminium foil and transported to crop physiology laboratory Fresh leaf discs (0.5g) were placed in 15-mL tubes containing 95% alcohol and the glass vials were sealed with Para film to prevent degradation of chlorophylls‟ by light. The homogenized sample mixture was centrifuge for 10,000 rpm for 15min at 20°C. The supernatant was separated and 0.5ml of each concentration level was analysed in triplicate for Chlorophyll-a and Chlorophyll-b at an absorbance of 649 nm and 664nm wavelength region, respectively, in spectrophotometer UV-2450 spectrophotometer (Hitachi Tokyo, Japan). Chlorophyll-a, Chlorophyll-b and total chlorophyll was determined by using the following equations (Lichtenthaler and Buschmann, 2001).

Ch a (μg/ml) =13.36 (A664) - 5.19 (A649)

Ch b (μg/ml) = 27.43 (A649) - 8.12 (A664)

Total chl (μg/ml) = chl a+chl b

Where; A = Absorbance, Ch a = Chlorophyll a, Chb = Chlorophyll b

Stomata anatomy: Stomata anatomy was measured by using modified protocol from Xu and Zhou (2008) at three months after the start of the treatment. A thin layer of transparent nail polish was uniformly used to stain the lower surface of fresh intact leaves. The polished surfaces were waited for 10 minutes until the nail polish dried and capture the epidermal imprint of the leaves, thereafter, a thin layer covering a surface on the leaves were peeled off using a clear tape and attached on the microscope slide. The imprinting was then look at using Automated Upright Leica Microscope DM5000 B with a 40x magnification lens fixed with a digital Leica DFC425/DFC425C image processing camera. Stomata number (per mm2), stomata width (µm) and length (µm) were measured from each sample.

Relative leaf water content: Three fully expanded leaves were collected from three plants at three months after the start of the treatment and leaf disks (9 mm in diameter) were immediately weighed (leaf fresh weight), there after the samples were immediately hydrated to full turgidity for 24 h by immersing the leaf disc in distilled water in a closed 15-ml falcon tube under room temperature. Then after, the weight of turgid leaf disc was measured to determine turgid mass (leaf turgid weight) after the water droplet was removed using tissue paper. Samples were oven-dried for 24 h at 75°C to obtain dry mass (leaf dry weight). Relative water content (RWC) was calculated as the following method developed by Turner (1981).

LRWC (%) = [(F.W – D.W) / (T.W – D.W)] * 100.

Where: F.W., Fresh weight; D.W., Dry weight; T.W., Turgid weight

3.5.3 Biochemical compound

Determination of proline concentration: At three months after the start of the treatment leaf samples from each treatment were collected for determining proline content. Using the method of Carillo and Gibon (2011) and Bates *et al*. (1973) 50 mg fresh leaf samples were placed in 1ml of ethanol and allowed to sit overnight at 4°C; in the following day the samples were centrifuged at 14000rp for 5minutes. 100 µL of reaction mix (1:60:20%) ninhydrin, glacial acetic acid and ethanol was pipetted to each sample. The sample was heated in water bath at 95 °C for 20 minutes. After cooling at room temperature the supernatant was centrifuged down quickly for1min at 2500 rpm. Then 100 µL of the supernatant was transferred to a micro plate reader with 96 wells and quantified the value at 520 nm absorbance using Multisken FC.

3.5.4 Biomass data

Fresh weight: One plant from each experiment was roughed and washed the root to remove the soil then weighed to know fresh weight of the seedling five months after planting or at the avocado seedling transplant age

Dry weight: Fresh weight measured plants was oven dried at 75° C for about 48 hours then the dried samples was weighed. Moisture content of each sample was calculated as percentage of fresh weight: (Fresh weight - dry weight / fresh weight) × 100- Lastly calculated weight of moisture from fresh weight of plants was subtracted in all pots of each treatment to know dry weight of plants.

3.6. Data analysis

All measured parameters were subjected to Proc GLM for analysis of variance (ANOVA) using statistical analysis system SAS software version 9.2 (SAS Institute 2008). The means were separate by using the Least Significant Differences (LSD) test at 5% level of significance. Pearson correlation analysis was used to define the relationship between variable.

4. RESULTS AND DISCUSSION

4.1. Morphological and Growth Parameters

4.1.1. Seedling height and Internode Length

The analysis of variance indicated that seedling height and internode length were significantly ($P < 0.001$) influenced by main effect of AM inoculation and moisture stress but they were not significantly ($P > 0.05$) influenced by the main factor race, two way interaction effects and three way interaction effect of arbuscular mycorrhizae, race and moisture stress (Appendix Table1).

The maximum (27.50cm) and (0.79cm) seedling height and internode length were measured from inoculated avocado seedlings and daily water treatments While, the minimum (24.18cm) and (0.71cm) seedling height and internode length were recorded from non-inoculated and 21 day water withholding respectively (Table 5). Furthermore the mycorrhiza inoculation increased seedling height and internode length by 12% and 10% respectively, with compared non-inoculated plants. Similar results have been shown in various reports indicating positive effects of different AMF species on the growth and development of avocado plants when compared to non mycorrhizal control (Rivera *et al.*, 2011). Alvarado *et al*. (2013) has shown that the seedling height of avocado seedlings treated with AMF was 50 to 54 higher than in the control plants. Moreover, the internode length of the inoculated plants were 1.08 times that of the non-inoculated plants which has also been clearly shown in another study that inoculation with mycorrhiza increased apple plant concomitantly with internode length and number (Jing *et al.*, 2022).

The increment on seedling height and internode length for plants inoculated by AMF might be due to phytohormones such as auxins and gibberellins which showed increased concentration and associated increase in seedling height (Barker and Tagu, 2000). Furthermore, Jing *et al*. (2022) showed that AMF regulates some metabolites and genes related to plant growth, such as hormone (IAA, CTK, and GA) synthesis and signaling and cell morphogenesis.

On the main factor effect of moisture stress the higher (29.88 and 0.88) cm as well as the lower(22.95 and 0.62)cm seedling height and inter node length was measured from daily and 21 days water treatments respectively on which the variation drops 23.2% and 29.5% for the seedling height and internode length respectively through increasing moisture stress levels. Hence there is no significance different between 7 and 14 days water treatment on seedling height and internode length. Main cause for the decrease in seedling height is due to decreased cell expansion, increased leaf shedding, and impaired mitosis under drought conditions (Yang *et al*., 2021). The result is harmony with Litvin *et al.* (2016) who found that seedling height and inter node length reduced in tomato plant through decreasing water availability. Similarly, Misra *et a*l. (2020) reported that there was a decrease in sugarcane height in drought (by 18.25%) as compared to normally grown canes.

Table 5. Effect of arbuscular mycorrhizae and moisture stress level on seedling height and internode length of two avocado races seedlings

AMF	**Seedling height(cm)**	**Internode length(cm)**
Inoculated	27.504^{a}	0.79^{a}
Non- inoculated	24.18 ^{b}	0.71^{b}
LSD	**1.05**	**0.005**
Water withholding		
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	**1.495**	**0.0781**
CV (%)	**6.94**	**12.44**

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

4.1.2. Leaf number, area, fresh and dry weight

Analysis of variance indicated that, Leaf number was significantly influenced by main factors moisture stress and arbuscular mycorrhizae at ($P < 0.001$)and two way interaction effect of arbuscular mycorrhizae with moisture stress at ($P< 0.05$). However, main factor of races and two way interaction effect of race with moisture and race with arbuscular mycorrhizae as well as the three way interaction effects of race, arbuscular mycorrhizae and moisture stress levels did not show significant difference ($P > 0.05$) on (Appendix table 1).

In this study high leaf number was counted from arbuscular mycorrhizae inoculated avocado seedlings grown under daily watering treatment and the lowest was from non-inoculated stressed plants which were water withholds for 21 days (Table 6). The inoculation of AMF increased leaf number by 14.6%, 14.8%, 10.6% and 13.6% in daily, 7 days 14 days and 21 days water withholding treatments, respectively, compared to non-inoculated. Similar findings was reported by Behrooz *et al.* (2019) who observed that the number of leaves in walnut seedlings inoculated with arbuscular mycorrhizae were increased significantly in both well watered and drought condition compared to non-inoculated walnut seedlings. This result also agrees with research result of Kamayestani *et al*. (2019) Mexican race inoculated with two commercial AMF inoculants scored high leaf number (48% and 37%) than non-inoculated. Similarly, Arbuscular mycorrhizae increased the average number of leaves in two grape cultivars compared to non-inoculated.

According to Hussain and Ali (2015) leaf number reduction on moisture stress condition is due to water stress and, furthermore, it reduces leaf formation, induces early abscission, senescence and consequently reduces the overall leaf number. Similarly Chartzoulakis et al. (2002) indicated the changes in the leaf growth characteristics such as leaf number where water-stressed plants had smaller number of leaves. This might be related to increased leaf unfolding rate which mainly affected by gibberellins (GA) and AMF are associated with increasing the levels of GA which in turn increase's number of leaves per plant (Jing *et al*., 2022).

Leaf area, leaf fresh weight and leaf dry weight of avocado seedlings were significantly ($P < 0.001$) influenced by the main factors and interaction effect of arbuscular mycorrhiza with moisture stress levels. Whereas, the interaction effect of races with moisture significantly ($P < 0.01$) and while, race with AMF and three way interaction effect of races, arbuscular mycorrhizae and moisture stress didn't show significant difference at ($P < 0.05$) (Appendix table 1 and 2).

On the interaction effect of AMF with moisture the highest value was measured from daily watering treatment with AMF inoculated seedlings, while the minimum value was obtained from 21 day water withholding treatment with non-inoculated seedlings for leaf area leaf fresh and dry weight respectively (Table 6).

In general, avocado seedlings inoculated with AMF had better performance compared to non-inoculated seedlings at all water withholding treatments. Interestingly, with increased moisture stress levels with 7, 14, and 21-day water withholdings, the leaf area, leaf fresh and dry weight of the plants grown under AMF inoculation were better than the non-inoculated ones. The inoculation of AMF increased by 43.9% , 32.5% and 41% on leaf area As well as, 29.9% ,29.6% and 24.2% on leaf fresh weight and 26.6%, 25% and 20% on leaf dry weight for water stressed 7,14 and 21 days water treatments respectively.

The result conform with the findings of Li *et al*. (2022) reported that AMF inoculation increased leaf area of stressed tea seedlings by 18.6% compared to non-inoculated tea seedlings. Similarly Kamayestani *et al*. (2019) observed that two grape cultivars exhibited changes in leaf size and weight, where, AMF inoculated plants had bigger leaves, higher fresh and dry weights as compared to non-inoculated plants. Likewise, Aslanpour and Omar (2019) revealed that AMF had a significant effect on the fresh weight of leaf and fresh and dry weight of leaves of white grape vines. The increment on plant leaf area, leaf fresh weight and leaf dry weight with AMF inoculated avocado seedlings could be due to the role of AMF to increase plant growth performance, improve soil-plant-water relations, enhance plant nutrient acquisition which leads to activation of biochemical and hormonal reactions, increase in weight of roots and leaves, weaker effect of drought stress and hence increased productivity of plants (Sharma *et al*., 2017). Furthermore, it could be due to increased carbon metabolism and accumulation in plant parts promoting growth (Jing *et al.*, 2022).

Table 6. Interaction effect of arbuscular mycorrhiza and moisture stress on leaf number, leaf area, leaf fresh and dry weight of two avocado races seedlings

AMF	**Water withholding**	**Leaf number**	**Leaf area (cm^2)**	**Leaf fresh weight (g)**	**Leaf dry weight (g)**
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
	21-days	13.01^f	537.3^d	10.3^e	3.e^c
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		**0.74**	**55.063**	**0.36**	**0.13**
CV (%)		**4.07**	**13.7**	**4.3**	**5.6**

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

Regarding the interaction effect of avocado races with moisture stress, the maximum leaf area, leaf fresh weight and leaf dry weight was measured from both Mexicana and Guatemalan races under daily water treatments. While, Mexicana race measured higher amount of leaf fresh weight and leaf dry weight under moisture stressed 21day water withholding treatments in relation to Guatemalan race as well as higher leaf area was also measured from Mexicana race on14 day water withholding treatment. A significant reduction of leaf area with increasing moisture stress levels was observed, reductions of 230.4, 216.25 cm2, and 514.38 cm^2 in the Mexicana race and 287.03 cm2, 480.52 cm2, and 552.02 cm^2 in Guatemalan race on 7-day, 14-day, and 21-day water withholding treatments. However, Mexicana race measured significantly higher leaf area on 14 days of water withholding treatment, which was 282.88 cm2 greater than the Guatemalan race. The mexicana race is considered as more stress tolerant since it have expanded leaf area in moisture stress condition which determines light interception and is an important parameter in determining plant productivity. Leaf area growth in addition, high-throughput phenotyping of plants often relies on optical methods in which leaf area growth is compared with estimates of photosynthesis derived from fluorescence signals. (Weraduwage *et al*., 2015).

This result is in agreement with the findings of Shao *et al.* (2008) who reported that during water stress, the total leaf area per plant decreased significantly in different plants and varieties. Leaf-area plasticity is important to maintain the control of water use in crops .Reduction in leaf area by water stress is an important cause of reduced crop yield through reduction in photosynthesis (Shao *et al*., 2006). The reduction in seedling height and leaf area under water stress may be associated with the decline in the cell enlargement and more leaf senescence (Kapoor *et al*., 2020).

Leaf fresh and leaf dry weight of the Mexicana race also measured higher in 7, 14 and 21 days water withholding treatments than the Guatemalan race. The leaf fresh weight increased by 6.5%, 17.4% and 9.7% and the leaf dry weight increased by 4.7%, 13.4% and15.8% in Mexicana race with 7, 14 and 21 days water withholding treatments respectively. Similar result was reported by Amalero *et al*. (2003) found that drought stress significantly affected leaf dry weight of potato cultivars.

Table 7 Interaction effect of race and moisture stress on leaf area, leaf fresh and dry weight of two avocado races at seedling stage

Races	**Water withholding**	**Leaf area(cm^2)**	**Leaf fresh weight (g)**	**Leaf dry weight (g)**
Mexicana	Daily watering	969.13^a	22.36^a	6.02^a
	7-day	738.73^b	15.94^b	4.03^b
	14-day	752.88^b	12.39^d	3.42^c
	21-day	454.75^c	9.52^e	2.97^d
Guatemalan	Daily watering	951.45^a	21.69^a	6.16^a
	7-day	664.42^b	14.89^c	3.84^b
	14-day	470.75^c	10.23^e	2.96^d
	21 day	399.43^c	8.59^f	2.50^e
LSD		**109.65**	**0.74**	**0.26**
CV (%)		**13.77**	**4.34**	**5.62**

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

4.1.3 Root number, root length, and root diameter

Root number and root length were significantly (P < 0.001) affected by the main factors, all two-way and three-way interaction effects of avocado races, arbuscular mycorrhiza and moisture stress, while leaf number didn't significantly (P > 0.05) influenced by the interaction effect of race with moisture (Appendix 3). The maximum root number was observed from daily watered Guatemalan race followed by inoculated Mexicana race under daily watering treatment. While the minimum value was recorded from stressed seedlings of both avocado races with AMF non-inoculated, the trend of response in Mexican and Guatemalan seedlings in relation to water holding interval and AMF inoculation is similar (Table 8). In both avocado races inoculated seedlings showed higher root number and length under all water withholding intervals as compared to non-inoculated treatments. However, on root number and root length the AMF inoculated Mexicana race performed better than Guatemalan under moisture stress condition.

The inoculation of AMF under drought stress condition increased the root number and tap root length of avocado seedlings. In the present study, under moisture stress conditions, the inoculation of AMF significantly increased root number by 38.5%, 36.5% and 37.6% in Mexicana and 32.3%, 26% and 28.8% in Guatemalan races with 21, 14 and 7days water withholding treatments, respectively, compared to non-inoculated treatments. This indicated that for plants grown under moisture stress the inoculation has helped to tolerate the water deficit by increasing their root number and length.

The present experiment also indicated that inoculation with AMF increased the root number and length to expand their contact surface with the soil, thus improving plant water uptake efficiently and in this way mitigating drought stress as compared to non-inoculated for both races. These results conform with the findings of Behrooz *et al*., (2019) stated that, root length, number and leaf fresh weight of walnut subjected to drought stress were declined significantly as compared to well-watered treatment, while the inoculation of AMF increased root length and number of walnut tree under drought condition. Abdelmoneim *et al*. (2014) also found that root number and root length in maize was increased when the plants were inoculated by AMF with compared to non-inoculated plants.

Root average diameter was significantly ($P < 0.001$) affected by main factors, two way interaction effect of race with moisture and three way interaction effects of avocado races, arbuscular mycorrhiza and moisture withholding interval, but not significantly affected by two way interaction effect of race with arbuscular mycorrhizae (Appendix 6). The maximum root diameter (8.03 mm) was observed from AMF inoculated Mexicana race under daily water treatment, whereas the minimum (1.80mm) was observed from non-inoculated Guatemalan race under 21 day water withholding treatments (Table 8). In stressed seedlings inoculation of arbuscular mycorrhiza significantly increases root diameter as compared to non-inoculated plants. These result coincides with Hussain *et al.*, (2021) revealed that AMF inoculum applied by seed coating and soil application recorded greater increase in root average diameter of maize crop under different moisture stress conditions. Similarly, Tomazelli, (2022) reported that root diameter was greater in plants inoculated with two AMF species (6.3% for A.colombiana and 10.3% for R.clarus) on St. Hill seedlings. The reduction of root average diameter in drought stress conditions may be due to deficit of water result in progressive changes in the structure of plant and its impact is particularly high because it is directly involved in the efficient use of water during the stress conditions.

Table 8. Interaction effects of arbuscular mycorrhiza, moisture stress and avocado races on root number, root length and average root diameter of avocado seedlings

Race	**Water Withholding**	**Root number(count)**		**Tap root length(cm)**		**Average root diameter(mm)**	
		inoculated	**Non inoculated**	**inoculated**	**non-inoculated**	**inoculated**	**non-inoculated**
Mexican	Daily	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		**2.26**		**1.63**		**0.25**	
CV (%)		**2.83**		**2.22**		**3.52**	

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

4.1.4 Root fresh and dry weight (g)

Root fresh weight and root dry weight was significantly ($P < 0.001$) affected by main factors, and two way interaction effect of arbuscular mycorrhizae with moisture. Whereas, race, the interaction effect of race with AMF and three way interactions effect of moisture, AMF and race did not have any significant ($P > 0.05$) effect on the root dry and fresh weight (Appendix Table 2). Regarding the interaction effect of race with moisture withholding the maximum root number was observed from daily watered avocado seedlings races inoculated with arbuscular mycorhzia, whereas the minimum was observed from water stressed with non-inoculated avocado seedling.

Furthermore, the increment in root dry and fresh weight was almost by 10-45% for both avocado races inoculated with AMF as compared to non-inoculated plants under all water treatment (Table 9). Especially, under moisture stress conditions (21 days water withholding), percent increase was 46.2 % and 38.77% in fresh and dry weight for AMF inoculated avocado seedlings. As Shao *et al*. (2008) reported that prolonged water stress reduced the biomass of fibrous roots in Avocado races, while inoculation with arbuscular mycorrhizae increased the biomass significantly under moisture stress conditions. Similar result was reported by Thakur and Shinde (2020) found that the mycorrhizal plants have shown more fresh and dry weight of root as compared to those of control in pea plants during water stress.

The cause for the increment in root dry and fresh weight could be due to increased overall growth in AMF inoculated plants resulted from increased water and nutrient absorption and subsequently improve plant growth and performance of root water absorption capacity (Masrahi *et al*., 2023).

Table 9: Interaction effect of arbuscular mycorrhiza and moisture stress on root fresh weight and root dry weight

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

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

4.2. Root anatomy

4.2.1 Root xylem vessel number and width

Xylem number and width was significantly ($P < 0.001$) affected by main factors, two way interaction effect of race with moisture. While remaining the two-way interaction effect of race with arbuscular mycorrhizae and AMF with moisture and the three-way interaction effect of races, arbuscular mycorrhiza and moisture were not significantly ($P> 0.05$) influenced. The maximum xylem number was observed from 21day water withholding Mexicana race seedlings whereas, the minimum was observed from daily watered Guatemalan seedlings. The result of this study is in line with Martin *et al*. (2017) found that xylem traits showed that pine species reacted to drought by building a xylem with larger lumens and narrower cell walls. Likewise, Cheng *et al.* (2016) reported that there was the increase in the number of metaxylem and secondary xylem on the rootstocks of peach trees inoculated with arbuscular mycorrhizae while the control plants showed a smaller number of metaxylem and secondary xylem that were also smaller in diameter compared to inoculated trees. The observed number of xylem was increased and the width was decreased while soil moisture content was decreased. This may be due to the formation of additional protoxylem strands metaxylem position when water availability is reduced (Ramachandran *et al*., 2020).

The increase in xylem number in moisture condition may be due to such as drought and oxidative stress promote xylem differentiation by modulating the interaction between cytokinin and jasmonic acid Jang and Choi, (2018). A decrease in the xylem diameter of the roots in response to drought is a mechanism to avoid the influence of embolism on the xylem. Ramachandran *et al.* (2020) also reported that the roots of soybean plants experience a reduction in the size of the xylem as a mechanism of plant tolerance in the face of drought stress.

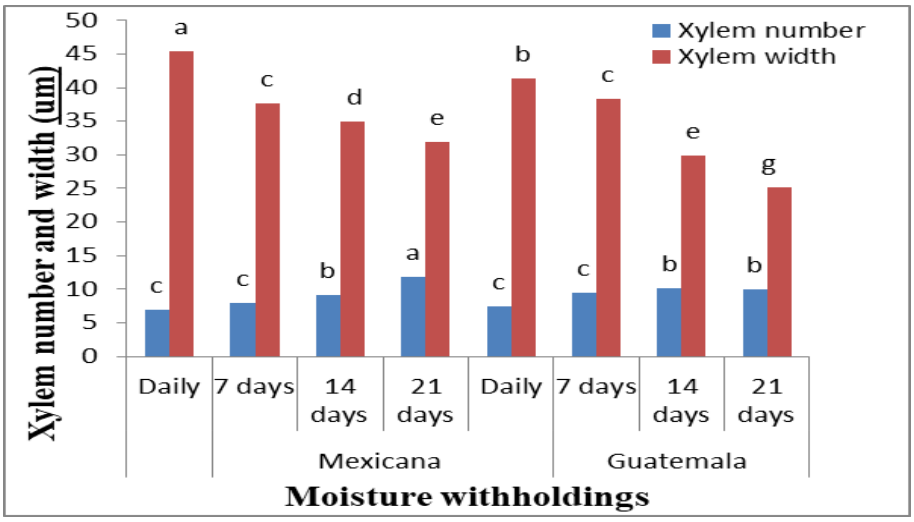
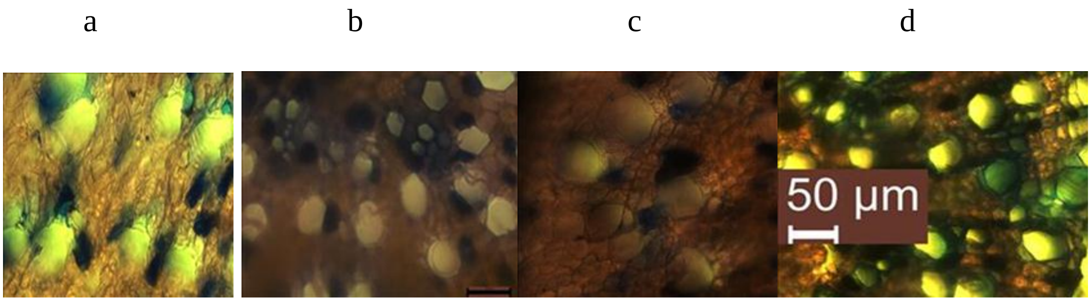


Figure 1. Mean value of xylem number and width of two avocado races as influenced by races and soil moisture withholding interval Means with different letter on the figure are statistically significant at P-values < 0.05. LSD (0.05), = 1.10, 1.76 and CV (%) 10.28, 4.21



a=Daily mexicana,b=21 days mexicana,c= Daily Guatemalan and d=21 days Guatemalan,

Figure 2: Pictorial representations of xylem numbers affected by moisture stress

4.2.2 Root AMF colonization rate

Root colonization with AMF was significantly (P < 0.001) influenced by main effect of arbuscular mycorrhizae, moisture withholding level and interaction effect of AMF with moisture withholding levels while other two way interaction effect of race with AMF, race with moisture withholdings and three way interactions effect of race, AMF and moisture withholding did not significantly (P < 0.05) influenced (Appendix table 7). The highest colonization rate (67.88%) was observed from daily water treatments while the lowest colonization rate (39.65%) was observed from stressed 21 days water withholding treatments (Table 10). The result of this study is in line with Abdel-Salam *et al*. (2018) revealed that mycorrhizal colonization was significantly decreased in damask rose root tissues as the level of the drought stress increased. The same result was reported by Prado-Tarango *et al.* (2023) stated that drought and competition resulted in lower colonization in *Artemisia* species. Similarly, Farghaly *et al.* (2022) revealed that the highest value of arbuscular colonization in wheat plants was found in the control AMF inoculated plants, and the lowest value was recorded in stressed plants.

Table 10: Interaction effect of moisture stress and arbuscular mycorrhizae on root mycorrhizae colonization rate

Water withholding	**Colonization rate (%) Inoculated**
Daily	67.88^{a}
7 days	60.93^{b}
14 days	55.63^{c}
21 days	39.65^{d}
LSD	**0.85**
CV (%)	**2.58**

4.3. Physiological and biochemical Parameters

4.3.1 Photosynthesis

Photosynthesis rate was significantly ($P < 0.001$) affected by main factors arbuscular mycorrhizae, moisture withholdings two way interaction effect of race with moisture and AMF with moisture and three way interaction effect of avocado races, arbuscular mycorrhiza and moisture withholding interval while the main factor race and two way interaction effect of race with AMF didn't significantly ($P > 0.05$) influenced (Appendix table 4). The highest photosynthesis rate was obtained from Guatemalan race inoculated by arbuscular mycorrhizae under daily water treatment followed by Mexicana with mycorrhizae inoculation under daily water treatments and the lowest mean value of photosynthesis rate was measured from Guatemalan without arbuscular mycorrhizae inoculation and 21 days water treatments (Figure 3). Photosynthesis rate in avocado races reduced significantly when seedlings were subjected to drought stress, but the inoculation of arbuscular mycorrhizae raised photosynthesis rate in all moisture intervals. The AMF inoculation increased photosynthesis rate by 37.3%, 39.4%, 24.5% and 40.5% in the

Mexicana race and by 40%, 26.9%, 32% and 69.8% in the Guatemalan race through increasing water withholdings (daily, 7 days, 14 days, and 21 days, respectively). However, the photosynthesis rate in stressed plants of mexicana race was maintained better with compared to Guatemalan.

The result of this study is in agreement with many studies that have found that AMF inoculation can improve photosynthesis capacity and chlorophyll content, such as studies on grapevine varieties that recorded that AMF inoculation maintained total chlorophyll and photosynthesis rate under drought stress conditions. Karoglan *et al*. (2021) assessed the influence of the application of mycorrhizal fungal inoculum on ‘grapevines leaf gas exchange and concluded that inoculated grapevines expressed improved leaf gas-exchange parameters in general. The mechanisms behind this action is due to the enhancement of plants drought tolerance by improving their gas exchange capacity, increasing chlorophyll fluorescence parameters, creating a greater osmotic adjustment capacity, increasing the scavenging of reactive oxygen species (ROS), and using signals for interactions between AMF and their plant hosts (Ye *et al*., 2022).

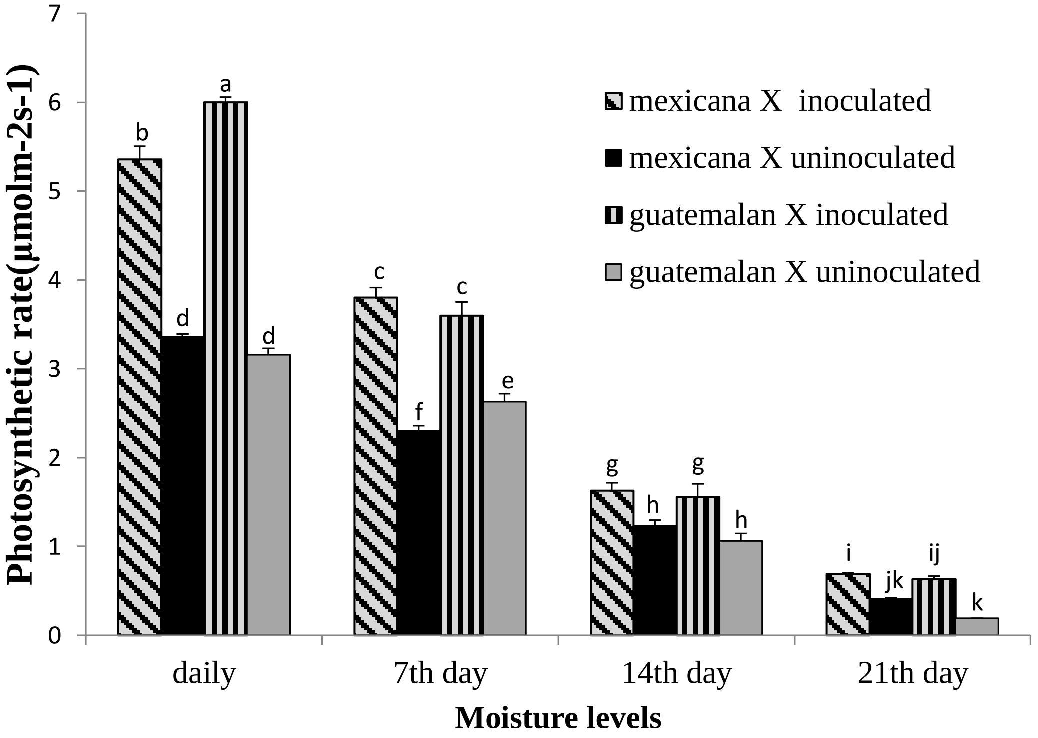


Figure 3: Mean value of photosynthesis rates of two avocado races as influenced by different arbuscular mycorrhizae and moisture stress. Means with different letter on the figure are statistically significant at P-values < 0.05. LSD (0.05), = 0.24 and CV (%)

4.3.2 Transpiration

Transpiration was significantly (P <0.001) affected by main factors arbuscular mycorrhiza, moisture withholding two way interaction factors race with moisture, AMF with moisture while the two way interaction effect of race with AMF and three way interaction effect of race, arbuscular mycorrhiza and moisture withholding interval significantly (P < 0.01) but the main factor race didn’t show significantly (P > 0.05) influenced (Appendix table 4). The maximum transpiration rate was observed from mexicana race treated arbuscular mycorrhizae with daily water treatment, followed by Guatemalan with arbuscular mycorrhizae inoculation under daily water treatment, while the minimum transpiration rate was obtained from Guatemalan which is grown under water stressed condion with non-inoculated seedlings (Figure 4). The result of the present study conforms with Hazzoumi *et al*. (2015) revealed that inoculation of the AMF improved transpiration, activated antioxidant enzymes, regulated cell osmotic state, and enhanced the drought tolerance of basil enabling its growth in fragile ecological environments. The transpiration rate of avocado races was reduced by water stress, but root hydraulic activity of inoculated seedlings was more than twice that of non-mycorrhizal citrus seedlings under well-watered and drought stress conditions (Graham and Syvertsen, 1984).

The increase of transpiration in all water withholding treatments with AMF inoculation may be due to Arbuscular mycorrhizal fungi can form symbiotic relationships with most plants and play an important role in plant growth and adaptation to various stresses and improve the growth of host plants due to direct H_2O and nutrient absorption and transportation through mycorrhizal hyphae (Ye *et al*., 2022). As Evelin *et al.* (2009) showed that AMF-inoculated plants regulate better their ABA level than no-inoculated ones, resulting in a suitable balance between leaf transpiration and root water absorption during drought stress and recovery.

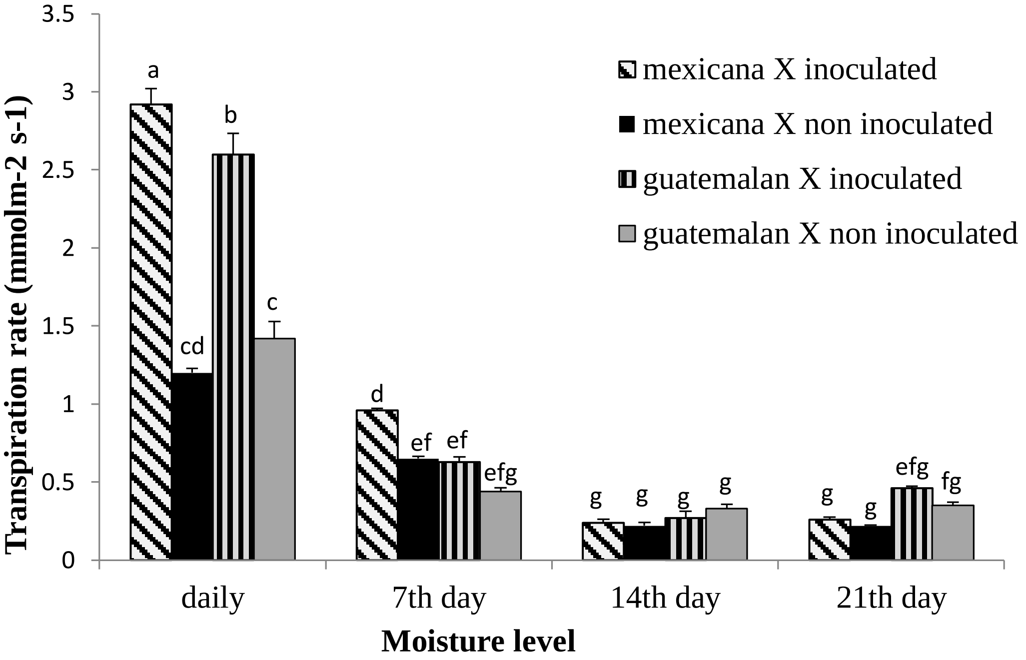


Figure 4: Mean value of transpiration rates of two avocado races as influenced by different arbuscular mycorrhizae and soil moisture stress. Means with different letter on the figure are statistically significant at P-values < 0.05 LSD (0.05), = 0.15 and CV (%) = 11.42

4.3.3 Water use efficiency

Water use efficiency was significantly ($P < 0.001$) affected by main factors arbuscular mycorrhizae, moisture withholding two way interaction effect of race with moisture, AMF with moisture while the main factor race and three way interaction effect of avocado races, arbuscular mycorrhiza and moisture withholding intervals are also significantly ($P < 0.05$) influenced (Appendix table 4). The maximum water use efficiency was recorded from mexicana races inoculated with AMF and grown under 14th day water treatment, while the minimum water use efficiency was observed from Guatemalan race which is grown under non- inoculated and 21th days water treatment (Figure 5). Water use efficiency was increased in all AMF inoculated seedlings at all water withholding treatments Compared to non-inoculated seedlings.

The result shows that mexicana race has higher water use efficiency than Guatemalan under drought stress condition this suggests that mexicana races is more drought tolerance than Guatemalan. Water use efficiency of mycorrhizal avocado seedlings was higher than non mycorrhizal avocado seedlings.

The possible mechanisms could be extensive absorption of water by external hyphae, stomatal regulation through hormonal signals and greater osmotic adjustment in mycorrhizal plants which promotes turgor maintenance even through at low tissue water potential and indirect effect on photosynthetic activity, proline accumulation in the mycorrhizal plants(Augé, 2001 ; Asrar *et al.* 2011). These mechanisms may be essential in adaptation of the mycorrhizal plants to drought conditions.

The result finding is in line with Guillermo *et al.* (2021) reported that there was higher water use efficiency in both levels of water stress (mild and sever) compared to control plants, with mild-WS plants displaying higher values which is due to that water-stressed avocado plants were able to maintain certain CO2 assimilation rates despite the low stomatal conductance values. Similarly, Asrar *et al.* (2012) reported that mycorrhizal inoculation improved water use efficiency of snapdragon plant under drought stress and well watered condition compared to non-inoculated plants

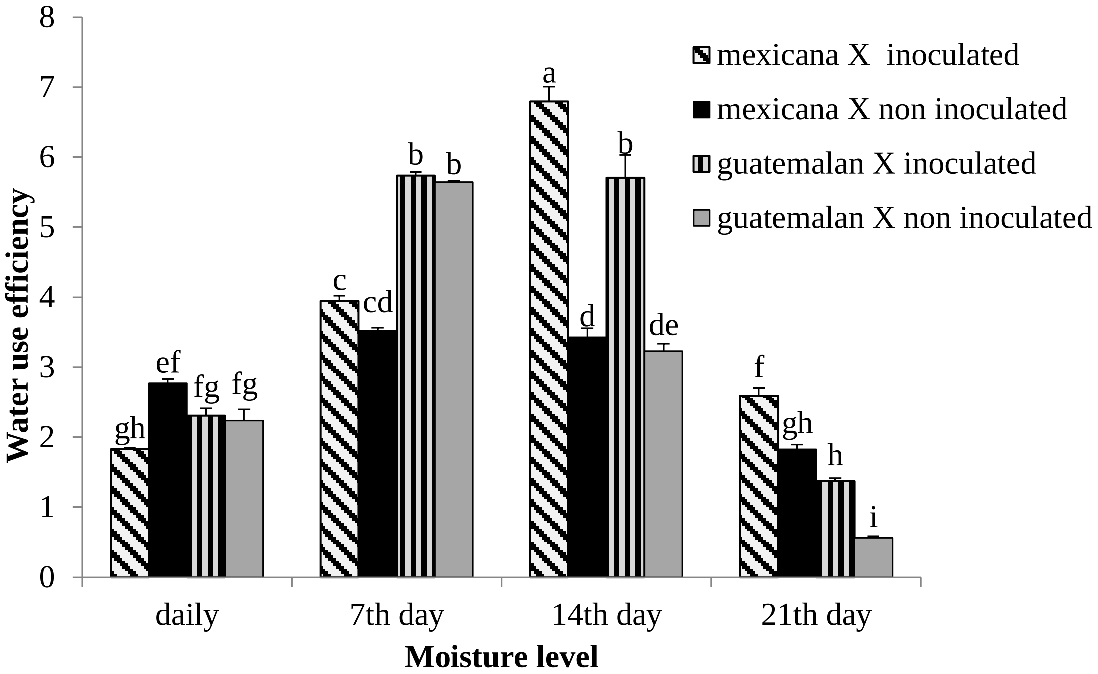


Figure 5: Mean value of water use efficiency of two avocado races as influenced by different arbuscular mycorrhizae and soil moisture stress. Means with different letter on the figure are statistically significant at P-values < 0.05 LSD (0.05),

4.3.4 Stomata conductance, stomata number and stomata width

Stomata conductance was significantly ($P < 0.001$) affected by main factors arbuscular mycorrhizae and moisture while the main factor race and two ways interaction effect of arbuscular mycorrhizae with moisture withholding interval significantly ($P < 0.05$) influenced, whereas it is not significantly ($P > 0.05$) influenced by the two way interaction effect of avocado races moisture withholding interval, avocado races with arbuscular mycorrhizae and three way interaction effect of race, AMF and moisture withholding intervals (Appendix table 5). The result indicated that the maximum stomata conductance was observed from daily water treatment with arbuscular mycorrhizae inoculated seedlings, while the minimum stomata conductance was observed from stressed with non-inoculated treatments (Figure 6).

Stomata conductance was increased in all AMF inoculated treatments at all moisture withholding levels compared to non-inoculated. The increase in percent was 31.2%, 23.15%, 26.25% and 41.07% higher for daily, 7 days, 14 days and 21 days moisture withholding AMF inoculated treatments compared to non-inoculated treatments respectively. It has been demonstrated that AMF participate in the uptake and transport of water in host plants and AMF species had a positive effect that has been observed on the water use efficiency and stomata conductance as compared to non-inoculated plants (Fernández-Lizarazo and Moreno-Fonseca, 2016). It has also been shown that higher gs rates in mycorrhizal plants are associated with low xylem-sap ABA concentrations and thus lower ABA content in the leaves (Ebel *et al.*, 2014).

This suggests that the stomatal conductance, transpiration rate and water potential of leaves can be higher in mycorrhizal plants under water deficit and inoculated with AMF *(Ye et al*., 2022). The same result was reported by Orivaldo *et al.*, (1999) stated that there was an increase in stomatal conductance in banana plants inoculated with AMF compared to control. Similarly, stomatal conductance increased in wheat races under normal, mild and stressed irrigations in AMF inoculated treatments compared to non-inoculated (Sayyahfar *et al*., 2018).

AM fungi also increases Zn and P concentrations in plant tissues, thereby positively affecting plant physiological processes such as photosynthesis, respiration, and stomatal conductance (Saboor *et al*., 2021).

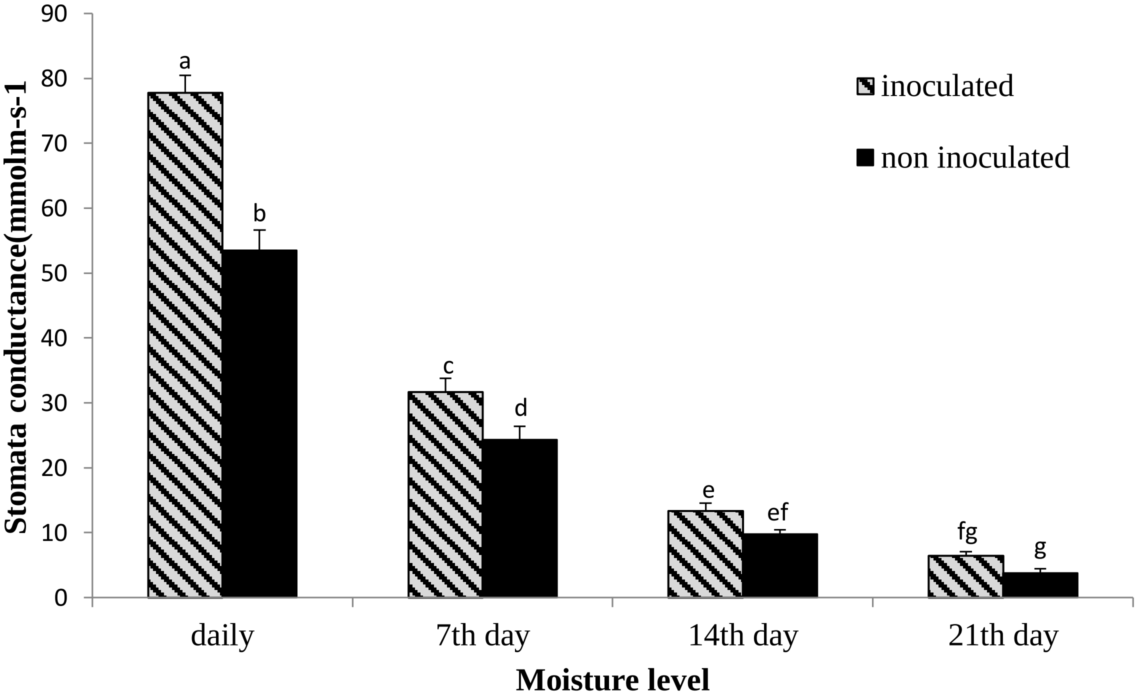


Figure 6: interaction effect of arbuscular mycorrhizae and soil moisture stress on stomata conductance. Means with different letter on the figure are statistically significant at P-values < 0.05 LSD (0.05), = 4.9 and CV (%) = 15.08

Analysis variance showed that stomata number was significantly ($p < 0.001$) affected by main factors arbuscular mycorrhizae and moisture withholding while it is not influenced by the main factor race and all two way and three way interaction effects (appendix table 6). The maximum stomata number was observed from arbuscular mycorrhizae inoculated seedlings than that of non-inoculated. The inoculation of arbuscular mycorrhizae increased stomata number by 8.3% than AMF non-inoculated avocado seedlings. Similarly high stomata number was counted on daily water treated seedlings while as stomata number was decreased as moisture stress level increases (Table 11).

Modification of stomatal density in response to drought varies between plant species, and is contingent on the severity of water deficit (Hasanuzzaman *et al.*, 2023). According to (Silva *et al.*, 2009) there was significant reduction on stomata density of GBU genotype of umbo tree with increasing moisture stress. The result of this study agrees with Ifeanyi and

Philippine, (2020) reported that the stomata number per leaf area and the size of stomata scored high values in arbuscular mycorrhizae inoculated cassava plants than non-inoculated. Similarly, Anosheh *et al.*, (2016) Found that stomatal number was decreased under more severe drought stress.

Table 11.The effect of main factors arbuscular mycorrhiza and moisture stress on avocado leaf stomata number at seedling stage

AMF	**Stomata number**
Inoculated	17.00^{a}
non-inoculated	15.58^{b}
LSD	**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	**1.12**
CV (%)	**8.27**

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

Analysis variance also showed that stomata width was significantly ($p < 0.001$) affected by main factors all two way interaction effects and also significantly ($p < 0.05$) on the three way interaction effects of races, AMF and moisture withholding interval (appendix table 6). The higher and lower stomata size was measured from Guatemalan race with AMF inoculated under daily water treatment and non-inoculated under 21 days' moisture withholding treatments respectively. The stomata size was decreased while increasing moisture withholding levels in all treatments but inoculation of AMF increased the stomata size compared to non-inoculated.

These may be due to plants close their stomata under drought stress condition to reduce water loss and this is mainly induced by ABA which is a key determinative for stomatal closure under drought conditions and colonization with AM fungal influences the accumulation of ABA in host plants compared to non-mycorrhizal ones (J. Zhang, 2022). Similar result was reported by Ouledali *et al.*, (2019a) revealed that AM-inoculation of the two studied olive races helped to some extent to maintain transpiration and stomatal conductance in AMF

Table 12**.** Interaction effect of arbuscular mycorrhiza, moisture stress and avocado races on stomata width at seedling stage

Race	**Water withholding**	**AMF**	
		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	**0.397**		
CV (%)	**2.73**		

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

4.3.5 Chlorophyll and leaf relative water content

Leaf chlorophyll content (Chla, Chlb and total chlorophyll) were significantly (P<0.001) affected by the main effects, two ways and three way interaction effect of avocado races, arbuscular mycorrhizae and moisture withholding interval . The result indicated that, the maximum leaf chlorophyll content (chl a, chl b and total chlorophyll) were recorded from Mexicana and Guatemalan races treated by AMF with daily water treatment, whereas the minimum was recorded from Guatemalan races under drought stress. The content of total chlorophyll (Chla + Chlb and T Chl) is significantly higher in mycorrhizal plants than in Non-inoculated seedlings. Furthermore, when comparing the total chlorophyll levels of these pigments between inoculated and non-inoculated seedlings, we see that mycorrhizal plants accumulate 7.2% and 8.8% more of chlorophyll under daily water treatment and 10.5% and 24.4% under 21 day water treatment in mexicana and Guatemalan respectively (Table 13). This result agrees with previous studies that arbuscular mycorrhizae inoculation increased Chlorophyll concentrations in leaves of mexicana race root stocks hence forth the inoculation of AMF were increased chlorophyll content by 19% with compared to those of the non-inoculated control (Alvarado *et al*., 2013). Similarly, Zhang *et al*. (2014) noted that the symbiotic association of mycorrhizal fungi with plant roots produces several changes or modifications at a physiological level; among which an increase in the chlorophyll content, photosynthetic capacity can be highlighted, due to a greater CO_2 fixation capacity.

Table 13: The interaction effect of arbuscular mycorrhiza, avocado race and moisture stress on chlorophyll a, chlorophyll b and total chlorophyll content of avocado seedlings.

Race	**AMF X moisture**	**chl a**	**chl b**	**TC**
Mexicana	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}
	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}
Guatemalan	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}
	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 -21days	13.24^{j}	1.76^{j}	15^{m}
LSD		**0.24**	**0.23**	**0.2**
CV (%)		**1.8**	**2.54**	**1.54**

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

Regarding leaf relative water content the analysis of variance indicated that LRWC was significantly ($P < 0.001$) influenced by main effects and the two ways interaction effect of avocado races and moisture withholding interval but they were not significantly ($P >0.05$) influenced by the two way Interaction effects of AMF with races, AMF with moisture withholding interval and three way interaction effect of avocado craces, AMF and moisture withholding interval (Appendix table 6). The higher leaf relative water content was measured from mexicana race in daily water treatment as well as stressed 14 and 21 days water withholding treatments which is 7.23%, 3.23% and 8.78% respectively higher than Guatemalan race (Table 14).

This is maybe due to Mexicana race showed better maintenance of higher RWC ensuring better hydration and more favourable internal water relations of tissue with a possibly higher pressure potential and showed better drought tolerance capacity (Seleiman *et al.*, 2021).

The result of this study consistent with Soltys-Kalina *et al.* (2016) reported that 3-week drought treatment decreased the leaf water content of the 18 potato cultivars in relation to control. Similarly, moisture stress significantly decreased relative water content in mungbean plants regardless of genotype (Relation *et al.*, 2023). Likewise, with Bolat *et al.*, (2014) reported that LRWC decreased with the increasing levels of water stress In two apple rootstocks. The negative effects of water stress on leaf RWC were reduced depending on the rootstock genotypes. Such a decrease in leaf RWC could have been due to unavailability of water in the soil (Bolat *et al.*, 2014) ,or root systems, which are notable to compensate for water, lost by transpiration through a reduction of absorbing surface (Gadallah, 2000).

Table 14: Interaction effect of avocado races and moisture stress on leaf relative water content of avocado seedlings

	Race	
Water withholding	**Mexican**	**Guatemalan**
daily	92.55^{a}	85.85^{b}
7 days	74.30^{c}	75.21^{c}
14 days	71.8^{d}	69.48^{e}
21 days	68.5^{e}	62.48^{f}
LSD	**1.25**	
CV	**1.42**	

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

4.3.6 Proline content

Proline content was significantly ($P < 0.001$) affected by main factors, two ways and three way interaction effect of avocado races, arbuscular mycorrhiza and moisture stress (Appendix table 4). The maximum proline content was measured from mexicana races inoculated with AMF and grown under 21 days water withholding treatment, while the minimum proline content was measured from Guatemalan race which is grown under non-inoculated and daily water treatment (Table 15). Proline content was enhanced by 12.6 and 9.2% on arbuscular mycorrhizae inoculated seedlings than non-inoculated seedlings under drought stress condition on Mexicana and Guatemalan races respectively.

This accumulation of proline in avocado seedlings had fundamental importance both to tolerate water deficits, and to aid in recovery after stress, providing greater gain in dry mass (Syvertsen, 2022). The result also agrees with Ruiz-Lozano *et al*. (1995) drought stress increased the proline content of lettuce especially on arbuscular mycorrhizae inoculated treatments under drought stress condition. Similarly, Higher levels of proline were observed in seedlings snapdragon plant under drought condition (Asrar *et al*., 2012).

The increment in proline content on inoculated treatments is because of proline biosynthesis has been associated with the glutamate or ornithine pathways which are both amino acids that were significantly increased in plants with AM fungi (Liu *et al*., 2022). Proline acts as an osmotolerant agent in addition to being an energy source which is a fundamental process for stress recovery and maintaining tissue water content under water deficit conditions protecting plant proteins and membranes from damage caused by excess reactive oxygen species (Sheteiwy *et al*., 2021)

Table15: Interaction effect of arbuscular mycorrhiza, avocado race and moisture stress on proline content

Water 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		**0.031**	
CV (%)		**2.89**	

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

4.4 Biomass parameters

4.4.1 Fresh and dry biomass dry weight

Biomass fresh weight and dry weight was significantly ($P < 0.01$) affected by main factors and two ways interaction effect of avocado race with moisture stress and arbuscular mycorrhizae with moisture stress, whereas it is not significantly ($P > 0.05$) influenced by the two way interaction effect of avocado races with arbuscular mycorrhizae and three way interaction effect of races moisture withholding and arbuscular mycorrhizae (Appendix table 7).

The maximum biomass dry and fresh weight (56.40 and 13g) was observed from mexicana grown under daily water treatment, while the minimum biomass fresh and dry weight was recorded from Guatemalan with 21 days water treatment (Table16). These findings are in conformity with those described by Kabir and Achakzai, (2009) varietal means had significant variations for biomass fresh and dry weight. Furthermore, he revealed that some varieties were ranked as drought tolerant and the other is as drought sensitive. Results regarding cumulative drought tolerance index indicated that maize cv. Azam could be rated as drought tolerant and cv. Agaithi-72 as drought sensitive.

Morphological parameters like fresh and dry weights have a profound effect in water-limited conditions. There was reduction one-third in fresh and dry weights of the Zizphus rotundifolia plant under drought conditions (Iraj *et al*., 2014) .

The performance and biomass production potential of trees depend on the maintenance of a higher physiological status and economical utilization of resources in agroforestry tree species. A decrease in total dry matter may be due to the considerable decrease in plant growth, photosynthesis and canopy structure, as indicated by leaf senescence during water stress in Abelmoschus esculentum. Changing resource pools (e.g., water or nutrient availability) may also affect the distribution of biomass.

Table 16.Interaction effect of moisture stress and avocado races on biomass fresh weight (BMFW) and biomass dry weight (BMDW).

Race	**Water withholding**	**BMFW**	**BMDW**
Mexicana	daily	56.46^{a}	13.49^{a}
	7-days	42.1^{b}	9.45^{b}
	14-days	35.8^{d}	8.17^{d}
	21-days	24^{f}	6.12^{f}
Guatemalan	daily	56.07^{a}	13.66^{a}
	7-days	39.05^{c}	8.86^{c}
	14-days	31.5^{e}	7.22^{e}
	21-days	24.94^{f}	5.72^{g}
LSD		**1.10**	**0.37**
CV		**2.43**	**3.50**

Means with different letter on the table are statistically significant at P-values < 0.05 based on the LSD (Least significance difference) comparison method, CV=coefficient of variance.

Regarding on the interaction effect of AMF and moisture stress the maximum biomass dry and fresh weight was observed from inoculated plants grown under daily water treatment, while the minimum biomass fresh and dry weight was recorded from non-inoculated with 21 days water treatment (Table 17). Inoculation of AMF fungi increased biomass fresh and dry weight significantly under both normal and drought stress conditions. Water stress had an effect on the dry and fresh weight of grapevines' shoots so that the average dry weight of shoot at a stress level of 25% was significantly lower than 75% level Highest fresh weight of root in grapevines inoculated with mycorrhiza funguses was significantly higher than grapevines without fungal infections (control subjects Considering the effect of drought stress on reduction in a dry matter of plants, water scarcity decreases nutrients uptake, transfer, and consumption at each growth step leading to lower carbon storage and dry matter (Aslanpour and Omar, 2019).

Table 17 Interaction effects of arbuscular mycorrhizae with moisture stress on biomass fresh weight and biomass dry weight.

Water withholding	**AMF**	**BMFW**	**BMDW**
Daily	Inoculated	62.88^{a}	15.15^{a}
	non-inoculated	49.65^{b}	12.01^{b}
7-days	Inoculated	47.85^{c}	10.55^{c}
	non-inoculated	33.30^{e}	7.76^{e}
14 –days	Inoculated	40.62^{d}	9.10^{d}
	non-inoculated	26.80^{g}	6.29^{g}
21- days	Inoculated	30.13^{f}	6.97^{f}
	non-inoculated	18.82	4.87^{h}
LSD		**1.10**	**0.37**
CV		**2.43**	**3.50**

4.5. Correlation Analysis

4.5.1 Associations between xylem traits physiological and biomass of avocado races with AMF inoculations and without inoculation under different soil moisture levels

Correlation analysis indicated that, the relationship of all physiological parameters had significant ($P \leq 0.001$) strong positive correlation (Table 16). Root xylem vessel number and width was positively and significantly correlated with all physiological and biomass traits

Table 18: Pearson Correlation coefficient (r) xylem among physiological and biomass of avocado races with AMF inoculations and without inoculation under different soil moisture levels from February 2022 to July 2022.

	XN	XW	BFW	BDW	Chl (a+b)	A	E	WUE	Gs
XN	1.0								
XW	0.95^{***}	1.0							
BFW	0.89^{***}	0.98^{***}	1.0						
BDW	0.89^{***}	0.98^{***}	0.99^{***}	1.0					
Chl (a+b)	0.94^{***}	0.99^{***}	0.98^{***}	0.98^{***}	1.0				
A	0.73^{***}	0.89^{***}	0.94^{***}	0.94^{***}	0.89^{***}	1.0			
E	0.63^{***}	0.79^{***}	0.86^{***}	0.87^{***}	0.80^{***}	0.94^{***}	1.0		
WUE	0.88^{***}	0.88^{***}	0.85^{***}	0.84^{***}	0.88^{***}	0.73^{***}	0.51^{***}	1.0	
Gs	0.65^{***}	0.84^{***}	0.90^{***}	0.91^{***}	0.84^{***}	0.97^{***}	0.97^{***}	0.60^{***}	1.0

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, all values were significantly and positively correlated at ($p \leq 0.001$).

4.5.2 Associations between stomata number and width parameters among physiological and biomass of avocado races with AMF inoculations and without inoculation under different soil moisture levels

Correlation analysis indicated that, the relationship of all physiological parameters with stomata number and width had significant ($P \leq 0.001$) strong positive correlation and Wue was not significantly ($p<0.05$) with all physiological and biomass traits (Table 19).

4.5.3 Associations between morphological and physiological parameters of avocado races with AMF inoculations and without inoculation under different soil moisture levels

Correlation analysis indicated that, the relationship of all morphological and physiological parameters is significantly ($P \leq 0.001$) strong positive correlation (Table 20).

Table 19: Pearson Correlation coefficient(r) stomata among physiological and biomass of avocado races with AMF inoculations and without inoculation under different soil moisture levels from February 2022 to August 2022.

	SN	SW	Chla	Chlb	Chl(a+b)	LRWC	A	E	WUE	Gs
SN	1.0									
SW	0.843***	1.0								
Chla	0.8325***	0.9546***	1.0							
Chlb	0.8622***	0.963***	0.9358***	1.0						
Chl	0.8578***	0.9726***	0.9893***	0.9764***	1.0					
LRWC	0.5978***	0.5944***	0.7356***	0.6371***	0.7097***	1.0				
A	0.8405***	0.9099***	0.9357***	0.8995***	0.9360***	0.6959***	1.0			
E	0.7235***	0.7302***	0.8390***	0.6779***	0.7869***	0.7505***	0.8799***	1.0		
WUE	-0.0314^{NS}	0.2006^{NS}	0.0989^{NS}	0.2648^{NS}	0.1682^{NS}	-0.1379^{NS}	0.0344^{NS}	-0.352^{NS}	1.	
Gs	0.8176***	0.8445***	0.9242***	0.8188***	0.8955***	0.7813***	0.9281***	0.9447***	-0.19NS	1.0

Where, SN= stomata number, SW= stomata width, Chla=Chlorophyll a, Chlb=Chlorophyll b, Chl= Chlorophyll (a+b), A=Photosynthetic rate, E=Transpiration rate, WUE=Water use efficiency, Gs=Stomata conductance, all values were significantly and positively correlated at ($p \leq 0.001$).

Table 20: Pearson Correlation coefficient(r) among morphological trials and physiological of avocado land races with AMF inoculations and without inoculation under different soil moisture levels from February 2022 to August 2022.

	LA	LN	LDW	PH	IL	RL	RN	RDW	ARD	XN	XW
Ch-a	0.79	0.96	0.94	0.83	0.73	0.48	0.81	0.95	0,93	-0.55	0.91
Ch-b	0.70	0.87	0.84	0.72	0.70	0.55	0.73	0.89	0.87	-0.50	0.90
Ch (a+b)	0.77	0.94	0.71	0.80	0.73	0.62	0.79	0.94	0.92	-0.53	0.92
RWC	0.64	0.72	0.77	0.65	0.58	0.42	0.66	0.70	0.79	-0.33*	0.81
SN	0.60	0.84	0.80	0.65	0.62	0.51	0.74	0.83	0.82	-0.56	0.75
SW	0.71	0.89	0.85	0.74	0.67	0.45	0.73	0.88	0.85	-0.53	0.86
A	0.84	0.93	0.93	0.88	0.73	0.53	0.82	0.88	0.89	-0.51	0.88
E	0.77	0.88	0.91	0.75	0.58	0.37	0.80	0.81	0.82	-0.50	0.74
WUE	0.13ns	-0.01ns	-0.09ns	0.05ns	0.20ns	0.31*	-0.05ns	0.05ns	0.10ns	0.13hs	0.19ns
PC	-0.44	-0.74	-0.77	-0.54	-0.61	-0.09	-0.50	-0.71	-0.64	0.74	-0.61
Gs	0.76	0.92	0.95	0.80	0.66	0.40	0.78	0.89	0.88	-0.61	0.83
BDW	0.87	0.97	0.98	0.85	0.76	0.61	0.90	0.97	0.96	-0.47	0.90

Where LA=leaf area, LN=Leaf number, LDW=leaf dry weight, PH=seedling height, IL=internode length, RL= root length RN= root number, RDW= root dry weight, ARD= average root diameter, XN= xylem number, XW= xylem width, Chl- a=Chlorophyll-a, Chl-b=Chlorophyll-b, Chl (a+b)= TC= total chlorophyll (a+b), LRWC=relative water content, A=Photosynthetic rate, E=Transpiration rate, WUE=Water use efficiency, PC=proline content, gs=Stomata conductance and BDW=biomass dry weight

5. SUMMARY AND CONCLUSION

5.1 Summary

Avocado (*Persea americana* M.) is economically important fruit crop in Ethiopia mainly produced in the Southern, South Western and Eastern part of the country which mainly produced by small scale farmers. However the production is hampered by the wide spread incidences of biotic and abiotic stresses which is aggravated in recent years due to climate change. Drought stress is estimated to affect nearly one-third of soils, thus, for supporting normal plant development. Drought occurs in almost all climatic regions, and it induces crop yield loss in a wide range of plants, while also increasing global tree mortality. Plants respond to stress in many ways, but manipulation of the root microbiome is one of the significant mechanism to deal with such stresses. One of these symbioses is with arbuscular mycorrhizal. Arbuscular mycorrhizal fungi facilitate host plants to grow vigorously under stressful conditions by mediating a series of complex communication events between the plant and the fungus leading to enhanced photosynthetic rate and other gas exchange-related traits. So this study was conducted to study biochemical, growth and physiological responses of arbuscular mycorrhiza inoculated avocado races under moisture stress conditions.

The result of my study found that growth parameters such as internode length and seedling height were influenced by main effect of races, arbuscular mycorrhizae and moisture. However, leaf number, leaf area; and leaf fresh ad dry weight, root dry and fresh weight, xylem length and xylem width was influenced by main and two way interaction effects of races with moisture and arbuscular mycorrhizae with moisture levels. The highest mean value of leaf area, root number and root length were observed from mexicana treated with arbuscular mycorrhizae under daily water treatment. Whereas the minimum was recorded from Guatemalan grown under uninoculated and 21 days water treatment. Moreover, Photosynthesis rate, water use efficiency, chlorophyll content, proline content, biomass fresh and dry weight were significantly influenced by the main, two ways and three way interaction effect of avocado races, arbuscular mycorrhizae and moisture levels. All growth, physiological and biomass parameters were significantly decreased under drought stressed conditions. However, arbuscular mycorrhizae inoculation significantly increased all those growth, physiological, and biomass parameters even under drought stress conditions as compared to uninoculated seedlings.

5.2 Conclusion

According to the results of this study, the mexicana race responded better to stress conditions in terms of leaf area, leaf fresh weight, leaf dry weight, root number, length, and average diameter, xylem width, and physiological parameters like photosynthesis, WUE, chlorophyll content, and total dry biomass.

As a result, drought stress negatively affected all morphological, physiological parameters except proline accumulation as stress level increased in both mexicana and Guatemalan races. However, the inoculation of arbuscular mycorrhizae considerably increased drought stress tolerance in both races compared to non-inoculated treatments. However, further research is needed under field condition with different avocado races before generalized conclusions can be drawn.

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7. APPENDECIS

Appendix Table 1: Analysis of variance of seedling height. Internode length, leaf number and leaf area of avocado races

Source of variation	**DF**	**PLH**	**INL**	**LFA**	**LFN**
Race	1	0.725^{Ns}	0.00003^{Ns}	138201***	0.441^{Ns}
AMF	1	132.335***	0.07521**	1620686***	60.750***
Moisture withholding	3	102.305***	0.14539***	590148***	119.352***
Race X AMF	1	4.260^{Ns}	0.00301^{Ns}	5702ns	0.368^{Ns}
Race X moisture	3	5.127^{Ns}	0.01116^{Ns}	42324**	0.947^{Ns}
AMF X moisture	3	4.782^{Ns}	0.01301^{Ns}	101313***	1.404*
Race X AMF X moisture	3	4.782^{Ns}	0.00343^{Ns}	19291^{Ns}	0.581^{Ns}
Error	32	3.219	0.00878	8649	0.395
CV (%)		**6.94**	**12.44**	**13.77**	**4.07**

The sign ns, * ** and *** indicates; non-significance different (P>0.05), significance difference (P<0.05), highly significant difference (p<0.01) and very highly significant difference (P<0.001) respectively, PLH=seedling height, INL=internode length, LA= leaf area length, LN= leaf number, AMF=arbuscular mycorrhizae fungi, and. DF= degree of freedom,

Appendix Table 2: Analysis of variance of leaf fresh and dry weight, root dry and fresh weight of avocado races

Source of variation	**DF**	**LFW**	**LDW**	**RDW**	**RFW**
Race	1	17.352^{NS}	0.0626**	0.0169^{NS}	0.945^{NS}
AMF	1	238.521***	0.6698***	9.2752***	1.300***
Moisture withholding	3	388.969***	11.5542***	14.3413***	277.922***
Race X AMF	1	0.001^{NS}	26.4426^{NS}	0.0602^{NS}	261.034^{NS}
Race X moisture	3	1.301*	0.1376**	0.0174^{NS}	1.435**
AMF X moisture	3	7.908***	0.2363***	0.1791**	2.592***
Race X AMF X moisture	3	0.180^{NS}	0.4019^{NS}	0.0274^{NS}	8.317^{NS}
Error	32	0.394	0.0659	0.0267	0.374
CV (%)		**4.34**	**5.62**	**5.34**	**3.77**

The sign ns, * ** and *** indicates; non-significance different (P>0.05), significance difference (P<0.05), highly significant difference (p<0.01) and very highly significant difference (P<0.001) respectively, LFW=leaf fresh weight, LDW=leaf dry weight RDW= root dry weight, RFW= root fresh weight, AMF=arbuscular mycorrhizae fungi and. DF=degree of freedom

Appendix Table 3 : Analysis of variance of root length, root number and root xylem number and root xylem width of avocado races

Source of variation	**DF**	**RL**	**RN**	**RXN**	**RXW**
Race	1	52.71***	54.19***	1.0208^{Ns}	175.567***
AMF	1	3275.26***	3657.52***	28.5208***	339.203***
Moisture withholding	3	37.8249***	1090.35***	28.6319***	505.832***
Race X AMF	1	233.73***	20.02**	1.6875^{Ns}	0.003Ns
Race X moisture	3	32.84***	4.08^{NS}	6.5208***	29.801***
AMF X moisture	3	65.37***	10.63**	0.3542^{Ns}	4.351^{Ns}
Race X AMFX moisture	3	85.64***	23.35**	1.1875^{Ns}	2.984^{Ns}
Error	32	0.96	1.85	0.8833	2.243
CV (%)		**2.24**	**2.83**	**10.28**	**4.21**

The sign ns, * ** and *** indicates; non-significance different (P>0.05), significance difference (P<0.05), highly significant difference (p<0.01) and very highly significant difference (P<0.001) respectively, TRL=tap root length, RN=root number RXN= root xylem number, RXW=root xylem width, AMF=arbuscular mycorrhizae fungi and DF=degree of freedom

Appendix Table 4:Analysis of variance of root length, root number and root xylem number and root xylem width of avocado races

Source of variation	**DF**	**A**	**E**	**WUE**	**Proline**
Race	1	0.0018^{Ns}	0.00590^{NS}	0.6907^{*}	0.0088***
AMF	1	15.0192^{***}	2.31090^{***}	4.8781^{***}	0.0438***
Moisture withholding	3	37.8249^{***}	8.21811^{***}	39.1742^{***}	0.2812***
Race X AMF	1	0.0514^{Ns}	0.08267^{**}	0.6244^{*}	0.0046***
Race X moisture	3	0.0901^{*}	0.10118^{***}	7.8524^{***}	0.00791***
AMF X moisture	3	2.7667^{***}	1.40023^{***}	2.9754^{***}	0.005***
Race X AMF X moisture	3	0.2226^{***}	0.04991^{**}	0.3782^{*}	0.00287***
Error	32	0.0216	0.00890	0.1248	0.00035
CV (%)		**6.25**	**11.42**	**10.17**	**2.89**

The sign ns, * ** and *** indicates; non-significance different (P>0.05), significance difference (P<0.05), highly significant difference (p<0.01) and very highly significant difference (P<0.001) respectively, A=photosynthesis, E=transpiration WUE= water use efficiency, AMF=arbuscular mycorrhizae fungi and. DF=degree of freedom

Appendix Table 5: Analysis of variance of chlorophyll a, chlorophyll b, total chlorophyll and stomata conductance of avocado races

Source of variation	**DF**	**Chla**	**Chlb**	**TC**	**Gs**
Race	1	0.986^{***}	0.4238^{***}	2.562^{***}	123.52^{*}
AMF	1	28.830^{***}	11.2617^{***}	75.375^{***}	1073.52^{***}
Moisture withholding	3	105.688^{***}	43.6969^{***}	277.293^{***}	8836.08^{***}
Race X AMF	1	1.129^{***}	0.3485^{***}	2.876^{***}	0.19^{NS}
Race X moisture	3	0.498^{***}	0.8997^{***}	2.725^{***}	38.74^{NS}
AMF X moisture	3	1.065^{***}	0.4873^{***}	1.372^{***}	307.41^{*}
Race X AMF X moisture	3	0.516^{***}	0.0821^{*}	0.870^{***}	14.52^{NS}
Error	32	0.022	0.0197	0.015	17.32
CV (%)		**0.80**	**2.54**	**0.50**	**15.08**

The sign ns, * ** and *** indicates; non-significance different (P>0.05), significance difference (P<0.05), highly significant difference (p<0.01) and very highly significant difference (P<0.001) respectively, chla=chlorophyll a, chlb =chlorophyll b TC= total chlorophyll content, gs=stomata conductance AMF=arbuscular mycorrhizae fungi and. DF=degree of freedom

Appendix Table 6: Analysis of variance of leaf relative water content, stomata length and stomata width of avocado races

Source of variation	DF	**LRWC**	**SN**	**SW**	**RD**
Race	1	150.17***	5.33333ns	0.2700*	0.9352***
AMF	1	354.80***	24.0833***	7.5208***	30.8802***
Moisture level	3	1243.82***	120.972***	42.6881***	29.6452***
Race X AMF	1	2.85^{Ns}	6.52E-30^{Ns}	1.3333***	0.0052^{NS}
Race X moisture	3	37.40***	4.55556^{Ns}	0.7417***	0.718***
AMF X moisture	3	2.83^{Ns}	2.52778^{Ns}	1.3469***	0.1385**
Race X AMF X moisture	3	0.82^{Ns}	1.88889^{Ns}	0.2506*	0.8824***
Error	32	1.13	1.81667	1.7546	0.0235***
CV (%)		**1.42**	**8.27**	**2.73**	

The sign ns, * ** and *** indicates; non-significance different (P>0.05), significance difference (P<0.05), highly significant difference (p<0.01) and very highly significant difference (P<0.001) respectively, LRWC = leaf relative water content, SN = stomata length SW = stomata width, AMF=arbuscular mycorrhizae fungi and. DF=degree of freedom.

Appendix Table 7: Analysis of variance of leaf relative Root colonization, Biomass fresh weight and Biomass dry weight of avocado races

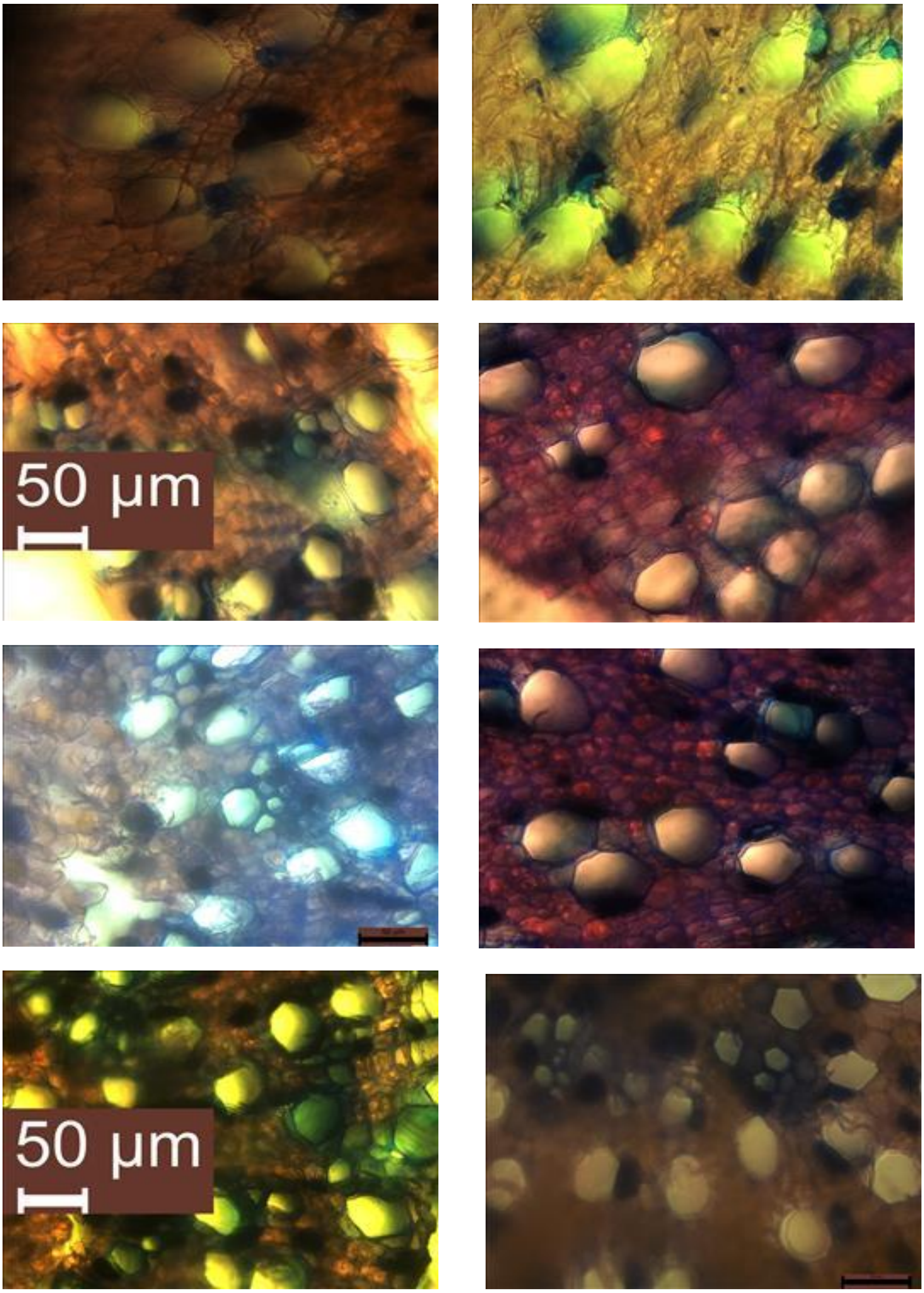
Source of variation	**DF**	**RC**	**BMFW**	**BMDW**
Race	1	0.5^{NS}	35.28***	2.328***
AMF	1	36437.9***	2100***	88.156***
Moisture withholding	3	401.7***	2157.53***	128.467***
Race X AMF	1	0.5^{NS}	0.11^{NS}	0.073^{NS}
Race X moisture	3	0.1^{NS}	17.58**	0.662***
AMF X moisture	3	401.7***	5.79**	0.596**
Race X AMF X moisture	3	0.1^{NS}	0.73^{NS}	0.15^{NS}
Error	32	0.5	0.89	0.101
CV (%)		**2.58**	**2.43**	**3.5**

The sign ns, * ** and *** indicates; non-significance different (P>0.05), significance difference (P<0.05), highly significant difference (p<0.01) and very highly significant difference (P<0.001) respectively, LRWC = leaf relative water content, SN = stomata length SW = stomata width, AMF=arbuscular mycorrhizae fungi and. DF=degree of

SKETCH OF BIOGRAPHY

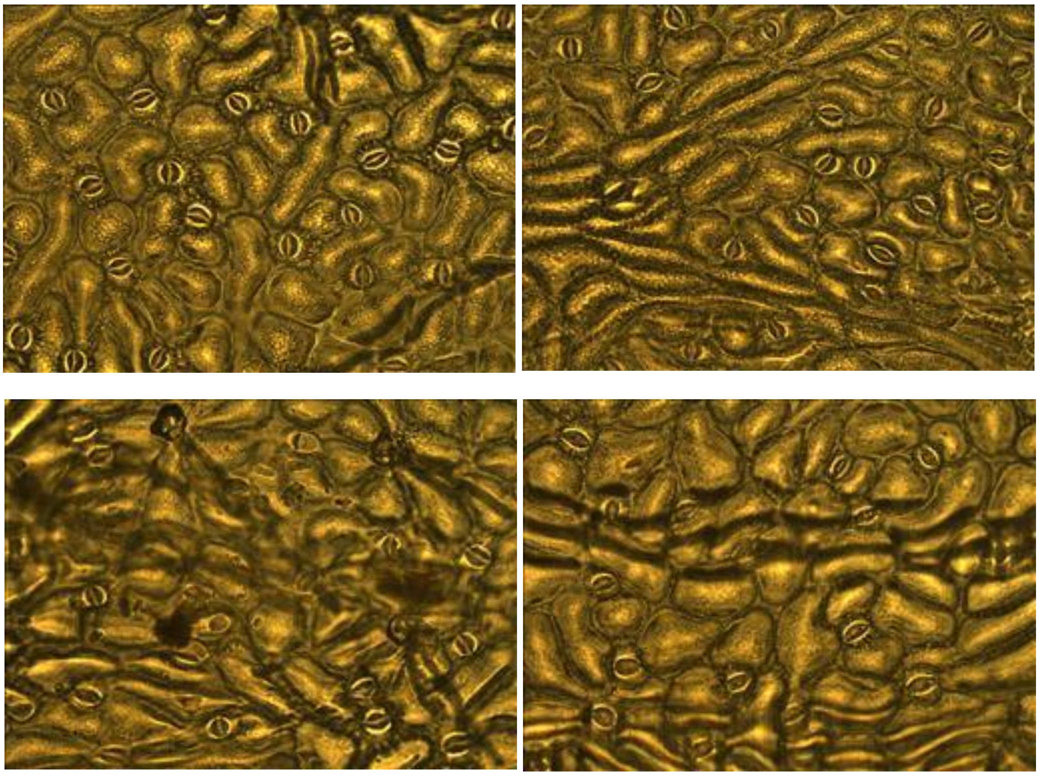
The author, Hiwot Kelbo Hado was born on February, 1992 GC at Wonji, Shewa Zone, Oromia regional state, Ethiopia. She attended her Primary school (1-4) at Wonji Shewa and (4-8) at Hosanna Haile Bubaamo elementary school.Then after, she attended her high school (9-10) at Yekatit 25/67 secondary school and (11-12) at Wachemo secondary and preparatory school. After completion of University entrance examination, she joined Hawassa University in 2009 to pursue her first Degree and graduated with BSc Degree in plant science in July 2011. After her graduation, she had worked at Haddiya Zone Soro District agricultural office for 3 years. At 2016/2017 she has been employed as a senior technical assistant at Wachemo University until she has got a chance to attend her Master's program. In 2019/2020, she joined the Graduate program in Horticulture at Hawassa University. Now she is ready to defend her MSc thesis as well.

Pictorial representatives of some activities

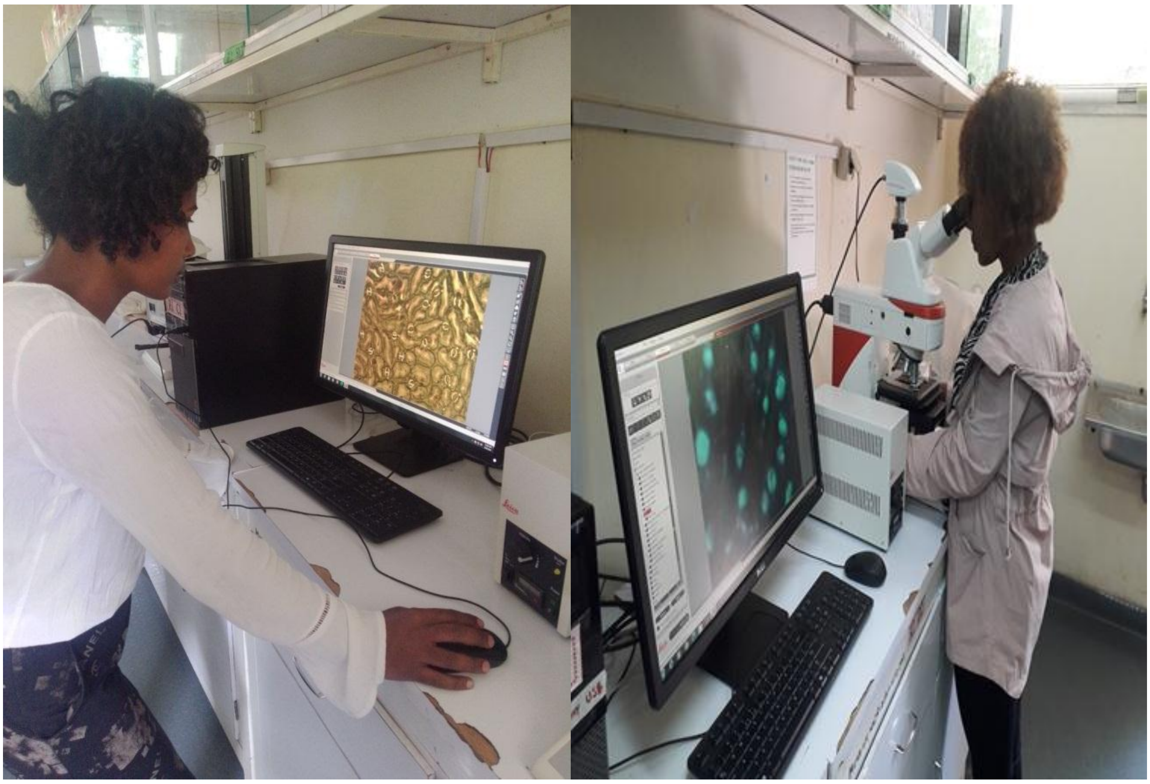


Guatemalan race **Mexicana cultivar**

Appendix Figure 1: root xylem anatomy of avocado under daily, 7 days, 14 days and 21days water treatments respectively for Guatemalan and mexicana races



Appendix Figure 2: stomata anatomy of avocado under daily, 7 days, 14 days and 21days water treatments



Appendix Figure 3: stomata and xylem anatomy of avocado

Appendix Figure 4: mycorrhizae crude inoculum preparation

Appendix Figure 5: avocado races under all treatments

Appendix Figure 6: trimming, embedding and staining

Appendix Figure 7: Chlorophyll extraction and leaf area measurement

Appendix Figure 8: Avocado roots AMF inoculated and not inoculated