Effectiveness of Indigenous Arbuscular Mycorrhizal Fungi Inoculum in Enhancing Tolerance of Sorghum (*Sorghum bicolor* L. Moench) to Water Stress

Kumbi Geda

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of

Master's in Applied Biology (Applied Microbiology)

Office of Graduate Studies

Adama Science and Technology University

June, 2023

Adama, Ethiopia

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DECLARATION

I hereby declare that this Master Thesis entitled "**Effectiveness of Indigenous Arbuscular Mycorrhizal Fungi Inoculum in Enhancing Tolerance of Sorghum (*Sorghum bicolor (*L*)* Moench) to Water Stress**" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation

_____ _____ _____

Name of the student Signature Date

RECOMMENDATION

We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled "**Effectiveness of Indigenous Arbuscular Mycorrhizal Fungi Inoculum in Enhancing Tolerance of Sorghum (*Sorghum bicolor (*L*)* Moench) to Water Stress**" prepared under our guidance by Kumbi Geda submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Applied Microbiology. Therefore, we recommend the submission of revised version of the thesis to the Department following the applicable procedures.

Major Advisor	Signature	Date
Co-Advisor	Signature	Date

APPROVAL PAGE

We, the advisors of the thesis entitled "**Effectiveness of Indigenous Arbuscular Mycorrhizal Fungi Inoculum in Enhancing Tolerance of Sorghum (*Sorghum bicolor (L)* Moench) to Water Stress**" and developed by Kumbi Geda; hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor	Signature	Date
Co-Advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis Kumbi Geda have read and evaluated the thesis entitled "**Effectiveness of Endogenous Arbuscular Mycorrhizal Fungi Inoculum in Enhancing Tolerance of Sorghum (*Sorghum bicolor (*L*)* Moench) to Water Stress** " and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Microbiology.

Chairperson	Signature	Date
Internal Examiner	Signature	Date
External Examiner	Signature	Date

Date Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

Department Head	Signature	Date
School Dean	Signature	Date
Office of Postgraduate	Signature	Date

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LISTS OF ACRONYMS

ABA Abscisic acid

AC Arbuscular colonization

AMF Arbuscular Mycorrhiza Fungi

ANOVA Analysis of Variance

ASTU Adama Science and Technology University

CMN Common mycorrhizal networks

DS Drought stress

HC Hyphal colonization

INVAM International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi

MRC Melkasa Agricultural Research Center

PGPB Plant Growth Promoting Bacteria

PVLG Polyvinyl-Lactic Acid-Glycerol

RCBD Randomized Complete Block Design

RWC Relative water content

SSA Sub-Saharan Africa

VC Vesicular colonization

ABSTRACT

Sorghum (Sorghum bicolour L. Moench) is an important cereal crop ranked second after maize as a staple food in Ethiopia. However, its production is threatened by low soil fertility and moisture content. Arbuscular mycorrhizal fungi (AMF) have shown their importance in nutrient and water uptake and mitigating drought stress resulting in better yields of crops. Hence, this study was initiated to investigate the effect of indigenous arbuscular mycorrhizal fungi to enhance growth of two selected sorghum varieties (Melkam and Local seed varieties) under drought conditions. AMF was propagated in trap culture. The experimental design was arranged in a randomized complete block design with three replications under greenhouse conditions. Four treatments were set up for sampling sites at Diredawa, Jigjiga and Harar field sites: sorghum with AMF inoculation at 80% soil field capacity (+AMF+80%FC), plants without inoculation of AMF (controls) at 80% soil field capacity (-AMF+80% FC), plants inoculation with AMF at 50% soil field capacity (+AMF+50%FC*) and plants with AMF inoculation at 25% soil field capacity (+AMF+25%FC). Data of plant growth parameters on sorghum seedlings, and AMF colonization percentage and AMF diversity and spore density were collected. Analysis of variance (ANOVA) was used to analyze data using SPSS software package (version 26.0) and significant means separated using Tukey's test at 5% significance level. In this study, the highest spore density and highest root colonization rate of AMF were recorded from trap culture of maize from Diredawa location. The result also showed that Melkam variety showed the highest shoot fresh and dry weights (14.73, 3.23g/plant, highest root fresh and dry weights (8.13, 2.16g/plant) and longest roots (39cm) at 80%FC. Relative water content, proline, total soluble sugar and total chlorophyll content were significantly (*p <0.05*) influenced by inoculation of AMF in both varieties at all soil water field capacity. A total of 24 AMF species belonging to 15 genera were detected from trap culture experimental soil of all locations. Gigaspora, Claroideoglomus, Funneliformis and Rhizophagus were the common genera detected in the trap culture. This study demonstrated that AMF inoculants had a good potential in improving sorghum growth under water stressed conditions. Such strategies could enable food production in marginal areas characterized by low rainfall and high temperatures. However, the application of present findings should be validated under field conditions.*

Keywords: Consortium of AMF sp., *inoculation* of AMF and Root colonization.

Chapter one

1. Introduction

1.1. Background of the study

Sorghum (*Sorghum bicolor L. Moench*) is the fifth most important cereal crop grown worldwide (Twomlow *et al*., 2004). It is widely used as human foods mainly as processed flour, fermented porridge, feed industries and bioenergy crop (Muui *et al*., 2013). Due to its ability to tolerate drought, it is mainly grown in dry and semi-arid areas (Younis *et al*., 2007). Sorghum is fairly adaptable and can grow well in temperate to warm weather conditions, fairly stable rainfall patterns, and well-drained fertile soil through the growth period. Sorghum production is constrained by soil fertility problems caused by ongoing climate change that has caused severe yield reduction and crop failure (Ntiyari and Gweyi-Onyango, 2021). This growth suppression can be alleviated by incorporating beneficial micro-organisms during sorghum production. Beneficial soil microorganisms perform a great role in sustainable crop production. They increase tolerance to abiotic and biotic stresses, involve in recycling of nutrients and mineralization of the soil organic matter (Hodge and Fitter, 2010).

Arbuscular mycorrhizal fungi (AMF) are among the helpful microorganisms that are necessary for sustainable production of agricultural crops (Vierheilig *et al*., 1998). They form symbiotic association with the roots of more than 80% of terrestrial plants (Gadkar *et al*., 2001). Both the host plant and the fungi benefit from this interaction since the host plant provides the fungi with food and shelter, while the fungi absorb nutrients from the soil through the extraradical fungal hyphae. It has been demonstrated that mycorrhiza promotes the uptake of immobile nutrients like phosphate, copper and zinc, as well as nitrogen (Abdel-Fattah and Mohamedin, 2000).AMF also promotes the formation of plant hormones, which increases the plant's tolerance to environmental stresses such salinity, heavy metal stress, and drought (Palta *et al*., 2010).AMF either directly (via glomalin secretion from a fungal structure) or indirectly (by increasing host plant tolerance) cause phytoremediation of Heavy metals(Muhammad *et al*., 2021) . It is established that plants with mycorrhizal inoculation grew more rapidly than plants without it (Smith and Read 2008).AMF remarkably increase nutrient provision to the host plant, increase drought resilience and defense against nematodes and fungal diseases

(Thygesen *et al*., 2004) In addition, hyphal network of mycorrhizal fungi and their glomalin products can enhance soil structure by creating stable soil aggregates.

In many regions, the availability of water is thought to be one of the key determinants limiting plant growth and production (Kramer and Boyer, 1997). These Crop productivity will be greatly impacted by the anticipated warmer and drier climate. The development of drought-tolerant crop varieties using microbial technologies such as arbuscular mycorrhizal fungi inoculant is therefore crucial in order to address this issue. Arbuscular mycorrhizal fungi are well known for their capacity to absorb water and mineral nutrients and boost plant growth. Effective nutrient uptake, however, depends on rhizosphere mechanisms like mycorrhizal inoculation in many barren soils. In addition, AMF are known to increase mineral uptake (Al Magrabi and Abdelmoneim, 2012), and to inhibit the growth of plant diseases (Azcón-Aguilar and Barea, 1997). In fact, AMF serve as an essential part of ecosystems by enhancing both plant productivity and environmental stability (Powell and Rillig, 2018)

Ortas *et al*., (1996) showed that growth of sorghum is stimulated by soil-beneficial microorganisms such arbuscular mycorrhizal fungi. Sisaphaithong and colleagues (2012) have stated that sorghum, barley, and wheat roots express plant genes for the development of fungal vesicles and arbuscular mycorrhiza-induced phosphate transporters. In general, higher plants are better able to respond to a variety of environmental challenges such drought, heat, salinity, and heavy metal contamination when arbuscular mycorrhizal fungi are inoculated (Garg and Chandel, 2011). Mycorrhizal inoculation has been shown to increase microbial populations in the rhizosphere of sorghum (Kumar and Fulekar, 2019), which may enhance the rhizosphere's microbial dynamic and improve soil quality (Ortaş, 2017).

1.2. Statement of the problem

Millions of people in South Asia and Sub-Saharan Africa (SSA) depend on sorghum as one of their main food sources. Sorghum is a native crop to Ethiopia, the sixth-largest sorghum producer in the world. It contributes 16.4% of the country's annual cereal grain production and is ranked third in terms of both total area coverage and productivity. It is also used to make "***injera***," which is Ethiopia's second-most popular food after "tef." (CSA, 2017). Nearly all of the Ethiopia's regions between 400 and 2500 metres above sea level grow sorghum. The three main sorghum-producing regions are Oromia, Amhara, and Tigray, accounting for 86% of the total area and 89% of the

production for the previous nine years (CSA, 2017). Sorghum makes up more than one-third of the cereal consumed in Ethiopia, and it is primarily farmed by subsistence farmers to suit their needs for food, income, animal feed, and building materials (McGuire, 2005).According to Eggen *et al*. (2019), a number of Africa's sorghum-producing regions are susceptible to drought stress, and future climates are expected to see more droughts (IPCC, 2013). As a result, drought has emerged as a serious obstacle to the growth and development of the sorghum crop, particularly in tropical areas (Xie, 2018).

Climate change effect on potential agricultural lands is anticipated to be marginalized by rising temperatures and a lack of moisture. As a result, in order to address the serious negative effects of water scarcity, contemporary water stress management strategies should concentrate on cutting-edge technology like microbial inoculants. It has been proposed that arbuscular mycorrhizal fungi (AMF) are essential for sustainable agriculture because they enhance plant water relationships by raising plants' capacity to withstand drought (Mehjin, 2016). In Ethiopia only a few research findings have been done on the function of arbuscular mycorrhizal fungi (AMF) on sorghum plants' improvement to drought resistance (Mohammed and Misganaw, 2022; Assefa, 2020). As a result, the objective of this study was to assess the impact of inoculating native arbuscular mycorrhzia fungi on the growth of sorghum (*Sorghum bicolor*) cultivated in a green house under both well-watered and water-stressed conditions.

1.3. Research questions

- What is the effect of indigenous AMF on growth condition (height, leaf number, leaf relative water content and fresh and dry weight) on the plant of two sorghum varieties?
- Which host is more suitable for Arbuscular mycorrhizia fungi (AMF) trapping?
- What is the effect of indigenous AMF on biochemical parameters (proline content, total soluble sugar content and chlorophyll content) of sorghum plants?

1.4. Objectives of the study

1.4.1. General Objective

The general objective of this study was to evaluate the effectiveness of greenhouse inoculation of indigenous arbuscular mycorrhizal fungi to enhancing the growth of S*orghum bicolor* under drought conditions in the eastern part of Ethiopia.

1.4.2. Specific objectives

The specific objectives of the present study are to:

- ✓ Determine the AMF spore abundance of the field soil inoculums.
- ✓ Determine the root colonization rates of trap culture and the inoculated sorghum plants.
- ✓ Determine a suitable host for trapping AMF from the collected soil samples to use them as potential propagules.
- ✓ Assess relative water content and growth (height, Number of life, root/shoot biomass, etc.) parameters of the AMF inoculated and non-inoculated sorghum plants under greenhouse conditions with different levels of water stress.
- ✓ Determine biochemical parameters (leaf proline content, total soluble sugar and chlorophyll) during drought stress in two sorghum varieties.
- ✓ Determine the physico-chemical properties of soil samples of the study sites.

Chapter Two

2. Literature Review

2.1. Economic Importance of Sorghum

Millions of people who live in about 30 nations in the subtropical and semi-arid regions of Africa and Asia rely on sorghum as a staple food. It is mostly used in traditional, smallholder farming as a source of food and fodder. Additionally, it serves as a feed crop in the high-input commercial farming sector and is quickly establishing itself as a biofuel crop (Hariprasanna and Rakshit, 2016).Numerous species and subspecies belong to the genus Sorghum. Grain sorghums, forage sorghums (for pasture and hay), sweet sorghums (for syrups and biofuel), and broomcorn are some of the different varieties of sorghum. The crop is suitable to hot, arid agroecologies where other food grains don't produce much or are even challenging to grow. Sorghum is a dual-use crop in these agro ecologies because both the grain and the stalks or stover are prized for human and animal sustenance, respectively. Grain is a crucial component of animal and bird feed in affluent nations like the United States, Japan, and Australia, as well as in certain developing nations like China and Mexico. Stover contributes up to 50% of the crop value in many developing countries, especially during drought years (ICRISAT & FAO, 1996).

The traditional smallholder farming sector, which is primarily practised as subsistence farming in Asia and Africa, and the modern high-input large-scale farming sector, which is primarily practised in industrialised nations and in Latin America, are the two distinct components of the sorghum-based global economy (Hariprasanna and Rakshit, 2016). Sorghum is grown largely for food by low-income farmers on the African and Asian continents in poor nations, where it occupies 42.12 million ha (FAO, 2015), or more than 80% of the world's sorghum acreage. The remaining 16–20% of the area, which is primarily in the industrialised world, is cultivated, particularly by massive industrial farms that primarily grow sorghum for animal feed. Because to the application of contemporary agricultural techniques, the yield levels in the latter sector are high. Together, Africa and Asia produce around 56% of the world's sorghum, compared to the Americas' 38% contribution from just 16% of the harvested land worldwide.

2.2. Sorghum production in Ethiopia

After maize, rice, wheat, and barley, sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most significant cereal crop in the world (Beyene *et al*., 2016). Worldwide, sorghum is ranked in the top 5 for cereal grains in production and acreage. The world produced 62.0 million metric tons of sorghum in 2020 up 3.98 million metric tons. Sorghum is a key staple food crop in Ethiopia, coming in second after maize in overall production. It is third in terms of productivity per hectare, after wheat and maize, and third in terms of farmed area, behind teff and maize covering a total land area of 1.8 million ha (Esubalew *et al*., 2022). It provides 300 million people in sub-Saharan Africa with a significant portion of their food security. It is planted in marginal, semi-arid, and drought-prone locations where other crops cannot thrive consistently. Ethiopia's intermediate, wet lowland, highland, and dry lowland agro-ecological zones are where sorghum is largely grown (Semahegn and Teressa, 2021). Ethiopia's highland agro-ecologies are characterised by high altitude (> 1900 masl), high rainfall (1000mm), and low temperatures. Sorghum is primarily grown in Ethiopia's intermediate, wet lowland, highland, and dry lowland agro-ecological zones High altitude (> 1900 masl), high rain falls (1000mm), and low temperature characterize Ethiopia's highland agro-ecologies.

In developing countries, including Ethiopia, more than 500 million people consume sorghum as their principal food source (Burke *et al.*, 2013). Sorghum is a gluten-free cereal used as a whole grain or processed into flour to provide essential nutrients including carbohydrates, protein, vitamins and minerals, and nutraceuticals such as antioxidants, phenolics and cholesterol lowering waxes (Perazzo *et al*.. 2014). In Ethiopia a total of 4.34 million tons of sorghum is being produced per annum. The mean yield level in the country is estimated at 2.4 t ha^{-1}. The crop is highly adapted to the lowland and drier parts of Ethiopia owing to its considerable drought resilience. Despite its ability to grow in the arid and semi-arid areas of sub-Saharan Africa including in Ethiopia, the yield and quality of sorghum is affected by a wide array of production constraints such as the use of low yielding traditional varieties.

The most significant production barriers to sorghum production in Ethiopia are drought stress and Striga damages (Gebretsadik *et al.* 2014). Sorghum production is severely hampered by drought, which is also regarded as the largest factor in agricultural plant yield loss (Besufekad and Bantte 2013), particularly in regions of the world with limited water supplies, such as sections of eastern and

southern Africa. According to Ejeta (2007), striga infestation is frequently associated with low soil fertility, which causes poor harvests and ultimately, starvation. Striga has a more noticeable effect in locations that are under moisture and nutrient stress.

2.3. Constraints to Sorghum production

In semiarid areas with limited precipitation, sorghum is a significant crop. It comes in at number seven among cereal crops in importance. Sorghum is a C4 plant that was created and domesticated in Ethiopia. People eat it as Injera, boiled gruel, malted beverages, beer, popped grain, and chips as well as use it as fuel and food. In Ethiopia, biotic, sociological, and abiotic restrictions affect the production and productivity of sorghum. Biological, social, and abiotic factors all affect how much sorghum can be produced. Ethiopia is unable to produce crops to their full potential due to a variety of socioeconomic factors, including a lack of funding, a lack of farmer-preferred crop varieties, a lack of improved seed systems, a lack of market connectivity, a lack of value addition, a lack of extension service support, and a lack of storage facilities. The three main abiotic factors that limit sorghum production, including in Ethiopia, are drought, insufficient soil fertility, and soil salinity (Dessi, 2018).

2.3.1 Biotic factors

Striga is a significant obstacle to the production of sorghum, particularly in Sub-Saharan Africa. Striga asiatica and Striga hermonthica are at least two species of Striga that have an impact on the production of sorghum (Jamil *et al*., 2021). In sub-Saharan Africa, Striga hermonthica is the most significant parasitic weed and one of the most detrimental biotic factors affecting sorghum productivity (Muchira *et al*., 2021). The resistant sorghum cultivars that have so far been created, however, have demonstrated a high level of resistance, largely due to reduced stimulant production (Belay, 2022). Resistance can be compromised over time and turn susceptible, highlighting the significance of ongoing efforts to create diverse forms of resistance. Sorghum landraces are crucial sources for the development of Striga resistance genes, according to reports from (Mengistu *et al., 2020*).An important fungus disease called anthracnose causes significant economic loss to crops. According to Stoop et al. (1981), the disease was originally identified in Togo in 1902. The majority of the world's growing regions for sorghum have since seen a small distribution of it. Most often, the illness affects sorghum in Africa, India, and the United States in a mild to severe form. Anthracnose

can cause symptoms including seedling blight, leaf blight, and head blight in different regions of the plant. Anthracnose on leaves can cause losses in photosynthetic photosynthesis of up to 50% or greater. On extremely vulnerable types, it may rise to 80% in extreme circumstances (Okosun *et al*., 2021).

Many countries in North and South America, Asia, and Africa are affected by severe rainy season sorghum diseases such grain mould. The illness is more severe there and in Asia, according to (Waliyar *et al.,* 2018), where white grain sorghum is produced in greater quantities. When plants mature in humid, tropical, and subtropical climates during the rainy season, improved short- and medium-duration sorghum cultivars suffer more. Late-maturing sorghums tend to prevent grain mould because they flower and fill grain in dry weather and are sensitive to photoperiod (Reddy *et al.*, 2020). Production losses might exceed 100%, depending on the cultivar and the current circumstances. Grain mould lowers the nutritive content of food and feed as well as the grain's cooking quality. Mouldy grains frequently contain mycotoxins, some of which are harmful to humans, animals, and poultry. Depending on the severity of the infection and the stage of grain development, grain mould symptoms might vary.

The extensively dispersed sorghum midge is one of the most harmful sorghum insect pests in southern America and Africa. Almost everywhere in the world where the crop is produced experiences it, with the exception of South-east Asia. As a result of the sorghum midge larvae feeding on the newly fertilized ovary, kernel development is prevented, which can result in severe direct grain loss. The glumes of a sorghum midge-infested spikelet squish firmly together because no kernel develops. Depending on the extent of the damage, a sorghum panicle infested by sorghum midge will frequently have different amounts of healthy kernels scattered among non-kernel bearing spikelets (Vercambre and Trouche, 2010).

2.4. Abiotic factors

2.4.1. Salinity

The most affected climate zones are those with arid and semi-arid climates, where salinity is a key contributor to soil deterioration in agricultural land globally (Tomazm *et al*., 2020). Salinity, in particular, interferes with the biochemical cycles of nitrification, denitrification, and the degradation

of organic residues in the soil (Singh, 2016). Additionally, salinity changes the physicochemical composition of the soil, which results in the loss of aggregate stability and the reduction of organic matter. This weakens the structure of the soil, reduces its ability to store and drain water, and increases the vulnerability of the soil to surface runoff and wind erosion (Okur and rçen, 2020).Salinity hinders some physiological processes, which leads to lower-quality yields, which has an adverse effect on plant growth. Na^+ in the soil can damage soil structure and lessen plants' ability to absorb water from the soil solution, which in turn can result in decreased plant growth, yield, and quality, according to Krishnamurthy *et al*. (2007). Na and Cl are very damaging elements for plants. Along with these consequences, salinity also causes mineral shortages, ion toxicity, and osmotic stress, all of which negatively affect physiological, biochemical, and physiological processes and, as a result, to variable degrees limit crop yield and output (Hamid *et al*., 2008).

2.4.2. Poor soil fertility

The loss of soil fertility is one of the major barriers preventing Ethiopia from expanding its agricultural sector. Due to high rates of erosion, the removal of biomass and animal waste from fields, and the inadequate application of inorganic and organic fertilizers, the nation's nutrient depletion rates are rising, as they are in many other East African countries (Teshome, 2023).Low soil fertility brought on by severe land degradation and nutrient depletion is a substantial crop production concern for Ethiopia (FAO, 2015).The inappropriate application of fertilizers, the complete removal of crop wastes, and continuous cropping methods all contribute to a negative balance between nutrient input and output (Amare and Melkamu, 2020). The emergence of superior cultivars without effective nutrition management exacerbates the issue of nutrients as yield-limiting variables. 2015, according to (Drechsel *et al*., 2015)Sorghum (Sorghum bicolor), the grain that is most frequently grown in SSA, is essential for guaranteeing food security in some of the world's poorest countries (FAO, 2020). In terms of both production volume and area cultivated, it ranks third among Ethiopia's grain crops in 2018–19. Although the nation's productivity is greater than the average for the world (FAO, 2020), there is a sizable yield productivity gap, which means that the actual yield is lower than the potential yield (Temane, 2017), and the yield penalty is 2.5 tonnes ha-1 (Schneider and Anderson, 2010). According to Habte *et al*. (2020), inadequate fertilizer application rates, inefficient nutrient utilization, imbalanced fertilization, and considerable geographical and temporal variability in crop response to fertilizers are the main causes of the yield gap.

2.4.3. Drought

Drought is defined as a prolonged shortage of plant available water, primarily due to insufficient rainfall or precipitation. It can also occur due to exceptionally HTs and low humidity driving the evapotranspiration in plants. Drought is one of the significant environmental factors affecting crop growth and productivity worldwide. Drought stress affects almost all the developmental stages of a plant; however, seed germination and early seedling growth phase (Patanè, 2013) and reproductive stages are highly sensitive and critical, including in sorghum (Prasad, 2019). Drought stress decreases carbon assimilation, stomatal conductance, and cell turgor, thereby reducing crop normal growth and development and limiting yield (Prasad, 2019). Visible moisture stress symptoms on crop plants include wilting of leaves and reduction in leaf area, bud/flower formation, sink numbers and overall growth and yield (Djanaguiraman, 2020).

2.4.4. Extreme Temperature

HT is another significant abiotic stress that lowers crop yields. It is thought of as HT stress when a temperature is above the physiological optimum and interferes with regular growth and development. Unexpected brief or lengthy episodes of HT stress, which can significantly reduce yield, are anticipated to happen more frequently in the future (IPCC, 2014). The air temperature is frequently much higher than the ideal (32 °C) for sorghum development and yield in dry and semi-arid parts of SSA and SA. Temperature increases above optimal might reduce the yields of food grain crops like sorghum (Prasad, 2019), wheat (*Triticum aestivum* L.), and others. Therefore, the anticipated rises in HT will seriously hinder the production of sorghum in semi-arid areas of SSA and SA. According to the IPCC (2014), HT stress is expected to happen more frequently, which might significantly reduce yield. According to the IPCC (2014), rising levels of carbon dioxide and other glasshouse gases might result in an increase in global air temperature of 3.7–4.8 °C by the end of the 21st century (Frank *et al*., 2015)

2.5. Role of phytobeneficial microbes

Many microbes are useful in farming and are currently being employed in the growth of sustainable food crops (Kassam *et al*., 2019). It has been demonstrated that beneficial microorganisms contribute to processes such as atmospheric nitrogen fixation, the breakdown of organic wastes and residues, the

detoxification of pesticides, the control of plant diseases and soil-borne pathogens, the enhancement of nutrient cycling, and the production of bioactive substances like vitamins, hormones, and enzymes that promote plant growth (Omotayo and Babalola).Plant-growth-promoting rhizobacteria (PGPR) and fungi (PGPF) are two types of microorganisms that have been employed to reduce abiotic stress (Fadiji *et al*., 2021). Studies have demonstrated that helpful microbes reduce abiotic stress by employing a variety of strategies, such as growth promotion by enhancing the production of phytohormones, siderophores, and phosphate solubilizers; lowering ethylene levels; and up regulating the expression of dehydration response and antioxidant genes (Ojuederie *et al*., 2019).Arbuscular mycorrhizia (AMF) has also emerged as an important approach to improve crop productivity under drought stress conditions.

AMF establishes a relationship with 80% of land plants and it induces pronounced impacts on plant growth and provides protection to plant from abiotic stress. Drought stress significantly reduces plant growth and development by inducing oxidative stress disturbing membrane integrity, plant water relations, nutrient uptake, photosynthetic activity, and photosynthetic apparatus and anti oxidant activities. However AMF can improve the plant tolerance against drought stress.AMF maintains membrane integrity; improve the plant growth under DS. Moreover AMF also protects the photosynthetic apparatus from drought induced oxidative stress and improves photosynthetic efficiency, osomolytes, phenols and hormone accumulation and reduces the accumulation of reactive oxygen(ROS) by increasing anti oxidant activities and gene expression which provide the tolerance to plants against drought stress(Haiying *et al*., 2022).

2.6. Arbuscular Mycorrhiza Fungi

Scientist A. B. Frank from Germany first used the term "mycorrhiza" in 1885. The term, which literally translates as "fungus-root," depicts the mutualistic relationship between a collection of soil fungi and higher plants (Ajit, 2008). The relationship is based on the plant component giving the fungal necessary chemical molecules like glucose. In exchange, the fungal component, which colonises the nearby soil as well as the root, aids the plant's ability to absorb nutrients and water by extending the root system.

Vesicular Arbuscular (VA) type endomycorrhizal connections are the most prevalent both geographically and within the plant kingdom. In order to complete their life cycle, mycorrhizas consume lipids and products of plant photosynthetic activity (Bago *et al*., 2000). The improvement of

water and nutrient intake from the nearby soil is just one way that AMF-mediated growth promotion works (Jung *et al*., 2012). Another way that it works is by protecting the plants against fungi that might cause disease. By forming soil aggregates and enhancing plant roots, AM fungi enhance nutrient cycling, soil quality, and erosion management. Additionally, AMF can affect plant biodiversity, provide protection from pests and diseases, and improve plant germination and survival rates. In addition, it improves crop output and quality, increases crop yield and tolerance to drought, reduces soil salinity, and promotes plant development in nutrient-poor soils or polluted settings (kanwal *et al*., 2015). As a result, AMF are essential endosymbionts that effectively contribute to plant productivity and ecological health. They are crucial for improving sustainable crop yields (Gianinazzi *et al*., 2010).

The sub-phylum Glomeromycotina of the phylum Mucoromycota is where the bulk of AMF species are found (Spatafora *et al*., 2016). In this sub-phylum, which also contains 25 taxa, four orders of AMF have been identified: *Glomerales, Archaeosporales, Paraglomerales, and Diversisporales* (Redecker *et al*., 2013). They use lipids and products from plant photosynthetic processes to complete their life cycle since they are obligatory biotrophs (Jiang *et al*., 2017).In addition to enhancing the plants' ability to absorb water and nutrients from the nearby soil, AMF-mediated growth promotion also protects the plants from fungi (Jung *et al*., 2012). As a result, AMF are essential endosymbionts that effectively contribute to plant productivity and ecological health. They are crucial for improving crops in a sustainable way (Gianinazzi *et al*., 2010).

2.7. Arbuscular mycorrhzia fungi (AMF) symbiotic characteristics

AMF and plants were known to coexist 400 million years ago (Selosse *et al*., 2015). Such connections are made through a series of biological processes that have a range of beneficial consequences on both agricultural and natural biotas (Van der Heijden *et al*., 2015). AMF's symbiotic partnership is a prime example of a mutualistic interaction that can control plant growth and development. Under the plant's roots, fungi have a mycelial network that enhances nutrient uptake that would not otherwise be possible. Even though they are from different species, the fungal mycelium colonises the roots of several plants, forming a common mycorrhizal network (CMN). With its considerable impacts on many plant communities, notably invasive species (Pringle *et al*.,

2009), and the fungal-mediated transport of phosphate (P) and nitrogen (N) to plants (Smith and Read, 2008), this CMN is regarded as a major component of the terrestrial ecosystem.

The mechanism through which nutrients are transferred from the soil to plants involves AMF. The primary pathway for nutrient exchange between the above- and below-ground portions of the ecosystem is provided by the common mycorrhizal networks (CMNs), which are created by root peripheral hyphae and play a significant regulatory function in the ecosystem's nutrient cycle (Cheng *et al*., 2012). AMF exchanges photosynthates and lipids necessary for growth and reproduction for water and soil mineral nutrients, particularly phosphate (He *et al*., 2019). The mutually beneficial symbiotic interaction between AMF and host plants can not only meet their growth requirements but also increase photosynthetic efficiency, foster growth and development, which increases host plant productivity and biomass accumulation (Jajoo and Mathur, 2021).

According to Augé *et al*. (2015), mycorrhizal symbionts can also help the host plant cope with abiotic challenges including drought and nutrient shortage. According to environmental factors, AMF symbionts can have a neutral, promoting, or inhibitory effect on plant growth (Facelli *et al*., 2010).The benefits of AMF on plants are well known, but further research is needed to fully understand how the AMF symbiosis with plant roots affects growth and reproductive performance under various culture settings. A green plant and a fungus have a symbiotic relationship known as a mycorrhiza. The plant produces organic molecules like sugars through photosynthesis and gives them to the fungus, which then gives the plant water and mineral nutrients like phosphorus that it has gotten from the soil. Mycorrhizas are found in vascular plant roots, but mycorrhiza-like relationships also exist in bryophytes (Jacott *et al*., 2017).

Mycorrhizia are commonly divided into ectomycorrhiza and endomycorrhizas. The two types are differentiated by the fact that the hyphae of ectomycorrhizal fungi do not penetrate individual cells within the root, while the hyphae of endomycorrhizal fungi penetrate the cell wall and invaginate the cell membrane (Marleau, 2011). Arbuscular mycorrhizae are characterized by the formation of unique structures; arbuscules and vesicles.AM fungi are obligate biotrophs and rely on their autotrophic host to complete their life cycle and to produce the next generation of spores (Figure2.1). The spores are able to germinate without the presence of a host, but the spores respond with an increase in hyphal branching and metabolic activity to root exudates (Bücking, 2008). Plant

roots release for example strigolactones that are able to induce pre-symbiotic growth of AM fungal spores

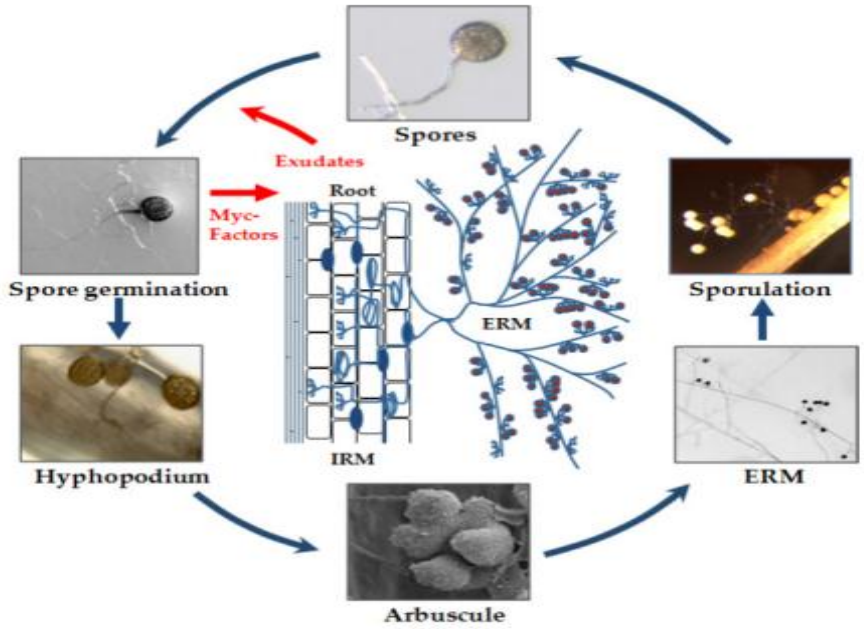

Figure 2. 1 The life cycle of an AM fungus and the several stages of AM development. IRM (intraradical mycelium) and ERM (extraradical mycelium) (Heike *et al*., 2012)

2.8. AMF and Nutritional Minerals

Increased nutrient uptakes are the result of AM fungus giving host plants access to nutrients that are otherwise inaccessible (Ardaka *et al*., 2011). AM fungi increase the availability of nutrients and encourage plant nutrient uptake by the host plant through the mycorrhizal uptake route (Figure 2.2). AM fungus increase the absorbing surface area and mobilize seldom accessible nutrients to aid plants in absorbing minerals like phosphate and nitrogen. In exchange, plant hosts give AM fungi a carbon source necessary for fungus growth.AM symbiosis is accomplished via chemical interaction between rhizosphere-dwelling bacteria and the roots of the host plant. In order to activate a common symbiosis signaling pathway shared by root nodulation symbiosis and AM symbiosis, AM fungi release Myc factors that can be recognized by plant potential receptors (Zhang *et al*., 2015). According to Mitra *et al*. (2019), it is clear that the inoculation of AMF can greatly raise the concentration of a variety of macro- and micronutrients. This increases photosynthate production and, in turn, biomass accumulation. AMF can increase the absorption of inorganic nutrients, particularly phosphate, in practically all plants (*Nell et al*., 2010).

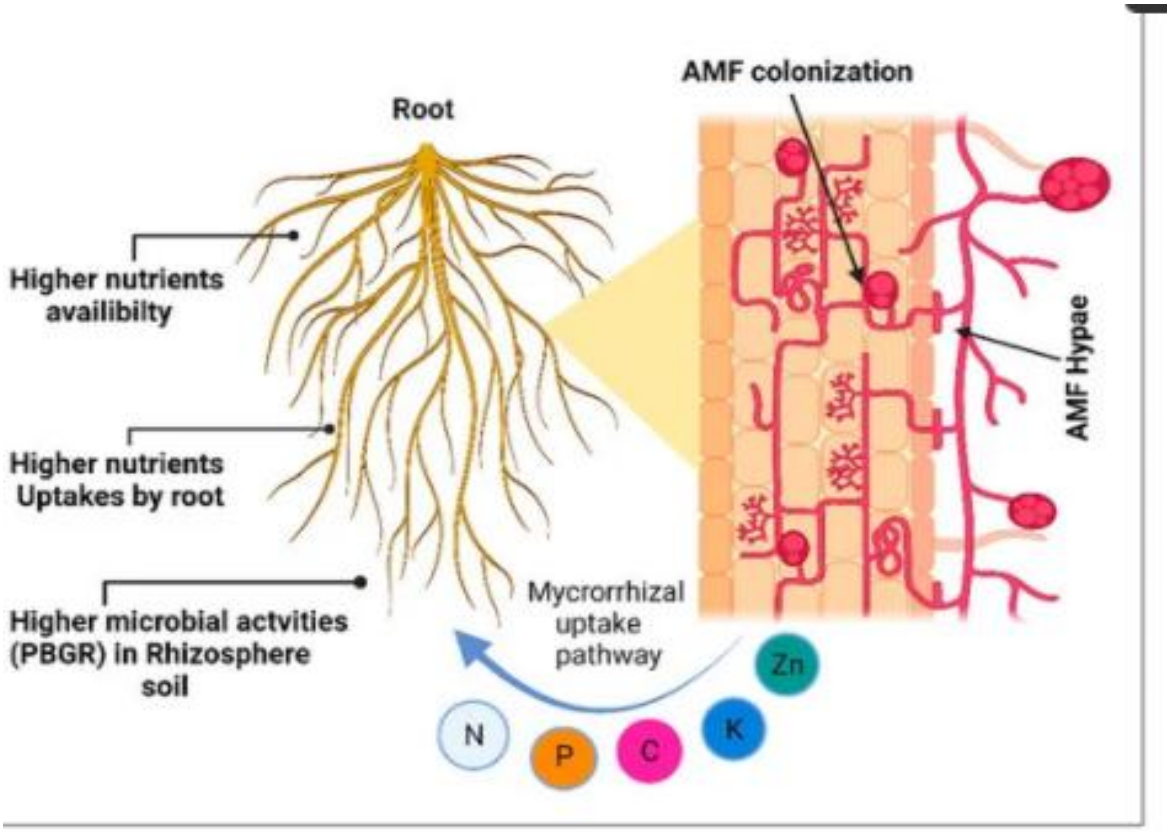

Figure 2.2. Shows the relationship between colonization and AM fungus spores, as well as the sharing and accessibility of nutrients in the plant's root (Khan *et al*., 2022).

2.9. Arbuscular Mycorrhizal Fungi as bio-fertilizers

Biofertilizers are substance that contains microbes, which helps in promoting the growth of plants and trees by increasing the supply of essential nutrients. They do not produce any pollution of any kind and are also environmentally beneficial. Utilizing biofertilizers in the soil helps to keep plants healthy and guards against disease. Among others, cyanobacteria, fungus, and bacteria are the primary suppliers of biofertilizers. These cultured bio fertilizers are applied to soil, seed, or both, depending on the situation, to boost the availability of plant nutrients. These fertilizers are very useful for soil health as well as for plant growth and development (Sadhana, 2014). Therefore, it is widely believed that AMF could be considered as a replacement of inorganic fertilizers in the near future, because mycorrhizal application can effectively reduce the quantitative use of chemical fertilizer input especially of phosphorus (Ortas, 2012).

2.10. Benefits of AMF in Alleviating Abiotic stresses

2.10.1. Drought

Numerous effects of drought stress on plant life include reduced transpiration rates and the development of oxidative stress (Hasanuzzaman *et al*., 2013). By altering enzyme activity, ion absorption, and nutrient assimilation, drought stress has a negative impact on plant growth (Ahanger and Agarwal, 2017). However, there is compelling evidence that AMF can reduce the effects of drought stress in a variety of crops, including wheat, barley, maize, soybeans, strawberries, and onions (Moradtalab *et al*., 2019).According to Augé (2001), the fundamental effect of AMF symbiosis in reducing water deficit is a combinatorial benefit liberated by nutritional, physical, and cellular effects. The mechanisms underlying the aforementioned phenomenon include increased nutrient uptake (Augé, 2004), water uptake by external hyphal mass articulation by increasing hydraulic conductivity rendering high water status for the host plants (Augé, 2004), osmotic adjustment (Porcel and Ruiz-Lozan, 2004), increase in antioxidant activity (Duc *et al*., 2018), and modification of hormonal balance (Chitarra *et al*., 2016). Key delays in gene expression and secondary metabolite synthesis have an impact on tomato plant yield and growth characteristics when water is not readily available in sufficient quantities (Giannakoula and Ilias, 2013).

2.10.2. Heavy Metals

Pollution from heavy metals (HMs) has received a lot of attention recently as a global environmental issue. One of the most important risks to the sustainability of the agro ecosystem has been identified as HMs contamination, a possible harm to the ecosystem and human health (Kamran *et al*., 2019). The process of environmental contamination caused by the release of hazardous materials into soil and water was drastically accelerated by anthropogenic activity. The safety of human health is seriously impacted by HMs because they are not biodegradable and because an excessive amount of them in the soil might enter the food chain, resulting in items like "cadmium rice" and "poisonous vegetables" (Gong and Tian, 2019).Once introduced to the human diet, HMs can accumulate in grains, join the food chain, and result in a variety of health issues for people (Chang *et al*., 2019). Once introduced, HMs stay in the environment for a long time. Abiotic stressors from heavy metals come in many different forms for agricultural plantations developing in unfavorable conditions. All of these factors not only slow down plant growth but also increase the risk of plant population extinction in the environment. Metal stress in plants disrupts plant metabolism and inhibits growth by

lowering the uptake of critical macro- and micronutrients (Zhao *et al*., 2019; Turan, 2020).Furthermore, HMs toxicities have an impact on plant cellular processes, impairing root development, upsetting regulatory processes, accumulating reactive oxygen species, impeding the uptake of vital nutrients, and causing membrane lipid peroxidation, which can in some cases lead to plant cell death (Saleem *et al*., 2020; Seneviratne *et al*., 2019).

The ability of these fungi to increase morphological and physiological processes that increase plant biomass and, as a result, uptake of significant immovable nutrients like Cu, Zn, and P and consequently reduce metal toxicity in the host plants, is the primary cause of AMF's strong effects on plant development and growth under extremely stressful conditions (Miransari, 2017). According to Audet (2014), increased growth or chelation in the rhizosphere soil may also result in metal dilution in plant tissues. According to AMF, Cd and Zn are bound in the mantle hyphae and cortical cells' ninth cell wall, preventing them from being taken up and enhancing growth, yield, and nutrient status (Garg and Chandel, 2012). Mycorrhizae can interfere with a plant's ability to absorb various metals from the rhizosphere and their movement from the root zone to the aerial parts (Li *et al*., 2015).

2.10.3. Extreme temperature

One of the most significant elements that has a detrimental effect on plant productivity is temperature stress (Zhu *et al*., 2011).The effects of heat stress, temperature swings, or severe temperatures include loss of plant vigour, slowed growth, senescence, browning of fruit, yield reduction, and lower biomass production. By increasing photosynthetic effectiveness, AMF enhances plants' ability to withstand high temperatures (Zhu *et al*., 2017). In order to boost photosynthetic rate and prevent damage to photosynthetic units, AMF modifies the root system in plants to absorb water at higher temperatures (Mathura, 2020).According to the research by Hajiboland *et al*. (2019), G. versiforme was substantially more adept than R. irregularis at reducing the cold stress felt in the winter and spring. Numerous metrics, including osmotic homoeostasis, plant growth, photosynthesis, and potassium uptake, were improved after the barley (Hordeum vulgare) was inoculated with AMF (Hajiboland *et al*. 2019). In order to mitigate climate change and tolerate a wide range of temperatures (both high and low), AMF is essential (Bender *et al*., 2014). By lowering the release of N_2O, it also enhances the intake and assimilation of N. The communities of AMF, however, may be impacted by excessive temperature, climatic, and seasonal changes (Ma, 2019).

2.10.4. Salinity

Stalinization of soils is a serious, worrying environmental issue that raises questions about the safety of the world's food supply. The salinity stress inhibits the plant's growth, which has an impact on all aspects of vegetative growth and absorption. There is proof that AMF occurs naturally in salty environments and that it helps plants that have been infected with it tolerate salt. AMF's contribution to salinity stress resistance depends on a number of factors, including its impact on the body's biochemistry, physiology, and nutrition. By altering a number of characteristics, such as absorption rate, salinity stress restricts plant development and productivity and leads to low yield output. (2017) Ahange *et al*. One method we have found to lessen the harm brought on by salinity is the prudent use of AMF (Santander, *et al*., 2019). According to numerous research, AMF increases plant yield under adverse circumstances (Talaat and Shawky, 2014).

AMF boosts photosynthetic rate, leaf water relations, and stomatal conductance in saline circumstances (Ait-El-Mokhtar *et al*. 2019). In Ocimumbasilicum under salt stress, AMF inoculation enhances gaseous exchange, photosynthetic rate, chlorophyll levels, and effective water utilization (Elhindi *et al*., 2017). Inoculated with AMF, Wang et al. (2018) found improved dry and fresh weights, N concentration in roots, and shoots during salt stress. Additionally, plants exposed to AMF exhibit significant hormone synthesis, including the production of salicylic and jasmonic acids as well as other inorganic minerals. In contrast to non-mycorrhizal plants, mycorrhizal inoculation of lettuce plants results in an increase in biomass production, an increase in N uptake, a high synthesis of proline, and changes in ionic relations, specifically a decrease in the accumulation of Na^+ (Santander *et al*. 2019). Additionally, plant growth facilitated by AMF encourages the modification of the polyamine pool (Kapoor *et al*. 2013). According to Aroca *et al*. (2013), lettuce plants inoculated with AMF exhibited an increase in strigolactone content that reduced the effects of salt stress. Ionic homoeostasis, the buildup of osmoregulatory substances such sugars, and a decrease in the Na and Cl concentrations are a few of the mechanisms that contribute to salinity tolerance.

2.11. The Effects of AM Fungi on the Chlorophylls, Carotenoid, and Photosynthesis Rate

An increase in photosynthetic rate may result in an increase in host plant metabolism and growth performance (Tariq, 2010). According to Smith and Gianinazzi (1988), AM fungus likewise did not improve host nutrient intake or speed up photosynthesis when exposed to low light. On the other

hand, cereal plants with AM fungus had increased chlorophyll content, photosynthetic rate, and enzymatic activity (Pepe *et al*., 2018). A better photosynthetic rate may also come from the enhancement of numerous enzymes during AM fungus inoculation, including phosphoenolpyruvate carboxylase (PEPC) and rubisco (Kaddes *et al*., 2019).

2.12. AMF and Sorghum yield

An important C4 crop produced for food, feed, and fibre is sorghum (Sorghum bicolor). Humans need calories and critical nutrients from grain varieties for food, which are especially significant as a subsistence crop in portions of Africa and Asia (Shakoor *et al*., 2014). The danger to food security in low- and middle-income nations is twofold: in order to stay healthy, one must consume enough calories as well as enough of the critical micronutrients zinc (Zn), iron (Fe), and vitamin A (Cakmak *et al*., 2017). The relationship with AM fungus benefits the host plant by enhancing the uptake of inorganic nutrients like P and Zn. Different species of AM fungus can heavily colonise sorghum types bred for biomass, and when they do, they often show favourable biomass responses (Watts-Williams, Emmett *et al*., 2019).AM fungus can increase the P, K, Zn, and Fe content of sorghum as well as its tolerance to salinity and drought stress (Caris *et al*., 1998; Cho *et al*., 2006). In both field and glasshouse experiments, many other cereal crops, including maize, rice, and wheat, exhibit positive growth responses to AM colonisation. However, when intervention techniques like tillage or rotation with a non-host crop, like canola, are taken into account, this is no longer the case (Zhang *et al*., 2019).

2.13. AMF Inoculum production and trap culturing

To get healthy AM spores for identification and to use as an inoculum to generate monospecific cultures, trapping is required (Gupta & Tuohy, 2019). Depending on all of the biotic and abiotic elements that affect growth, reproduction, and the division of colonisation and sporulation, trap cultures produce extremely varied results (INVAM, 2020).A trap culture can support a variety of AMF species because it comprises spores, hyhae, and colonised root fragments as an inoculum. Schalamuk and Cabello (2010), on the other hand, found that the inoculum used in trap cultures favoured higher growth of the Glomeraceae family while yielding less spores from other families.

2.14. Application and formulation of AMF Inoculum

Inoculum is small amount of propagules of mycorrhizal fungi which is used to initiate colonization in plants. The Mycorrhizal propagules comprises of spores, sporocarps, and auxiliary cells mycelia and infected root fragments. To generate inoculum, the understanding of both the spore, sporocarps, which are the main reproductive structure and the mycelia and auxiliary cells which make up the vegetative structures is vital. The AM enhances the absorption of water and nutrients, mainly P. It also increases the tolerance of plants to biotic and abiotic stresses, as pathogens, drought and high salinity. Besides that, the AM plays a critical role in the functional and successional processes of plant communities as soil formation, management and nutrient cycling.

AMF (Arbuscular Mycorrhizal Fungi) inoculum can be applied in sorghum production to promote plant growth and yield. Here are the steps for the formulation and application of AMF inoculum in sorghum production: **1.** Select the suitable strain of AMF inoculum for sorghum production. **2.** Prepare the inoculum carrier, which can be a combination of vermiculite, sand, and soil. **3.** Sterilize the carrier medium to eliminate any potential pathogens that may cause diseases. **4.** Mix the AMF inoculant with the inoculum carrier. **5.** Inoculate the roots of sorghum seedlings with the AMF inoculum during planting. **6.** Water the plants after inoculation. **7.** Monitor plant growth and soil nutrient availability to evaluate the effectiveness of AMF inoculum (INVAM, 2020).

CHAPTER THREE

3. MATERIAL AND METHODS

3.1. Description of the study areas

Soil samples for the study were collected from the Eastern part of Ethiopia namely Harar, Dire Dawa and Jigjiga (Figure 3.1).The study areas represent semi-arid to arid lowland agro-ecologies known for their sorghum production (CSA, 2012). Harar is part of east Hararghe zone and function as the administrative capital of Harari Region which is one of the eleven National Regional States of Ethiopia. Harar is located at 526 km far from Addis Ababa in East direction at a latitude of 8°500′9°15'N and longitude of 9°36′N 41°52′ East and situated at an altitude of 1850 m above sea level (m.a.s.l). The annual rainfall of the area is between 834 and 1300 mm. This area experiences a binominal rainfall pattern with a long rainy season from June to September and short rainy season from March to April, while the annual temperature ranges from 21 to 26°C (CSA, 2012). The soils of the area are sandy type and annual temperature ranges from 21 to 26 °C (CSA, 2012).

Dire Dawa is located at about 515 km to the east of Addis Ababa. The area is found between 9°27′ and 9°49' N latitude and 41°38′ and 42°19′ E longitude. The altitude of Dire Dawa Administration (DDA) ranges from 960 m.a.s.l in the northeast to 2450 m.a.s.l. in the southwest. The mean annual rainfall of the area varies from 550 mm in the lowland northern part to 850 mm in the southern mountains with average 640 mm. The monthly mean minimum and maximum temperature ranges from 14.5°C to 34.6°C, respectively (CSA, 2012).

Jigjiga is the capital of Ethiopian Somali Region which is among the eleven Regional States of the country. It is located at 615 km from Addis Ababa in east direction with an altitude ranging from 1660 to 1850 m.a.s.l. It is geographically located at 8^{o} 44'N longitude and 40^{o} 22′E latitude with mean minimum and maximum temperatures of around 17°C to 30°C, respectively. According to National Meteorological Service Agency reports, the mean annual rain fall is 660 mm and is bimodal in its distribution (CSA, 2012).

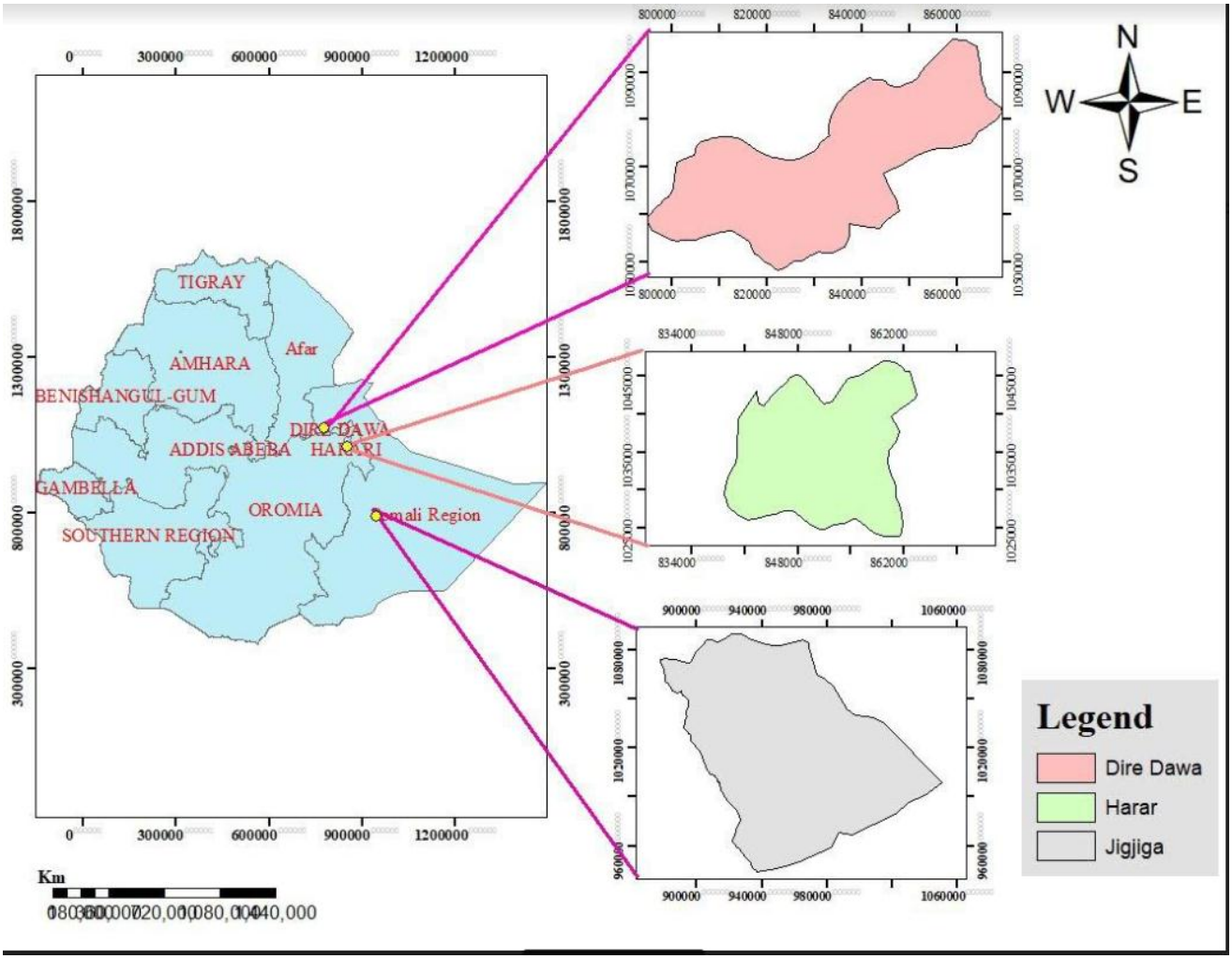

Figure 3. 1 Map of study area, Eastern Ethiopia (Diredawa, Harar and Jigjiga).

3.2. Soil samples collection

Soil Samples were collected in February, 2022 from three study sites on which sorghum was grown in eastern Ethiopia (Harar, Diredawa and Jigjiga). Three separate sample locations were established from each location. A composite soil samples was taken from a depth of 10-30 cm per location From Barren land. The samples were collected in alcohol sterilized plastic bags and were brought to the Microbiology laboratory of Adama Science and Technology University (ASTU) for subsequent activities. Soil physico- chemical characterization was undertaken at Bishoftu Agricultural Research Center following standard procedures and methods.

3.3. Soil Physicochemical Characteristics

The physicochemical properties of sample soils were analyzed as follows. Soil pH was measured potentiometrically using a digital pH-meter in the supernatant suspension of 1:2.5 soil: water. Soil texture was measured with the Boyacus hydrometer method (g/cm) using USDA textural triangle (Gee & Bauder, 1986). The total organic carbon was determined by using the method of Walkley & Black (1934), and available phosphorus was determined by Olsen methods (Olsen, 1954). In the Olsen procedure, $NaHCO_3$ at nearly constant pH of 8.5 was used as extracting solution. The total N content was determined using the Kjeldahl method by oxidizing soil samples with sulfuric acid and converting the N compounds into NH_4^+ as ammonium sulfate following the method of Hinds & Lowe (1980).

3.4. Establishment of Trap culture

In order to obtain many healthy and infective AMF propagules for inoculation, a trap culture was established in the greenhouse at ASTU for three months (March to June 2022), following the method of INVAM (2020). The trap cultures in pots were set up in three per site for all three soil samples from sorghum plots (Jigiga, Harar and Diredawa). A total of 18 pots (nine pots for wheat of abay and nine for maize-of melkassa 4 varieties) were established. Seeds of wheat (abay variety) and maize (melkassa 4) from Melkasa Agricultural Research Center (MRC) were selected as appropriate trap plants for their ability to induce high spore density, diversity, and species richness according to INVAM (2020).

The seeds were surface sterilized with 0.5% sodium hypochlorite solution for 15 minutes and repeatedly washed with sterile water, and sown at a two cm depth in each plastic pot with VOLUME OF 4kg filled with 3kg of mixed soil: autoclaved sand sample (1:1; v/v) irrigated for three days before sowing and covered with sterilized sand. Pots were irrigated daily as needed. During the growing phase, no fertilizer was added. All seedlings were grown in the greenhouse under natural light and temperature conditions for three months. Finally, watering was reduced during the final weeks of the third months to maximize spore production. At the end of third month, the plants were cut near the base, and the cultures were air-dried, analysed for AMF properties and stored in zipped plastic bags at room temperature for 30 days before inoculation (INVAM, 2021).

3.5. Determination AMF Spore Abundance and Diversity of the Trap culture

The abundance and diversity of AMF in the trap culture was examined to determine the initial mycorrhizal status of the inoculum after three months. Soil samples from the trap culture were air-dried and sieved through a 2-mm sieve to remove coarse debris. AMF spores from the soil samples were extracted by the wet sieving and decanting method (Gerdemann & Nicolson, 1963) followed by centrifugation in water and in a 60% sucrose solution (Brundrett *et al*., 1996).One hundred grams of soil inoculum was suspended in one liter of tap water in each batch and left to settle the heavier fractions to settle for two to three minutes.

Then, the liquid fraction was decanted through four stacked sieves of decreasing pore size: 500, 180, 90 and 63 µm. The first sieve was used to catch large pieces of floating organic debris, and the second sieve was used to capture some AMF sporocarps, and the third and fourth also to capture AMF spores and fine particles, respectively. The contents of the 180, 90, and 63 µm sieves were then put into a centrifuge tube containing water and centrifuged at 2000 rpm for five minutes. After pouring off the supernatant, 60% sucrose was added to the pellet and mixed it carefully, then centrifuged for one min at 2000 rpm.

The supernatant was carefully poured through a 63 µm sieve and washed with tap water to remove the sucrose. Finally, spores, spore clusters, and sporocarps were carefully washed and transferred to a plastic Petri dish for examination under the stereomicroscope. Enumeration of spore numbers per gram of dry soil was undertaken according to INVAM, http://invam.caf.wvu.edu.Thereafter, the healthy looking AMF spores were mounted on the slides in Polyvinyl Alcohol, Lactic acid Glycerol (PVLG) and Melzer's reagent (1:1) according to Brundrett *et al*.(1996) using soft forceps. A compound microscope (DN-300M, Taiwan) was used for the identification of spores based upon synoptic descriptions and specialized mwebsites (www.zor.zut.edu.pl/ Glomeromycota; invam. caf.wvu.edu).Isolation frequency (IF) is (the number of samples in which a given species was isolated / the total number of samples) × 100%. Relative abundance of spores (RA) is (the number of spores in a given species / total number of spores) × 100%. The dominant AMF species were determined according to relative abundance (RA>5%) and isolation frequency (IF >50%) (Li *et al*., 2007).

3.6. Assessment of AMF root colonization

The root samples were washed several times in tap water and cut into segments about 1–2 cm long. About 0.5 g of root segments were cleared in 10 % (w/v) KOH at 90^{o}C for 1 hour in water bath. And roots were further bleached with alkaline hydrogen peroxide (10% H_2O_2) for 3 minutes at room temperature. Thereafter, the roots were treated with 2% HCl (v/v) for 15-20 minutes at room temperature and finally stained with 0.05% w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at 90^{0C} for one hour in water bath. Samples were drained and washed thoroughly with distilled water at the end of every step except with the HCl treatment. The samples were left in distaining solution (lactoglycerol) for more than two days in a dark room.

Finally, roots were mounted in PVLG mountant on microscopic slides and covered with 24×24 mm cover slips (Wongchalee & Pukahute, 2012). AMF colonization was assessed from cleared and stained roots according to McGonigle *et al*. (1990). A total of 150 intersections were taken for each subsample to estimate percent AM root colonization under a compound microscope at a magnification of 100X. The presence of arbuscular colonization (AC) and vesicular colonization (VC) were calculated by dividing the count for the arbuscules and vesicles categories, respectively by the total number of intersections. Hyphal colonization (HC) was calculated by dividing the proportion of nonnegative intersections to total number of intersections.

3.7. Experimental design and AMF inoculation

The experimental factors were two Sorghum varieties (Melkam from Melkassa agricultural research center and the Local seeds from common market were grown under different water regimes (80%, 50% and 25%) field capacity of soil with AMF and Without AMF. The experiment was set up in a Randomized Complete Block Design (RCBD) with three replications in a greenhouse with total of 24 experimental treatments as shown in Table 3.1.The amount of water applied was determined following the procedure described by Million Eshete *et al*. (2005):

1. Dry weight of the soil was taken (Md).

2. The polyethene plastic bag was weighed alone (Mb).

3. Then, the soil was saturated with water.

4. Left for 24hours until no water drips from underneath.

5. The bag with soil was then weighed at field capacity; this gives wet mass of the soil with the bag used (Mwb).

6. Then, weight of pot was subtracted from wet mass of the soil with plastic bag which gave wet mass of the soil (Mw), Mwb-Mb = Mw. 7. Mw-Md= amount of water at field capacity (FC). Md- mass of dry weight of soil, Mb- mass of plastic bag, Mwb- mass of soil with plastic bag.

To maintain the soil water content near to the desired field capacity, all pots were weighed every day and water loss was replenished up to the end of the experiment. The trait measurements were made on the fully expanded leaves from the top of the plant soon after weighting the pots and replenishing water loss.

Table 3.1 Experimental design: treatments allocation

No	Sample source	No	Treatments	Melkam Var.	Local seeds
1	Diredawa	1	+AMF+80%FC	X	X
		2	-AMF +80%FC	X	X
		3	+AMF +50%FC	X	X
		4	+AMF+25%FC	X	X
2	Jigjiga	1	+AMF+80%FC	X	X
		2	-AMF +80%FC	X	X
		3	+AMF +50%FC	X	X
		4	+AMF+25%FC	X	X
3	Harar	1	+AMF+80%FC	X	X
		2	-AMF +80%FC	X	X
		3	+AMF +50%FC	X	X
		4	+AMF+25%FC	X	X

Legend: +AMF: - with inoculants -AMF: - without inoculants. FC:-Field Capacity

3.8. Inoculation of Arbuscular mycorrhizia Fungi (AMF)

Based on higher root colonization and spore abundance rhizospheric soil from maize was used as AMF inoculums for the sorghum. The trap culture based inoculum was prepared in a soil based system by mixing the root fragments (colonized root, mycorrhiza mycelium, vesicles and spore) with a steam sterilized soil mix (soil: sand, 1:1).AMF inoculum from Diredawa (Consortium of AMF inoculum,(24.24 spores g-1 of soil), Harar (22.23 spores g-1 of soil) and 22.48 spores g^{-1} of soil) propagules were inoculated.Three hundred gram of air-dried AMF inoculum was added to the slightly acidic soil obtained from Holeta area(with physiochemical characteristics of pH 4.72, total nitrogen

content of 0.2% and 2.06ppm of phosphorus content) (Zerihun Belay & Fassil Assefa, 2011) The experiment had two factors, AMF (seedlings treated with AMF and non-AMF (control) seedlings. Inoculation with AMF was applied to the soil before sowing the seeds. Another group of seedlings was established under the same conditions but without AMF (control). Cultures were grown in a greenhouse for at least three months under different water regime (80%FC, 50%FC and 25%FC) (Table 3.1).

3.9. Measurement and evaluation of plant growth parameters

3.9.1. Determination of fresh and dry weight of leaves, root and shoot

The plants fresh weights were recorded after harvesting. Fresh weight of the roots and shoot were obtained by separation from one another and weighing the parts by using a digital balance scale. The roots were thoroughly washed with tap water and the root and shoot were placed in paper bags and oven dried for 48 hrs at 70ºC. The dry shoot weight (DSW) and dry root weight (DRW) of each plant were then determined by weighing the oven dried shoots and roots.

3.9.2. Plant height and Leaf Number

Plant height was measured from the top of the soil (base of the plant) to tip of the youngest leaves by use of ruler from the three tagged plants in each experimental treatments. Leaves were counted on the main stem from plants in each experimental pot. Data were recorded at the end of vegetative growth.

3.10. Determination of relative water content (RWC)

Relative water content was measured according to Smart and Bingham (1974). Fresh weights of harvested leaves (FW) were measured and then put in a Petri dish with distilled water for 5 hours. The turgor weight (TW) of leaves was measured and then put into an oven using paper bag for 24 hours at 70^{o}C. Lastly, dry weights (DW) were measured. Relative water content was calculated with the following formula.

Relative water content (%) = (FW-DW) / (TW-DW) x 100 FW= fresh weight, DW=dry weight, TW= turgor weight (Smart and Bingham (1974).

3.11. Proline content

Proline content was determined by using acid ninhydrin reagent by the method of Bates *et al*. (1973). Fresh plant sample (0.25 g) was homogenized with 10 ml of 3 % aqueous sulfosalicylic acid and the homogenate was filtered using Whatman No.2 filter paper. The filtrate was used for proline concentration determination. Two milliliter of the filtrate was mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube and allowed to react for 1 hour in a water bath at 100^{o}C. It was then cooled, producing brownish color. The reaction mixture was extracted with 4 ml of toluene, mixed vigorously in a test tube and stirred for 15-20 seconds until separate layers were formed. The content from each tube was transferred to funnel. Chromophore or upper toluene layer containing the color complex due to proline ninhydrin reaction was separated from aqueous phase to other test tube and warmed at room temperature and absorbance was read at 520 nm by spectrophotometer (Genesys 10 UV, Thermo Electron Corporation, USA).

3.12. Total chlorophyll content

Photosynthetic pigments were extracted and estimated according to the formulae of Lichtentaler and Wellburn (1985) slightly modified by Dere *et a*l. (1998). A hundred mg of fresh leaf material from all the treatments were homogenized with mortar and pestle using 5ml of 100% (v/v) acetone. The green slurry was filtered through filter paper and centrifuged at 2500 rpm for 10 minutes. The supernatant was added into test tubes. The optical density was recorded at 662 nm, 645 nm and 470 nm against 100% (v/v) acetone as blank in spectrophotometer (Genesys 10 UV, Thermo Electron Corporation, USA). Chlorophyll and carotenoids contents were expressed in mg per g fresh weight of the leaves. Chlorophyll a, chlorophyll b and total carotenoid level present in the pigment extract were determined by employing the following formulae:

$$Chl.a = 11.75A_{662} - 2.350\ A_{645}$$

$$Chl.b = 18.61\ A_{645} - 3.960\ A_{662}$$

$$Cx+c = 1000\ A_{470} - 2.270\ Chl.a - 81.4Chl.b/227\ y$$

Total chlorophyll= (Chl.a + Chl.b)

(Chl.a = chlorophyll a; Chl.b = chlorophyll b; Cx+c AW = Absorbance at W nm wavelength)

3.13. Total soluble sugar (TSS)

Total soluble sugar in sorghum leaf samples was determined according to Dubois *et al*. (1956) with some modifications (Tiwari, *et al*., 2016). About 200 mg of fresh leaf tissues were homogenized in 5 ml of 80% methanol and was incubated in water bath at 70 ^{o}C for 30 min After incubation, 1ml of extract was mixed with 1 ml of 5% phenol and 5 ml of 95% H_2SO_4 and further incubated in dark for 15 min. Absorbance was then measured at 490 nm using spectrophotometer (Genesys 10 UV, Thermo Electron Corporation, USA).

3.14. Data Analysis

The analysis was performed using the SPSS software package (version 26.0). The AMF spore abundance and root colonization of the field soil and trap culture of the inoculum data; and the growth response (plant shoot height, root height, number of leaves, dry and fresh weight of root and shoot, Leaf proline content, RWC, Total chlorophyll and Total soluble sugar) data were subjected to one-way analysis of variance (ANOVA) using the treatments as factor. Tukey's honestly significant difference (HSD) post hoc test was used for pair-wise multiple mean comparisons tests. All the tests of statistical significance were decided at $p < 0.05$. And data are reported as means ± SE, based on three replicates.

CHAPTER FOUR

4. RESULTS

4.1. Physicochemical Analysis of Soil Sample

The soil samples from each sampling site (field) was slightly basic, with Diredawa soil being more basic with a pH of 7.83, while Jigjiga and Harar soil samples showed pH of 7.75 and 7.64, respectively (Table 4.1). For the three sampling locations, the sandy soil texture dominated the soil sample used for the production of AMF inoculums in this trial. The soil organic matter, total nitrogen and available P concentration ranged from 2.09% to 3.25%, 0.08% to 0.16 and 3.14 ppm to 4.54 ppm, respectively. And also Harar soil contained 0.16, Jigjiga soil 0.12, and Diredawa 0.08% of total nitrogen. Soil from Diredawa contained comparatively high phosphorus (4.54ppm), while soil from Jigjiga and Harar had 3.99ppm and 3.14ppm, respectively. AMF spore density of the rhizosphere soils sampled from Eastern Ethiopia (Diredawa, Jigjiga and Harar) ranged from 22.23 to 24.48 spores g^{-1} soil. The highest average spore density of 24.24 spores' g^{-1}recorded from Diredawa, and the lowest of 22.23 spore's g^{-1}counted from Harar soil samples.

Table 4. 1. AMF spore abundance and physico-chemical parameters of field soil samples (Diredawa, Jigjiga and Harar).

							Texture			SD(gm^{-1})
sample Location	pH	% OC	% OM	% TN	AV.P (Olsen)	ppm	% clay	% silt	% sand	
Diredawa sample	7.83	1.21	2.09	0.08	4.54		11.6	3.2	85.2	24.24±0.19[a]
Harar sample	7.64	1.48	2.55	0.16	3.14		25.6	33.2	41.2	22.23±0.24[b]
Jigjiga sample	7.75	1.89	3.25	0.12	3.99		21.6	7.2	71.2	22.48±0.24[b]

TN- total nitrogen OC- organic carbon Av.p –available phosphorus SD- spore density OM- organic matter lowercase letters represent significant difference at 0.05 level.

4.2. Assessment of AMF Root Colonization and Spore Density of the Trap Culture

The average number of AMF spore density enumerated in the trap culture ranged from 26.80 from sampling area of Harar for wheat plant to 32.99 g^{-1} of Maize from Dire Dawa (Table 4.2). There was a significant ($p < 0.05$) difference in AMF spore density among the areas of sampling and plant species (maize and wheat). The highest spore densities were found associated with maize from Dire Dawa soil (33.99 g^{-1}) while wheat from Harar soil recorded the lowest (26.80 g $^{-1}$ soil) AMF spore density. All seedlings of maize and wheat in trap culture were colonized by AMF. The percentage of AMF root colonization significantly ($p < 0.05$) differed between plant species and sampling area (Diredawa, Harar and Jigjiga) ($p < 0.05$) (Table 4.2). Average hyphal colonization ranged between 71 and 89.66%. The highest hyphal colonization was found in maize (89%) from Diredawa. The lowest colonization was in wheat species from Harar (71.86%).

Table 4. 2 AMF spore abundance & percentage of root colonization in trap cultures.

Sampling location	Variety of plant	SD (gm^{-1})	HC%	VC%	AC%
Diredawa	Maize	32.99±0.38^{a}	89.66±0.20^{a}	35±0.57^{a}	8.66±0.33^{d}
	Wheat	28.98±0.34^{c}	74.66±0.33^{e}	12.43±0.29^{e}	13.33+0.33^{c}
Harar	Maize	32.54±0.41^{a}	86.23±0.62^{b}	29.83±0.39^{b}	17.20±0.61^{b}
	Wheat	26.80±0.81^{c}	71.86±0.46^{e}	25.53±0.39^{c}	23.40±0.61^{a}
Jigjiga	Maize	30.03±0.60^{b}	82.66±0.33^{c}	19±028^{d}	12.20±0.41^{c}
	Wheat	27.32±0.54^{c}	77±0.57^{d}	26.53±0.39^{c}	16.53±0.39^{b}

Note- SD- spore density, AC- arbuscular colonization, VC – vascular colonization, and HC- hyphae colonization. Different lowercase letters in the same column represent significant difference at 0.05 level; Mean values followed by the same letters in the same column are not significantly different at $p<0.05$. Data are average of three biological replicates along with standard error bars.

4.3. Diversity of AMF from trap culture

A total of 24 AMF species belonging to 15 genera were identified in trap culture from all sampling location (Diredawa, Jigjiga and Harar) for maize as a host plant (Figure 4.1).Four species belonged to

Gigaspora, three to Funneliformis and *Pacispora*, two each *to Claroideoglomus*, *Rhizophagus*, and *Paraglomus* and one each to *Glomus, Acualospotra, Scutellospora, Dentiscutata, Septoglomus, Entrophospora, Diversispora* and *Racocetra.* The Isolation frequency (IF) and relative abundance (RA) of AMF species varied greatly among sampling sites (Table 4.3). The genera *Claroideoglomus* (all species), *Funneliformis (F. mossae*, and *F. geosporum), Paraglomus* (*P. occultum), Rhizophagus (R. aggregatus)* and *Gigaspora* (*G. gigantea*) were distributed in all the sampling sites. On the basis of IV (importance values), the AMF species *Claroideoglomus claroideum, Claroideoglomus, etunicatum, Funneliformis mosseae, F.geosporum, Gigaspora gigantean* and *Rhizophagus aggregatus* were categorized into the dominant genera.

Table 4.3.Morphologically identified arbuscular mycorrhizal fungi, their frequency of occurrence and relative abundances in rhizosphere soil of trap culture for Maize.

AMF species	Diredawa	Harar	Jigjiga	IF%	RA%	IV%	Category
Claroideoglomus							
C. claroideum (N.C. Schenck & G.S. Sm.) C. Walker & A. C (2010)	X	X	X	100	2.7	51.35	Dominant
C.etunicatum C. Walker & A. Schüßler (2010)	X	X	X	100	2.09	51.04	Dominant
*Diversisporatortuosa(*C. Walker & A. Schüßler (2004)	X		X	66.6	1.62	34.11	common
Funneliformis							
F.mosseae C. Walker & A. Schüßler (2010)	X	X	X	100	3.2	51.6	Dominant
F.geosporum C. Walker & A. Schüßler (2010)	X	X	X	100	2.5	51.25	Dominant
Funniliformis caledonius Walker & A. Schüßler (2010)							Common
Rhizophagus	X			33.33	0.46	16.89	
R.aggregatus (C. Walker, 2016)	X	X	X	100	1.6	50.8	Dominant
R.diaphanus P.A. Dang. (1896)		X	X	66.6	1.3	33.95	Common
Gigaspora							
G.gigantea Gerd & Trappe (1974)	X	X	X	100	3.2	51.6	Dominant
G. caledonius Gerd & Trappe (1974)	X			33.3	0.93	17.11	Common
G.margarita Gerd & Trappe			X	33.3	0.69	16.99	Common

(1974)							
G. rosea Gerd & Trappe (1974)			X	33.3	0.69	16.99	Common
Scutellospora pellucida(Nilson and schenck	X			33.3	0.23	16.76	Common
Dentiscutata rubra	X			33.3	0.27	16.78	Common
Paraglomus							
P.occultum(J.B. Morton & D. Redecker, 2001)		X	X	66.6	1.8	34.2	Common
P.laccatum (J.B. Morton & D. Redecker, 2001)		X		33.3	0.24	16.77	Common
Pacispora							
P.robiginia Sieverd. & Oehl (2004)		X		33.3	0.34	16.82	Common
P.scintillans Sieverd. & Oehl (2004)		X	X	66.6	1.35	33.97	Common
P.franciscana Sieverd. & Oehl (2004)			X	33.3	0.13	16.71	Common
Septoglomus constrictum (Sieverd., G. A. Silva & Oehl, 2011)		X		33.3	0.15	16.72	Common
Entrophospora nevadensis			X	33.3	0.16	16.73	Common
Acaulospora koskei (Gerd. & Trappe, 1974)			X	33.3	0.13	16.71	Common
Racocetra coralloidea F.A. Souza & Sieverd. (2008)			X	33.3	0.14	16.72	Common
Glomus sp. (Tul. & C. Tul. (1845)		X		33.3	0.5	16.9	Common

Note: - IF- isolation frequency RA- relative abundance and IV- importance values of AMF species in soil and trap culture of Eastern Ethiopia.

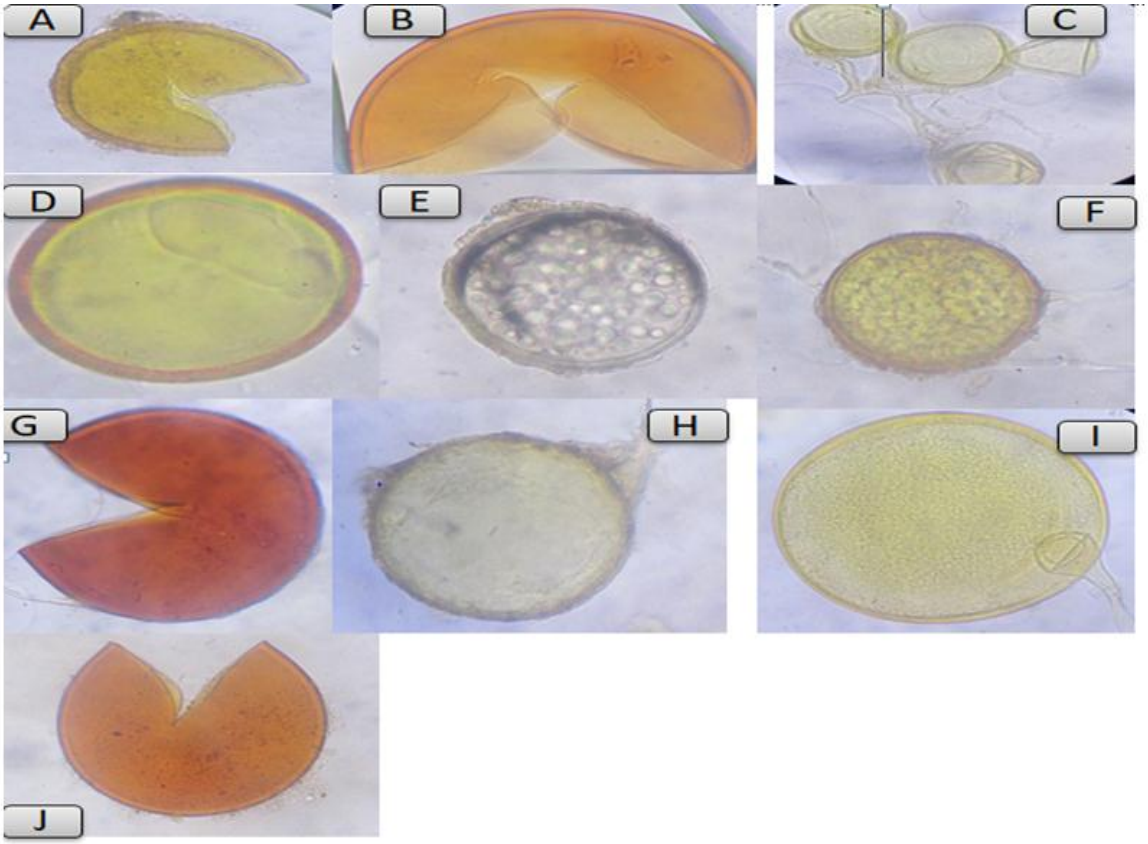

Figure 4.1. Some AMF species identified from rhizosphere soil of trap culture of maize plant. All photos are from slides made in PVLG. a) *Claroideoglomus claroideum* b) *Pacispora robiginia*, c) *Rhizophagus aggregatus* d) *Acaulospora koskei* e) *Rhizophagus diaphonus f)Claroideoglomus_etunicatum) g)Septoglomus constrictum) h) Funneliformis mosseae i)Gigaspora gigantea* j) *Funneliformis geosporum*

4.4. Effects of AMF Inoculation on the growth of sorghum

Sorghum can be highly colonized by arbuscular mycorrhizal (AM) fungi, and the plant-fungal association can lead to improvements in biomass (Table 4.4) The growth performance of the two types of sorghum varieties (Melkam and Local) was significantly ($p < 0.05$) different, especially in the height increment and root biomass. Furthermore, this study showed that Melkam varieties had a higher rate of growth performance than Local varieties in almost all treatments. However, both sorghum varieties showed no significant ($p < 0.05$) difference in leaf number for all sampling locations. The differences in growth parameters of the two sorghum varieties are shown in (Table 4.4).

Table 4.4 a Effect of AMF inoculums on growth performance of two sorghum Varieties Melkam variety.

Sampling Area	Treatments	RFW(g)	RDW(g)	SFW(g)	SDW(g)	SH(cm)	LN	RL(cm)
Diredawa	T1 (80%) +	7.46±0.18^{abc}	2.16±0.40^{ab}	14.73±0.43^{a}	3.23±0.38^{a}	93.33±0.24^{a}	5.33±0.33^{a}	39±0.25^{abc}
	T2 (80%) -	3.66±0.14^{i}	1.43±0.12^{bc}	7.8±0.25^{e}	0.76±12^{bc}	66.33±0.45^{f}	4.66±0.33^{a}	30.33±0.33^{abc}
	T3 (50%) +	6.66±0.30^{abc}	1.93±0.39^{ab}	13.16±0.87^{abc}	1.3±0.05^{bc}	92±0.28^{ab}	5.66±0.33^{a}	38.46±0.44^{abc}
	T4 (25%) +	5.13±0.46^{def}	1.5±0.20^{ab}	12.10±.0.26^{abc}	0.77±0.35^{c}	86±0.46^{bc}	4.74±0.00^{a}	36.5±0.57^{abc}
Harar								
	T1 (80%)+	8.13±0.24^{a}	2.16±0.17^{ab}	14.3±0.50^{abc}	2.7±0.37^{ab}	75±0.50^{d}	4.67±0.33^{a}	33.16±0.27^{abc}
	T2 (80%)-	5.53±0.49^{cde}	1.43±0.21^{bc}	10.97±0.36^{abc}	2±0.16^{bc}	61.33±0.33^{g}	4.33±0.66^{a}	31.33±0.85^{cd}
	T3 (50%)+	7.9±0.58^{ab}	1.93±0.12^{ab}	13.63±0.85^{abc}	2.3±0.15^{b}	73.56±0.11^{d}	5±0.00^{a}	33.89±0.69^{abc}
	T4 (25%)+	6.96±0.53^{abc}	1.5±0.14^{ab}	11.63±1^{abc}	2±0.17^{b}	75±0.24^{d}	5±0.00^{a}	35.66±0.38^{abc}

Jigjiga								
	T1 (80%) +	5.76±0.39^{bc}	1.2±0.05^{bc}	11.3±0.63^{abc}	1.7±0.10^{bc}	86.5±0.53^{bc}	5.33±0.00^{a}	31.33±0.41^{cd}
	T2 (80%) -	3.7±0.40^{h}	0.7±0.15^{c}	8.9±1.68^{cd}	2.13±0.29^{ab}	70±0.34^{f}	4.67±0.66^{a}	28.97±0.32^{f}
	T3 (50%) +	4.43±0.53^{g}	1.13±0.20^{bc}	11.3±0.60^{abc}	3.2±0.41^{ab}	79.33±0.48^{cd}	6±0.00^{a}	33.45±0.54^{abc}
	T4 (25%) +	3.46±0.67^{i}	0.6±0.11^{c}	12±0.83^{abc}	2.16±0.23^{ab}	81±0.28^{cd}	5.4±0.57^{a}	34±0.68^{abc}

Note: SH-Shoot Height, RH-Root Height, SFW-Fresh Weight of Shoot, RFW-Fresh Weight of Root, SDW-Dry Weight of Shoot, RDW-Dry Weight of Root and LN-Leaf number The same letters in the row indicate not significantly different values based on one-way ANOVA and Tukey's honestly significant difference (HSD) post hoc test ($p< 0.05$).T1: +AMF+80%FC ; T2:-AMF+80%FC; T3: +AMF+50%FC; T4: +AMF+25%FC

Table 4.4 b Effect of AMF inoculums on growth performance of Local sorghum Varieties.

Sampling Area	Treatments	RFW(g)	RDW(g)	SFW(g)	SDW(g)	SH(cm)	LN
Dire dawa	T1 (80%) +	6.7±0.15^{abc}	1.2±0.10^{bc}	7.7±0.60^{e}	1.1±0.10^{bc}	77±0.35^{d}	4.66±0.66^{a}
	T2 (80%) -	4.09±0.34^{g}	0.7±0.28^{c}	6.5±0.34^{f}	1.73±0.23^{bc}	71.67±0.52^{e}	4.46±0.66^{a}
	T3 (50%) +	4.86±0.32^{def}	1.13±0.26^{bc}	9.5±0.73^{bc}	1.76±0.06^{bc}	78.33±0.27^{cd}	4.66±0.33^{a}
	T4 (25%) +	4.43±0.34^{f}	1.19±0.17^{bc}	10.2±0.41^{abc}	1.16±0.13^{bc}	76.33±0.58^{d}	4.63±0.33^{a}
Harar							
	T1 (80%) +	6.16±0.17^{abc}	1.76±0.08^{ab}	7.8±1.15^{e}	1.33±0.13^{bc}	72.66±0.88^{e}	5.33±0.33^{a}
	T2 (80%) -	4.6±0.32^{ef}	0.66±0.08^{c}	6.3±0.55^{g}	0.46±0.08^{c}	69.33±0.32^{e}	4.33±0.66^{a}
	T3 (50%) +	5.4±0.34^{cde}	1.66±0.12^{ab}	8.36±1.53^{d}	0.93±0.08^{bc}	82±0.56c^{d}	5±0.00^{a}
	T4 (25%) +	5.66±0.71^{cde}	1.73±0.17^{ab}	10.7±1.23^{abc}	1.26±0.14^{bc}	75.33±0.58^{d}	5±1^{a}
Jigjiga							
	T1 (80%) +	4.36±0.49^{g}	1.1±0.05^{bc}	8.73±0.14^{d}	0.9±0.11^{bc}	72.33±0.45^{e}	5.33±0.66^{a}
	T2 (80%) -	3.53±0.29^{i}	0.6±0.05^{c}	4.46±1^{h}	0.76±0.12^{bc}	60±0.62^{g}	5.4±0.57^{a}
	T3 (50%) +	4.46±0.43^{f}	1.2±0.23^{ab}	8.73±0.14^{d}	0.9±0.11^{bc}	81.33±0.25^{cd}	4.66±0.33^{a}
	T4 (25%) +	3.9±0.05^{h}	1.21±0.23^{ab}	7.67±0.49^{e}	1.66±.021^{bc}	76±0.50^{d}	4.33±0.33^{a}

Note: SH-Shoot Height, RH-Root Height, SFW-Fresh Weight of Shoot, RFW-Fresh Weight of Root, SDW-Dry Weight of Shoot, RDW-Dry Weight of Root and LN-Leaf number The same letters in the row indicate not significantly different values based on one-way ANOVA and Tukey's honestly significant difference (HSD) post hoc test ($p< 0.05$).T1: +AMF+80%FC ; T2: AMF +80%FC; T3: +AMF+50%FC; T4: +AMF+25%FC.

AMF inoculation significantly ($p<0.05$) influenced fresh and dry weight of root. Inoculated treatments had the highest root fresh and dry weight of (T1) (8.13 g, 2.16 g), respectively from Diredawa by melkam variety but non-inoculated plants displayed significantly low root fresh and dry weight (T2) (3.53g 0.6g), respectively for Local variety from Jigjiga location. There were significant ($p<0.05$) differences in plant root fresh and dry weight between the two sorghum varieties (Table 4.4). The lowest root fresh weight is 3.53g measured from Jigjiga of local variety, while Melkam variety recorded the highest root fresh weight of 8.13 g from Harar. Similarly, root dry weight showed significant differences in which melkam variety showed 2.16 g dry weight both in Diredawa and Harar but 0.6 g was recorded from sample of Jigjiga of local variety. In addition, at 25%FC (T4) gave greater dry and fresh weight (6.96 and 1.73g) than control treatments (T2) (5.53 and 0.6g).

Inoculation of AMF to sorghum in general led to significant ($p<0.05$) increase in shoot fresh and root dry weight for all the sampling sites. Greater shoot fresh and dry weights (14.73, 2.23g) were recorded for sample from Diredawa with melkam variety while non inoculated control treatments(T2) gave lowest shoot fresh and dry weights(4.46, 0.46g) for local varities from Jigjiga for fresh weight and Harar for dry weight. The study also demonstrated that improved melkam variety performed better than local variety in terms of shoot fresh and dry weight with AMF for (T1, T3 and T4).Both root fresh and dry weights increased with the amount of water applied. Accordingly, 80% water field capacity recorded the highest root dry and fresh weights throughout the growth period.

AMF caused a significant ($p <0.05$) increase in the root length of sorghum. The highest root length of(T1) 39cm was recorded in Diredawa of melkam variety inoculated with AMF but the treatment without AMF recorded the lowest root length(T2) (28.6cm) from the same location with local variety. Likewise, 25%FC (T4) water regime gave higher root length when inoculated across all the sampling sites than the control treatment (T2) for both melkam and local sorghum varieties.

Results of this study revealed that sorghum varieties were significantly varied (<0.05) in terms of the root length. Local sorghum variety recorded the lowest root length of 27.33cm from sample of Diredawa but the highest length recorded for melkam variety from Diredawa (Table 4.4). Water regimes significantly ($p<0.05$) influenced roots growth of sorghum plant with the highest root length from the sample of Diredawa and this happened in the plants under high moisture level (80%).

Application AMF showed taller height than the non-AMF plant for both sorghum varieties. It was observed that the seedlings inoculated with AMF at 80%FC (T1) showed significantly greater response to height of plant over the uninoculated control (-80 %FC) and other treatments. And also at 25%FC inoculated treatments recorded higher result for all locations than the control treatments at 80%FC without AMF inoculation for both sorghum varieties. The results revealed that sorghum varieties were significantly ($p <0.05$) different from each other in terms of plant height (Table 4.4). Melkam sorghum variety recorded the highest plant height of 93.33cm from Diredawa when inoculated with AMF at 80% FC (T1). Local sorghum recorded the lowest plant height of 60 cm from Jigjiga (T4). Further, there were significant ($p<0.05$) interactions between sorghum varieties, water regimes, AMF inoculation on plant height of sorghum. Lowest plant heights of 60 cm were recorded at water regime (80%FC) with local sorghum variety without AMF.

There was no significant ($p<0.05$) difference in case of leaf number for all the AMF inoculation and water regimes compared to plants that were not inoculated. However, the highest number of leaves (T3) was recorded from Jigjiga with melkam variety but lowest number of leaves (for soil sample from Jigjiga with local variety. Melkam sorghum variety from Jigjiga under 50%FC+AMF recorded the highest number of leaves (6) compared to the lowest 25% water regime (4).

4.5. Determination of relative water content (RWC)

Results of study showed that AMF treatment significantly affected the relative water content of sorghum varieties under drought stress (Figure 4.2). AMF-inoculated plants had greater RWC values in drought conditions for all the sampling sites for both varieties. Maximum RWC was recorded (84.30%) from Diredawa for Melkam variety, while the lowest (39.47%) RWC resulted from non-inoculated (Control) treatment from Jigjiga sampling site for local variety.

At 25% soil field capacity (T4) also resulted in greater RWC for all sampling site with maximum (82.13 %) from Diredawa while none inoculated or control treatments (T2) recorded lowest result relatively. In addition, Melkam sorghum Variety showed greater RWC for sampling site of Diredawa while on average local varities showed greater RWC than Melkam Variety (Figure 4.2).

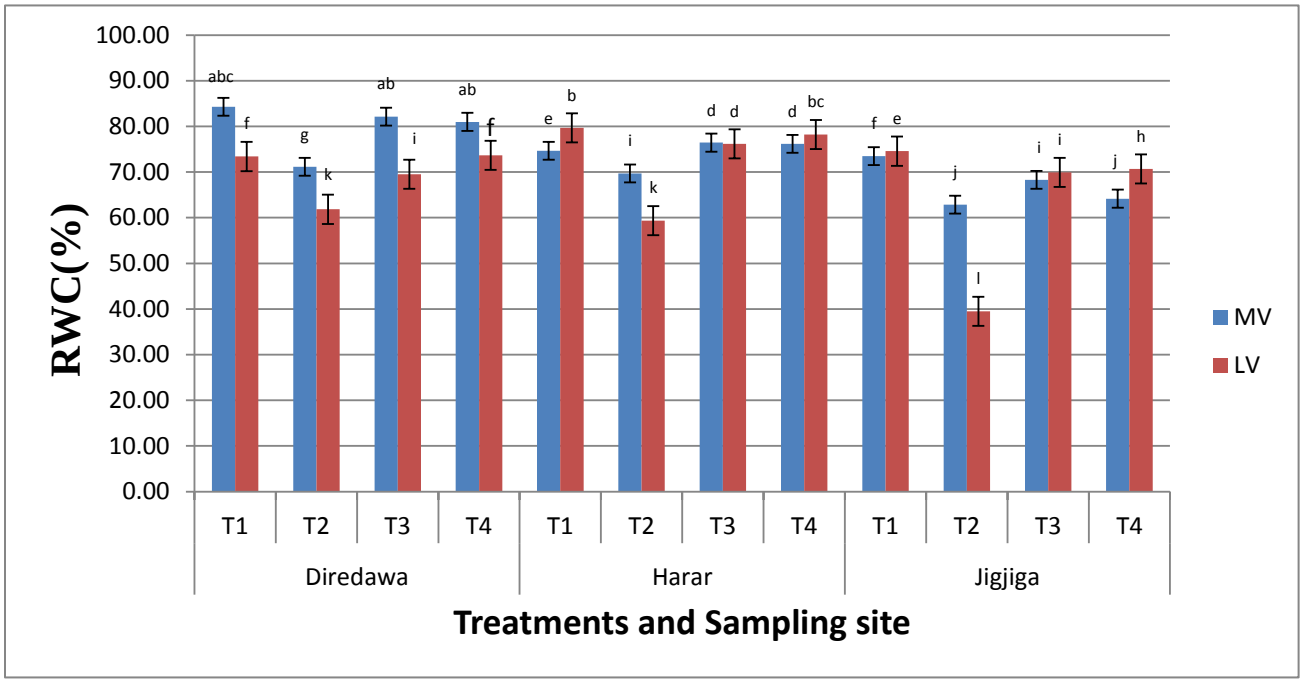

Figure 4.2.Mean relative water content (±SE) of Melkam and Local varieties under well-watered, water deficit, and different inoculation treatments. T1 (80%FC+AMF), T2 (Control), T3 (50%FC+AMF) and T4 (25%FC+AMF).MV (melkam Variety) and LV (Local Variety). Data are average of three biological replicates along with standard error bars

4.6. Leaf Proline Contents

The study showed that the two sorghum varieties differed significantly in proline content, particularly under AMF and drought treatment (Figure 4.3). Inoculation of AMF increased the proline content under well-watered conditions with significant difference ($p<0.05$). Accumulation of proline in higher plants is an indication of disturbed physiological condition, triggered by biotic or abiotic stress condition. Free proline content can increase upon exposure of plants to drought, salinity, cold, heavy metals, or certain pathogens. Determination of free proline levels is a useful assay to monitor physiological status and to assess stress tolerance of higher plants. Interestingly, the two sorghum

varieties (Melkam and Local) had different responses to drought stress, both with and without AMF treatment. Melkam sorghum variety with AMF inoculation produced the highest proline content (34.80±0.23mM/g fresh leaf weight) for sample from Diredawa at 25% FC of soil. Low accumulation of proline was recorded (6.40±0.1123mM/g leaf fresh weight) for soil sample from Harar with local variety at water regime of 80%FC without AMF inoculation.

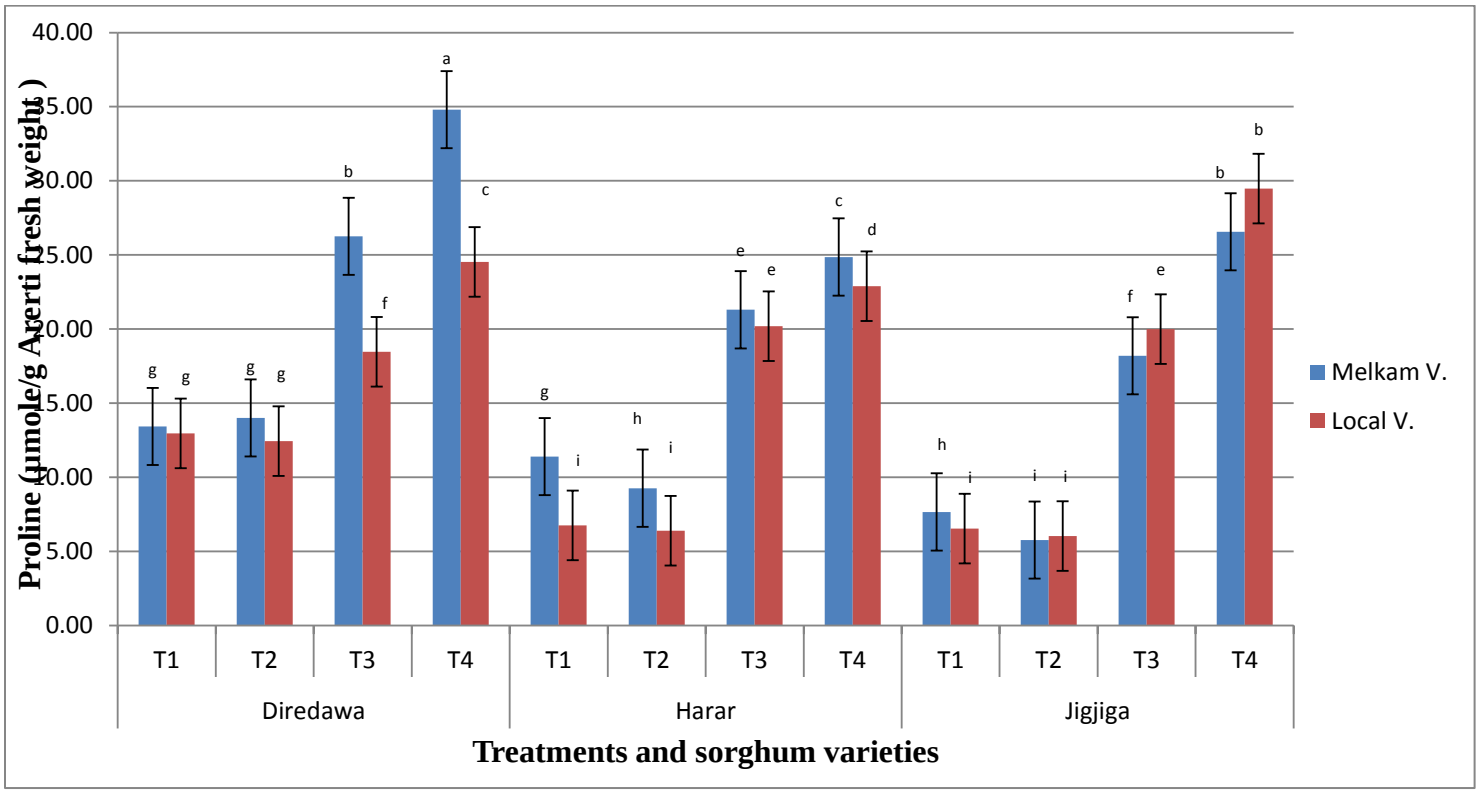

Figure 4.3. Mean proline content (µmole/g fresh weight) (±SEm) sorghum varieties under well-watered, water deficit, and different inoculation treatments. T1 (80%FC+AMF), T2 (Control), T3 (50%FC+AMF) and T4 (25%FC+AMF).MV (melkam Variety) and LV (Local Variety). Data are average of three biological replicates along with standard error bars.

4.7. Total chlorophyll

Both melkam and local sorghum varieties were significantly improved when inoculated with arbuscular mycorrhizal fungi ($p<0.05$) for the content of total chlorophyll (Figure4.4).The highest amount of the total chlorophyll of sorghum (39.51±0.24 mg g^{-1} FW) was obtained from inoculation with AMF with 80%FC conditions from Diredawa for melkam variety. The lowest amounts of total

chlorophyll (18.64±0.30 mg g^{-1} FW measured under no inoculation with AMF at 80%FC (T2) of soil from Harar. On the other hand, at 25%FC with AMF gave greater chlorophyll content (37.66, 30.16 mg/g fresh weight) while control treatments gave lowest total chlorophyll (T2) (18.68, 20.31 mg/g fresh weight), for melkam and local varieties respectively.

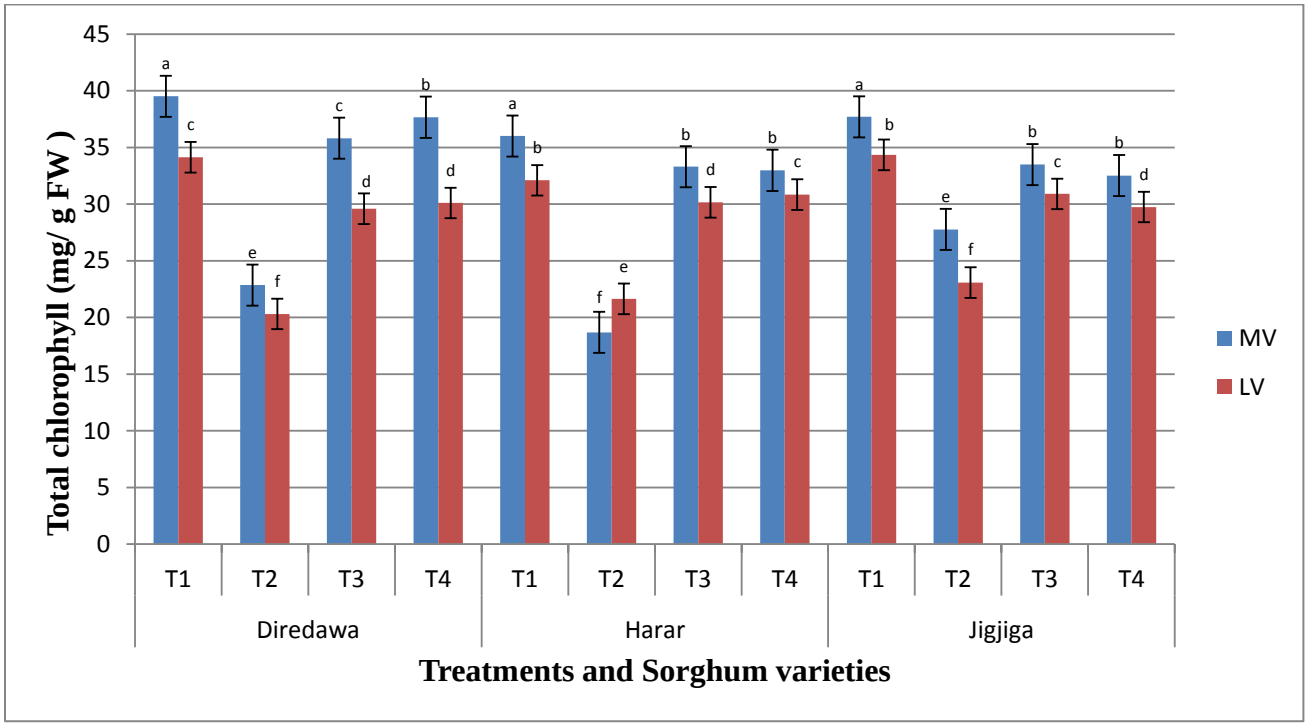

Figure 4.4: Mean total chlorophyll content (mg/g fresh weight) (±SEm) of sorghum varieties under well-watered, water deficit, AMF inoculation treatments and Non AMF Inoculation. T1 (80%FC+AMF), T2 (Control), T3 (50%FC+AMF) and T4 (25%FC+AMF).MV (melkam Variety) and LV (Local Variety). Data are average of three biological replicates along with standard error bars.

4.8. Total soluble sugar

AMF Inoculation had significant effect on Melkam and local ($p< 0.05$) on total soluble sugar under well-watered and water deficit conditions (Figure 4.5) Total soluble sugar in well-watered control (80%FC) condition for uninoculated plants was found low in comparison to AMF inoculated treatments. Maximum sugar content was observed in melkam sorghum variety (84.69±0.41mg/g fresh weight) from Jigjiga at soil field capacity of 80 %(T1) while minimum total soluble sugar was recorded from local variety (28.4mg/g fresh weight) from Harar at field capacity of 80% from non-inoculated treatments (T2). Figure 4.5).Moreover, application of AMF resulted in greater total soluble

sugar content (72.21,64.94mg/gFW) at 25%FC than 80%FC without AMF(control treatments) with values of 58.10,36.60mg/gFW) for Melkam and Local varieties, respectively(Figure 4.5)

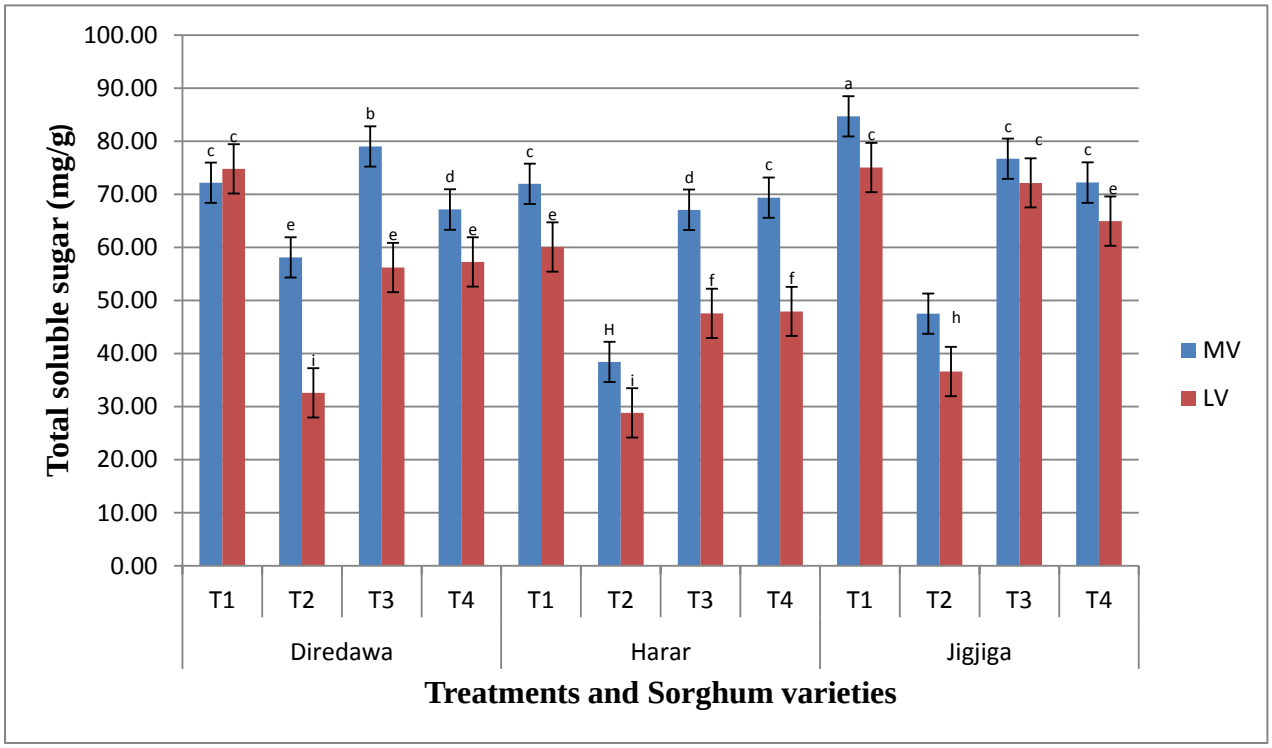

Figure 4.5. Mean total soluble sugar (mg/g fresh weight) (±SEm) of Sorghum varieties under well-watered, water deficit, and different inoculation treatments. T1 (80%FC+AMF), T2 (Control), T3 (50%FC+AMF) and T4 (25%FC+AMF).MV (melkam variety) and LV (Local variety). Data are average of three biological replicates along with standard error bars.

4.9. Root colonization

Melkam variety recorded the highest root colonization with intensity of 89.16% for sample from Diredawa at 25%FC+AMF, while the lowest intensity of 0% was recorded in both melkam and local varieties for all the sampling locations under (T2) control conditions (Table 4.6). Melkam varieties recorded highest result (89.16%, 82.84% and 84. 33%) for location of Diredawa, Harar and Jigjiga, respectively. Local variety resulted in the highest root colonization at 25%FC for Diredawa (83.06%), Harar (84.86%) and Jigjiga (86%) locations. Variation in the amount of applied water significantly ($p<0.05$) affected sorghum roots colonization intensity at 50% of water field capacity gave the highest root colonization intensity throughout the study period for all locations and also for both.

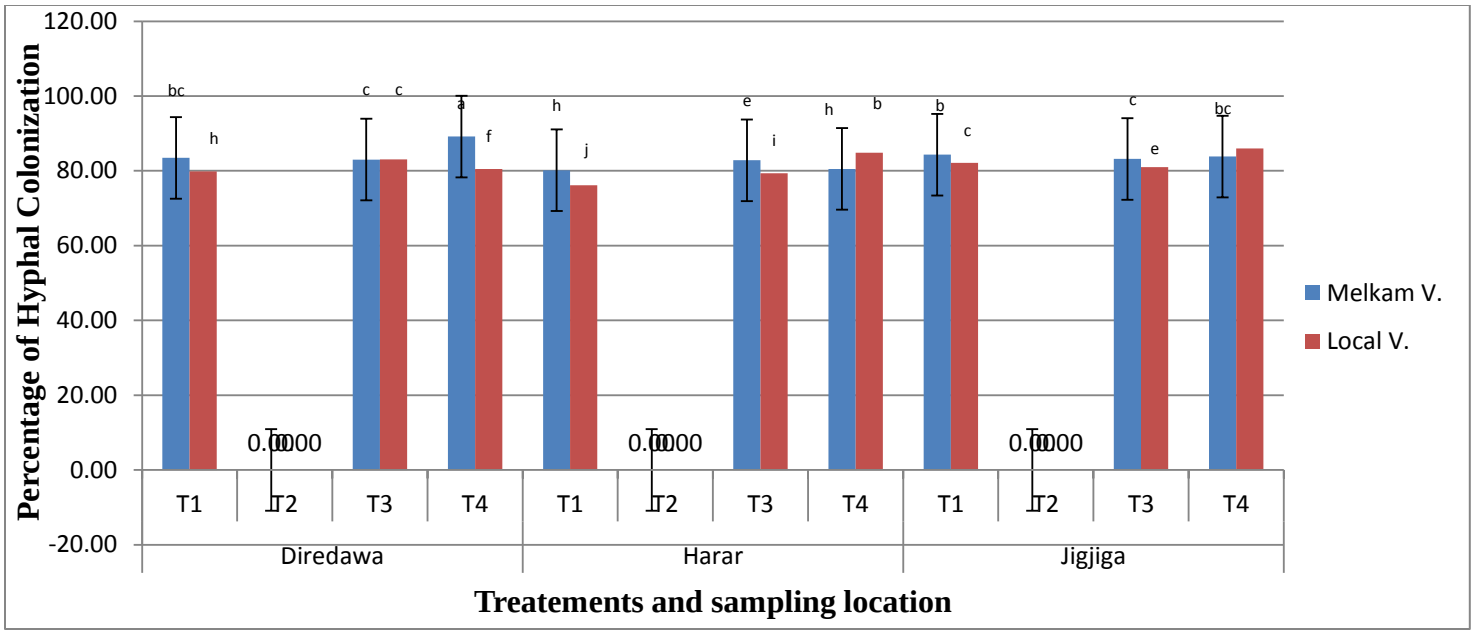

Figure 4.6 comparing the mean of hyphal root colonization of Sorghum varieties under well-watered, water deficit, and different inoculation treatments. T1 (80%FC+AMF), T2 (Control), T3 (50%FC+AMF) and T4 (25%FC+AMF).MV (Melkam Variety) and LV (Local Variety). Data are average of three biological replicates along with standard error.

CHAPTER FIVE

5. Discussion

In the present study, AMF inoculants from rhizosphere soil of Sorghum plots were collected from Diredawa, Harar and Jigjiga. Spore density was checked prior to the preparation of trap culture. Application of AMF inoculants which were isolated from rhizosphere soil of Diredawa showed better influence on the growth of sorghum. The results showed that the highest average spore density of AMF was recorded in the rhizosphere soil of Diredawa (24.24 spore's g^{-1} soil) followed by 22.48 spore's g^{-1}soil of Jigjiga. Similarly, in this study after trap culture maize considerably had the greatest average AMF spore density and root colonization from all sampling sites. In general, trap culturing enhanced spore abundance but decreased AMF species richness which in line to Belay *et al*. (2015).The results of our research show that AMF spore density decreased with higher levels of drought stress. The result of this experiment revealed that the level of sorghum root colonization in inoculated sorghum plants with AMF was significantly higher than those of non-inoculated plants.

For all sampling sites, trap cultures allowed for the identification of 24 different species from 15 different genera. All study sites consistently yielded samples from the genera Glomus, *Funneliformis, Septoglomus, Claroideoglomus, Gigaspora, Acaulospora, Paraglomus, Diversispora, Pacispora,* and *Rhizophagus. Diversispora tortuosa, Rhizophagus Claroideoglomus etunicatum, Funneliformis mosseae, Funneliformi geosporum, and Claroideoglomus claroideum* are frequently found in the soil of the Diredawa and Jigjiga sites. Belay *et al.,* (2020) determined that *Claroideoglomus* and *Funneliformis* were dominating genera because they were present in all types of land use. The genera *Glomus, Paraglomus, Rhizophagus, Acaulospora* and *Gigaspora* were categorized as common, while *Claroideoglomus etunicatum, G.gigantea* and *Glomus* sp dominated the soil of Harar. Hontoria *et al*. (2019) have specified that genus *Glomus* is generally reported from cultivated lands. This is attributed to their better adaptation to tilled soils and also their high production of spores and mutual benefits by means of plant roots that allow faster reestablishment through myclia. This Genus is also known to have greater survival under extremely oligotrophic conditions (de Araujo *et al*., 2018).The high incidence of *Glomus* and *Funneliformis* spp. has been also associated with their capacity to produce more spores in a shorter time than genera such as *Gigaspora* and *Scutellospora* (Bever *et al*., 1996:

Oehl *et al*., 2009). On the basis of IV (importance values), the genera Gigaspora, *Funneliformis, Rhizophagus* and *Claroideoglomus* were categorized into the dominant genera (Chen *et al*., 2012).

The results showed that all the inoculation treatments significantly increased all the growth parameters compared to non-inoculated for all the sampling locations with Melkam sorghum variety that showed the highest growth parameters compared to the local variety for all water regime. The result of our study are also In line with Chen *et al*. (2020).There is a positive correlation between AMF colonization and total fresh biomass, the number of leaves, root and shoot height, fresh shoot and root mass, dry shoot and dry root mass, as well as the number of leaves and the height of the roots and shoots. This showed that this growth advantage was probably caused by the vast extracellular hyphae AMF network, which supplies water and nutrients to roots and increases their range of absorption. Arbuscular mycorrhizal fungi boost plant height (shoot length) and biomass in sorghum plants, according to research by (Kchikich *et al*., 2021). This is because they help plants absorb nutrients more effectively.

There were significant differences ($p < 0.05$) in the fresh and dry weight of root among sorghum varieties, soil moisture regimes and AMF inoculation. Melkam variety had superior root fresh and dry weight from Harar and Diredawa sampling sites with inoculated treatment and 80% FC (Table 4.2). The study demonstrated that improved variety (Melkam) performed better than local variety in terms of root dry and fresh weight. AMF treatment can increase root biomass higher than stem biomass with 24.2% in stems and 29.6% in roots. Wu *et al*. (2022) have reported that the increase in biomass by AMF treatment depended on the organ type and functional group of the host plant. The current findings are consistent with Moreira *et al*. (2018) who reported higher root dry and fresh weight in inoculated plants than those not inoculated. This could be attributed to enhanced root hydraulic conductance and improved water absorption and nutrient by plants through extended extraradical mycelia (Hajiboland *et al*., 2018).AMF caused a significant ($p < 0.05$) increase in the root length of sorghum, however, the treatments without AMF recorded the lowest root length for both varieties. Likewise, Janos (2007) has reported that mycorrhizal fungi effectiveness is represented by the difference in growth between plants with and without mycorrhiza as a property of the interaction between plant and fungal species. This indicated that sorghum plant responded positively to AMF resulting in enhanced root growth under stressed conditions.

There were significant ($p < 0.05$) differences in plant shoot fresh and dry weight between the two sorghum varieties (Table 4.2). Melkam variety recorded the highest shoot dry weight of 14.73 g when grown in soil from Diredawa with 80% FC and AMF inoculation. Infected plants had a higher shoot fresh and dry weight than uninfected plants, which may be due to better hydraulic conductance dynamics (Chelangat *et al*., 2021a). On the other hand, the enhanced phosphorus and other nutrient intake are frequently associated with the increased growth impacts of the AMF-inoculated plants (Abdel-Salam *et al*., 2018). A primary method for increasing drought tolerance in crops inoculated with AM fungus, according to Giri *et al*. (2007), who have provided evidence to back the aforementioned claim. Similar to this, Moradtalab *et al*. (2019) found that AMF inoculation significantly boosted biomass output by the plant due to better water quality and an increase in photosynthesis.

Results of this study revealed that sorghum varieties were significantly varied ($p < 0.05$) in terms of the root length. Melkam variety recorded the highest root length of 39 cm at 80% FC with inoculation of AMF for soil of Dire Dawa. AMF caused a significant increase in the root length of both sorghum varities, such that the treatments without AMF recorded the lowest root length. AMF extra radical hyphae enhance nutrient uptake therefore improving plant growth (Lehmann and Rillig, 2015).The shoots and roots of the seedlings treated with AMF increased in height and mass but also showed significantly higher colonization by AMF and spore density than control. This indicated that trees inoculated by AMF improved in all aspects of the growth parameters directly by the support of the AMF.

This result is in agreement with a report by Kumar *et al*. (2014), who stated that AMF (*Glomus fasciculatum)* inoculum improved directly plant height, plant spread, and number of branches; reduced disease incidence; and increased tuber dry mass of Coleus forskohlii. To Lee *et al*. 2015 AMF Inoculation increase length of shoot and root which similar to our result. This result is similar with the previous report by Lee et al. (2015) that mycorrhizal inoculation increased maize shoot and root length compared to non-mycorrhizal plants. This result is similar with the previous report by Lee et al. (2015) that mycorrhizal inoculation increased maize shoot and root length compared to non-mycorrhizal plant Arbuscular mycorrhiza inoculation gave the highest plant height in all sampling location, and the data was significant at $p < 0.05$ as shown in Table 4.3.

Melkam variety recorded the highest plant height of 93.33 cm for sample of Diredawa whereas local seeds variety recorded the lowest plant height 61.33cm from Harar. Water application influenced plant height significantly ($p < 0.005$) in both growing seeds (Table 4.2). Maintaining soil moisture at 80% water field capacity with inoculation of AMF was superior the plant height for all location .Sorghum plants supplied with AMF inoculum exhibited increased growth in plant height than the plants which had no AMF inoculum. These results correlated with those of Xie *et al*., (2018) who observed that mycorrhiza inoculated plants have more growth and better physiological status (stomatal conductance, water use efficiency and photosynthetic rate) compared to crops without inoculum. In addition, where soil moisture is reduced, plants with AM fungi inoculum show greater plant height than non-inoculated. This could be because of the adjustment of abscisic acid levels and regulation of stress responsive genes in the plant caused by AMF (Moreira *et al*., 2018). Also, AMF is also a natural symbiont that can provide essential nutrients to the host plants, thereby increasing growing of the plant under well water and stressed soils (Begum *et al*., 2019).

Melkam variety had the highest leaf number and plants fewer than 50% water regimes recorded the highest number of leaves compared to the lowest 25% water regime (Table 4.3). AMF inoculated sorghum plants exhibited a significantly higher number of leaves compared to plants that were not inoculated. Sorghum plant inoculation with AMF was found to increase the leaves number of the host plant. The results are corroborated with those of Wang *et al*. (2019) who verified that AM inoculated sorghum plants of both cultivars had improved plant biomass. These increments were attributed to the beneficial effect of AMF in enhancing water transport through regulation of aquaporins genes expression, therefore, helping in the transport of water as well as solute thereby improving water and nutrient status of plants (Zuccarini and Savé, 2016).Other research by Moradtalab *et al*. (2019) show that infection by AMF considerably increased leaf area, chlorophyll content, photosynthesis rate and photochemical efficiency of plants under drought

Relative water content (RWC) is reliable parameter for quantifying plant drought stress response and as an indicator of plant water status (Yan *et al*., 2016). Relative water content in uninoculated AMF and inoculated for both sorghum varieties demonstrated that there was significant alteration in relative water content ($p<0.05$).The application of AMF enhanced RWC especially under severe stress with 50 and 25%FC of the soil the highest relative water content of sorghum leaves for melkam variety.. In our study, the results show that the sorghum plants inoculated with AMF had a higher

RWC in all irrigation treatments in comparison with those that were not inoculated. The relative water content of a leaf indicates the amount of water in the plant and the ability of the plant to survive under conditions of drought stress. These results are in agreement with those found by Nxele *et al*. (2017). In our study, the improvement in the water relationship of mycorrhizal plants is probably attributed to the higher water uptake due to changes in the root morphology stemming from the expansion of the surface absorbing area of the roots by mycorrhiza hyphae (Cheng *et al.*, 2004; Darbani *et al*., 2017). Barros *et al*. (2018) Assert that AMF connections enhance plant water status at the level of the entire plant as well as demonstrated by high leaf relative water content, which is consistent with our experimental findings. AMF also enhances water and nutrient intake, which enhances drought tolerance by influencing several physiological and biochemical processes (Diagne *et al*., 2020). As resut Inoculation of AMF increase leaf relative water content.

The significant increase in proline content observed in Melkam and local variety ($p<0.05$) under water deficit condition compared to the control condition in inoculated and uninoculated conditions (Figure 4.3). There was significant interaction between stress levels and variety in proline content. Accumulation of proline has been established as a parameter of choice for stress tolerance (Jaleel *et al*. 2007) and it is supposed to play an adaptive role in plant stress tolerance (Hayat *et al*., 2012). In the present study, it was observed that the proline content was higher for the severe and moderate drought stress treatments than non-stress treatment.

The high content of proline can be used as an indicator that the plant was under stress. When drought occurs, the water content in the cells will decrease, so it will trigger cell damage. Plants will try to minimize the impact of damage by several mechanisms, one of which is the accumulation of proline. Proline plays as an osmolyte in the cell that can maintain the osmotic balance in the cell under stress conditions, particularly drought (Havrlentová *et al*. 2021). The accumulation of proline in plants inoculated with AMF during drought stress conditions was the mechanism by which AMF improved the host plant's resistance under stress conditions. (Ruiz-Lozano, 2003) have demonstrated similar findings, demonstrating that water stress caused proline buildup in various plant leaves As an osmotic regulator, proline is a substance that helps stressed plants tolerate water stress by preserving turgor through osmotic adjustment.

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Both Melkam and local seeds varieties chlorophyll contents of leaf (a, b and total

chlorophyll) significantly improved due to inoculation of AMF compared to the uninoculated controls. The results showed that increasing drought stress levels significantly decreased the chlorophyll content of the sorghum plants, while all the treatments of inoculation with the bio-fertilizers (AMF) improved the chlorophyll content. The highest amounts of total chlorophyll (39.51 mg g^{-1} FW respectively) were obtained from the inoculation with AMF under irrigation of 80%FC (non-stress) conditions.

The contents of total chlorophyll (chl.a + chl.b) and carotenoid under water deficit condition were reduced. However, the parameters were more enhanced in inoculated plants than control plants. The enhanced chlorophyll content may increase the photosynthetic efficiency of inoculated sorghum plants and thus maybe important for tolerance to drought stress and the reduction in the content of chlorophyll (a, b, and total) in sorghum leaves under irrigation 25% FC and 50% FC (severe and moderate drought stress) compared to irrigation 80% treatment (non-stress) is probably due to the oxidative damage to the chloroplast lipids (membrane lipids), pigments and proteins. Increasing the chlorophyll content (this study) under drought stress through the inoculation with bio-fertilizers was probably due to the fact that AMF play an important role in chlorophyll formation by increasing the pyridoxal enzyme activity. This enzyme plays an important role in the synthesis of α-aminolevulinic acid as a major compound in chlorophyll (Kahil *et al*., 2017). Consistent with these results, Rahimi *et al*. (2019) also reported that the highest content of chlorophyll in cephalaria was obtained through the interaction of AMF and *Azotobacter*.

Inoculation of AMF significantly improved the total soluble sugar content of both sorghum varieties. Maximum sugar content was observed in Melkam variety at 80%FC for Jigjiga location (84.69mg/g fresh weight) and minimum was observed in local seeds (28.84 mg/g fresh weight) at 80% FC for Harar sample with no inoculation of AMF. Also at 25% FC for all sampling sites showed higher TSS than non inoculated treatments. Study results in agree to (Parida and Das, 2005) Carbohydrates, such as sugars (glucose, fructose, sucrose, fructans) and starch, play important roles in osmoprotection, osmotic adjustment, and radical scavenging, in addition to carbon storage .Plants can accumulate soluble sugars to adjust the osmotic potential and to maintain higher turgor pressure, which constitutes an important plant protection mechanism against osmotic stress (such as salinity and drought) (Evelin *et al*., 2009).

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The study revealed application of AMF significantly affects the growth and development of both Melkam and local sorghum varieties by affecting fresh weight and dry weight of leaves, root, shoot and RWC. Under drought conditions, AMF inoculation caused a significant difference in response to proline accumulation, total sugar and total chlorophyll contents in both sorghum varieties. The experimental soil (trap culture) was dominated by genera *Gigaspora, Claroideoglomus, Funneliformis* and *Paraglomus.* Furthermore, the study concluded that the soil native AMF diversity was very high which helps the plant to make association with it. AMF inoculation improved growth of sorghum in all treatments for both varieties but Melkam variety showed better result than local in all the treatments, while none AMF treatments (T2) shows less growth relatively than AMF inoculated. Overall, soil samples from Diredawa site showed better result than Harar and Jigjiga.

6.2. Recommendations

During this study, the capacity for survival and growth of plant seedlings in the field, as well as combinations of AMF and other Biofertilizers (PGPB), were not explored. As a result, it will be critical to compare their efficacy under different climatic conditions and against additional field experiments, including plant growth promoting bacteria combinations in the future. Furthermore, delivery of the inoculums resulted in increases in all growth parameters; however, no investigation of the effect of these inoculums on nutrient uptake was carried out during this experiment. As a result, the inoculums impact on these parameters should be determined. The current findings must be supported by addition field research

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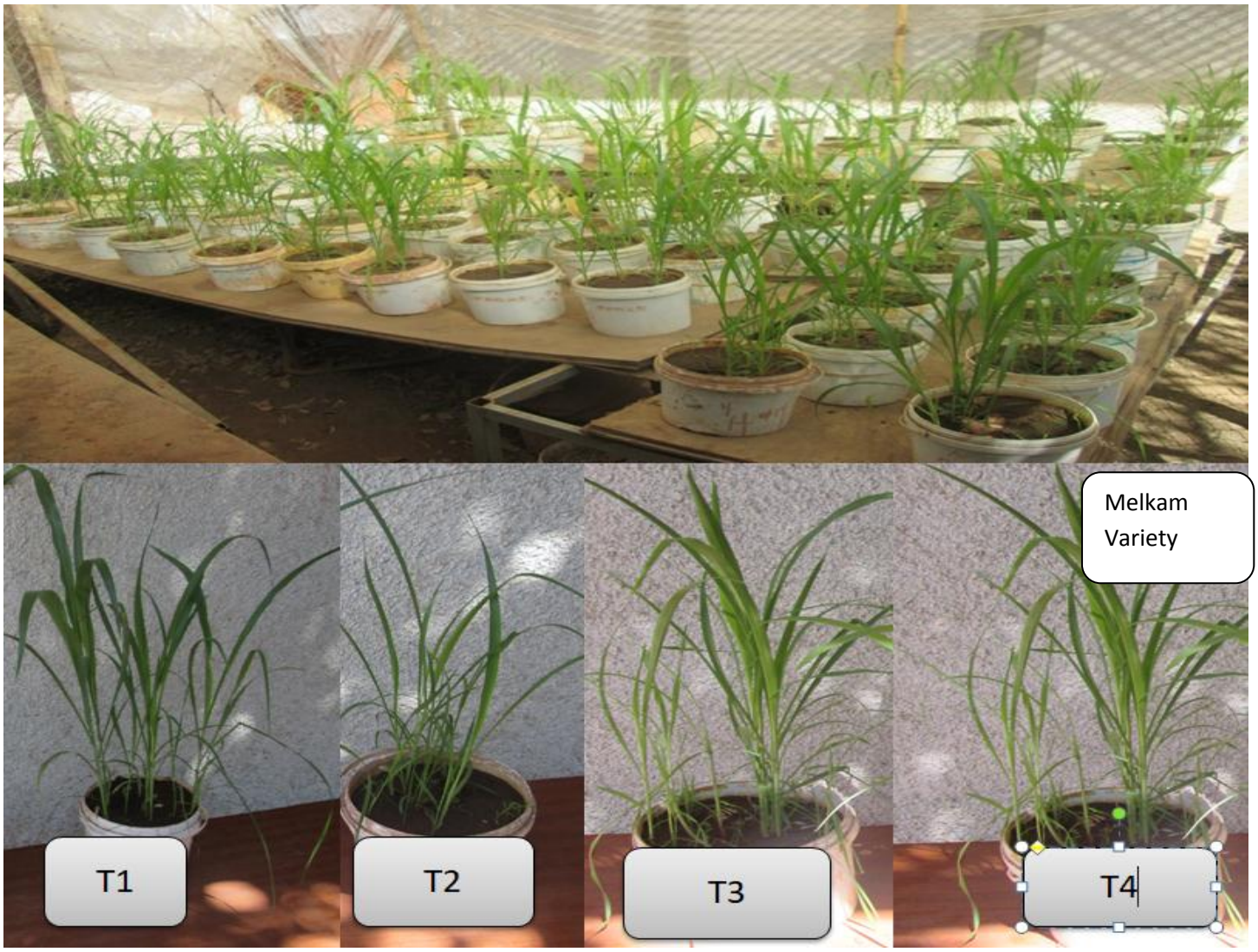
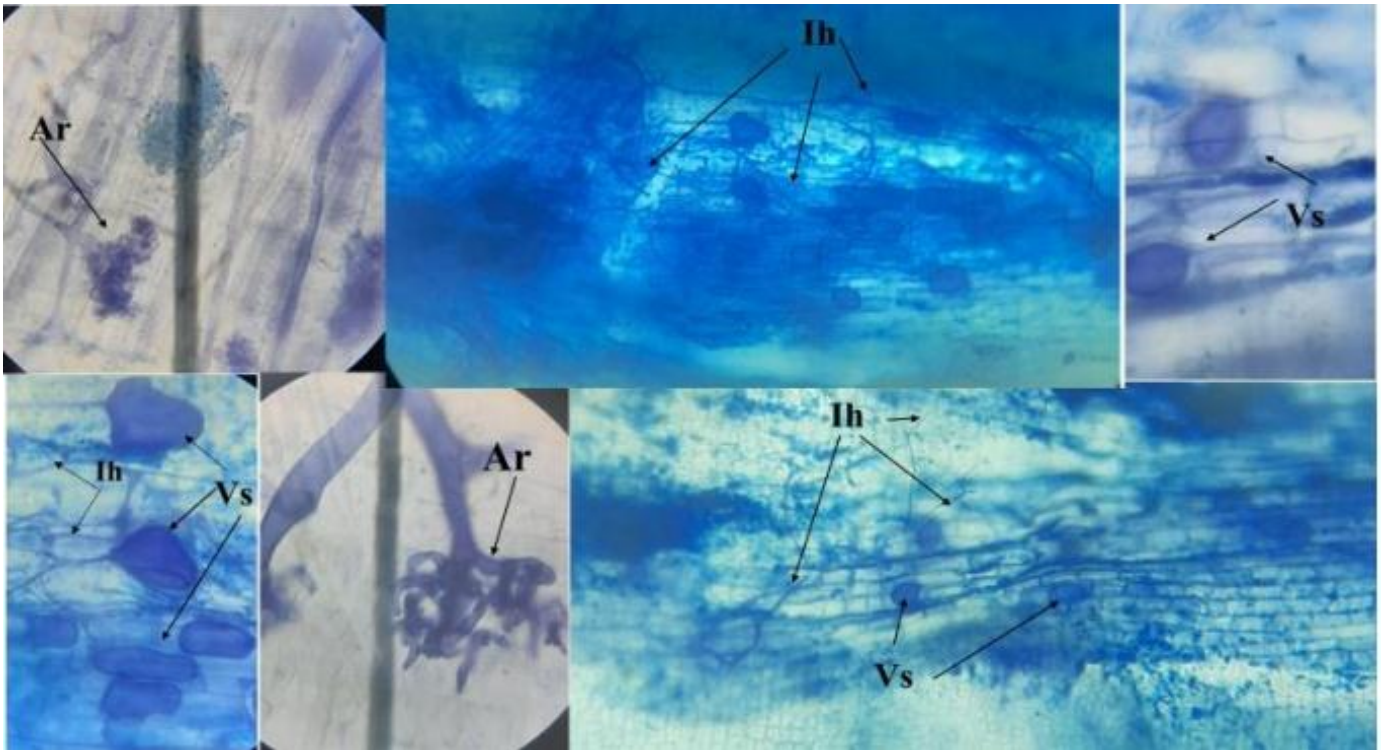
8. APPENDIXCES

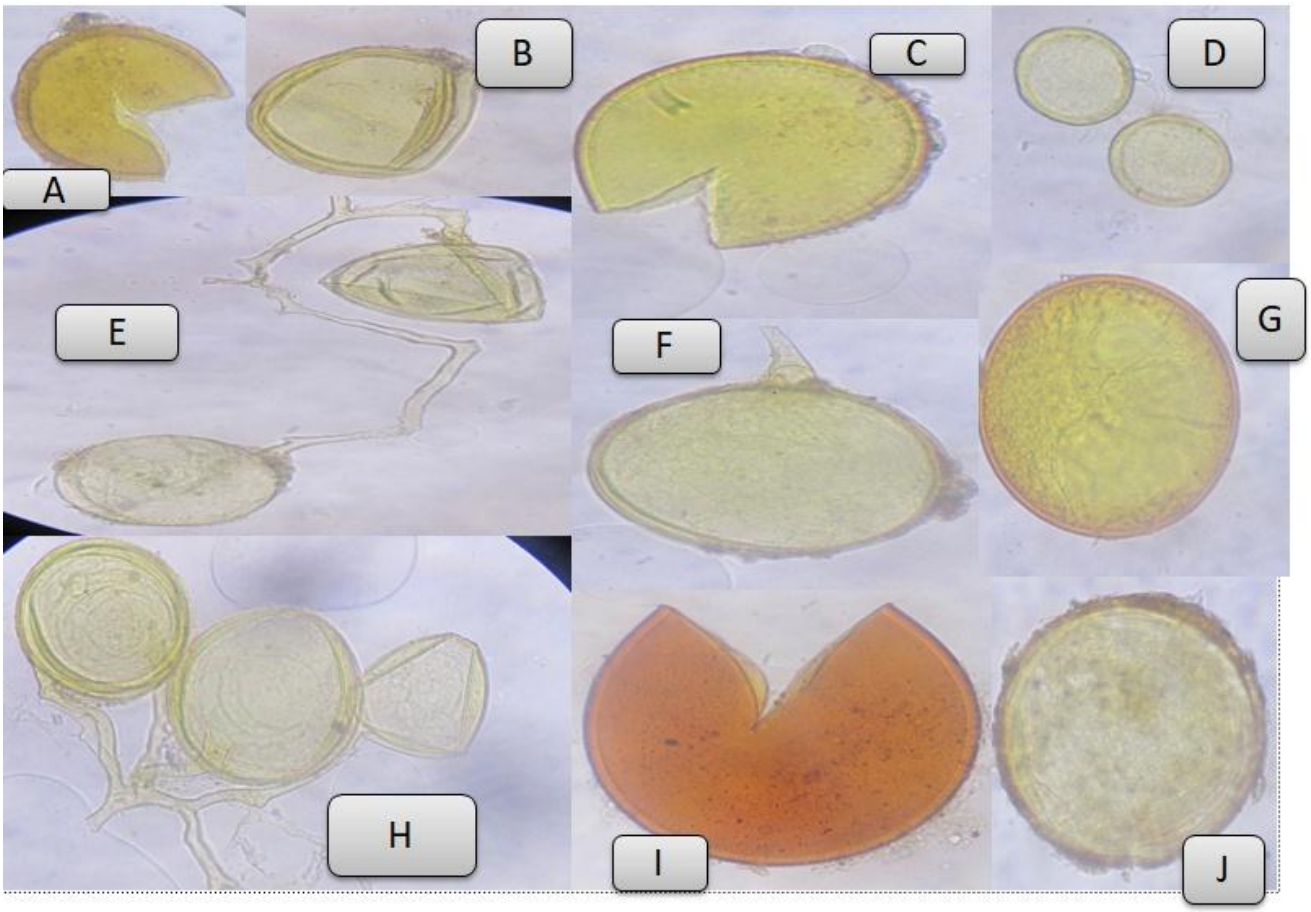
Appendix 1. Typical images demonstrating the growth response of two sorghum varieties to AMF and non-AMF (negative control) inoculums at various water regimes.



Appendix 2. Microscopic picture of root colonization. Intraradical hyphae (Ih), Arbuscule (Ar), and vesicles(Vs)



Appendix 3. Microscopic images of a few morphotypes were taken and used to identify spores from the AMF inoculum of maize plants grown in trap cultures at all sampling sites. The images were all made with PVLG slides**.**



A and C(*Claroideoglomus claroideum*) B(*Funneliformis geosporum*) E and H(*Rhizophagus aggregates*) F(*Funneliformis coronatum*) G(*Gigaspora margarita* I(*Funneliformis geosporum*) J(*Pacispora Scintillans*).

Appendix 4. Representative pictures to give further illustration of practical works beginning from trap culturing to the end of laboratory session**.**

A and B (Trap culturing of Maize and wheat), C (Pot setup before planting the sorghum), D (Growing of the sorghums), E,(Spore density and diversity identification) F(Enumeration and extraction of AMF spore under stereomicroscope), G(Root staining) andH(Estimation percent AM root colonization under a compound microscope).

Appendices 5: One-way ANOVA Result analysis output of the plant growth parameters in both sorghum varieties from SPSS software.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Root fresh weight	Between Groups	137.924	23	5.997	12.008	.000
	Within Groups	23.971	48	.499		
	Total	161.895	71			
Root dry weight	Between Groups	1463.739	23	63.641	.952	.537
	Within Groups	3207.220	48	66.817		
	Total	4670.959	71			
Root length	Between Groups	792.893	23	34.474	9.042	.000
	Within Groups	183.000	48	3.813		
	Total	975.893	71			
Shoot fresh weight	Between Groups	491.448	23	21.367	8.909	.000
	Within Groups	115.127	48	2.398		
	Total	606.575	71			
shoot dry weight	Between Groups	35.322	23	1.536	1.867	.034
	Within Groups	39.493	48	.823		
	Total	74.815	71			
shoot Length	Between Groups	5710.797	23	248.296	241.199	.000
	Within Groups	49.412	48	1.029		
	Total	5760.209	71			
Relative water content	Between Groups	5601.614	23	243.548	183.148	.000
	Within Groups	63.830	48	1.330		
	Total	5665.444	71			
Leaf number	Between Groups	19.208	23	.835	1.227	.269
	Within Groups	32.667	48	.681		
	Total	51.875	71			

Appendices 6: One-way ANOVA Result analysis output of Leaf relative water content, leaf proline content, total chlorophyll and root colonization in both sorghum varieties from SPSS software.

ANOVA

Relative water content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5978.059	23	259.916	192.125	.000
Within Groups	64.936	48	1.353		
Total	6042.995	71			

ANOVA

Leaf Proline content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4956.025	23	215.479	212.152	.000
Within Groups	48.753	48	1.016		
Total	5004.778	71			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Diredawa	Between Groups	997.590	7	142.513	351.013	.000
	Within Groups	6.496	16	.406		
	Total	1004.086	23			
Harar	Between Groups	770.546	7	110.078	284.706	.000
	Within Groups	6.186	16	.387		
	Total	776.732	23			
Jigjiga	Between Groups	417.389	7	59.627	125.115	.000
	Within Groups	7.625	16	.477		
	Total	425.014	23			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Hayphae	Between Groups	77570.669	23	3372.638	4720.093	.000
	Within Groups	34.297	48	.715		
	Total	77604.967	71			
Vescle	Between Groups	2359.775	23	102.599	103.623	.000
	Within Groups	47.525	48	.990		
	Total	2407.300	71			
Arbuscule	Between Groups	967.829	23	42.080	66.503	.000
	Within Groups	30.372	48	.633		
	Total	998.200	71			

Post Hoc Tests