IMPROVING SMALLHOLDER FARMERS' RESILIENCE TO CLIMATE CHANGE THROUGH ON-FARM MYCORRHIZAL PRODUCTION

Report to the

Water Research Commission

by

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EXECUTIVE SUMMARY

Background And Motivation

The Water Research Commission’s water utilization in agriculture programme supported this project on improving smallholder farmers' resilience to climate change through on-farm mycorrhizal production. Climate change has been identified by the government, scientists, NGO’s and industry as one of the major challenges that South Africa will face in the twenty-first century. Drought is one of the most important factors limiting crop productivity today, resulting in significant yield losses in many agricultural crop production areas. Plant tolerance to abiotic stresses caused by climate change can be increased by mycorrhizal fungi, reducing the risk of plant extinction and allowing time for plant dispersal and adaptation. Mycorrhizae is a broad category of mutualistic relationships formed between plant roots and fungi. Although most plants can grow without these fungi, plants colonised with mycorrhizae grow much better than those that do not. These symbionts are critical in environments with limited water and nutrients because mycorrhizae can significantly increase water and nutrient uptake compared to plants that do not have these associations. Commercial mycorrhizae formulations are available, but they are generally too expensive for smallholder farmers to use.

Project Aims

The project aimed to create an on-farm mycorrhizal production system for smallholder farmers by using locally adapted mycorrhizae to create mycorrhizal inoculum. When these farmers inoculate their crops with mycorrhizae, they can achieve higher yields with lower inputs. Inoculating crops with mycorrhizae can reduce crop water and fertiliser needs, making farming more sustainable. This will help to conserve South Africa's limited water resources while lowering fertiliser inputs, thereby protecting the environment. This will help to support the National Water Resources Management Strategy, which recommends that water resources be used more effectively in all sectors, especially agriculture, which is South Africa's largest water user. The second aim was to characterise the on-farm produced AMF inoculum to determine the most efficient method of production and determine species richness. The produced AMF were then inoculated onto two crops and the growth and yield were evaluated under different watering regimes. The final aim of the project was to implement the on-farm production at a smallholder farm.

Importance of Arbuscular Mycorrhizal Fungi

Water is vital for life, supporting ecosystems, agriculture, and industries. Its availability depends on natural resources and management strategies (UNESCO and UN-Water, 2020). However, water scarcity, caused by droughts and flooding, is likely to be intensified by climate change, which will impact agriculture and national economies (IPCC, 2021; Vörösmarty et al., 2010). For example, South Africa faces severe water shortages due to low rainfall, affecting subsistence farming and contributing to food security (Chaves et al., 2016).

Indigenous drought-tolerant crops are promising for improving water use efficiency, particularly for resource-poor households. These crops require minimal inputs, adapt well to drier conditions, and offer better yields in water-scarce regions (Nyathi et al., 2016). Additionally, understanding cultivation strategies can improve resilience to climate stressors.

AMF play a critical role in enhancing plant drought tolerance by improving water and nutrient uptake. AMF symbiosis helps plants withstand stress by boosting water efficiency, photosynthesis, and nutrient exchange, particularly phosphorus (Augé, 2004; Brachmann and Parniske, 2006; Tang et al. 2022). This relationship relies on carbon exchange between the plant roots and fungi, optimizing plant energy and nutrient balance (Smith and Smith, 2012). AMF, which form symbiotic associations with approximately 80% of plant families, improve nutrient and water uptake, soil aggregation, microbial biomass, and plant resilience against pathogens, nutrient depletion, and heavy metal contamination (Smith and Read, 2010; Gianinazzi et al., 2010).

In Sub-Saharan Africa (SSA), low soil fertility necessitates inorganic fertilisers to boost crop yields. However, their use is linked to high production costs, environmental issues such as nitrate leaching and phosphate runoff, and long-term soil degradation that increases dependency on chemical inputs (Mukhongo et al., 2016).

AMF offer a sustainable alternative to inorganic fertilisers by enhancing soil fertility and crop yields. AMF improves nutrient uptake, plant health, and productivity, benefiting smallholder farmers who struggle to afford fertilisers. Despite these advantages, AMF adoption remains limited in SSA due to low awareness and the lack of local production units, with most inoculants imported from Kenya and South Africa, driving up costs (Ijdo et al., 2011; Mukhongo et al., 2016).

By fostering AMF interactions and drought-tolerant crops, agriculture can adapt to climate challenges, improving food security and livelihoods in water-scarce regions. The increased uptake of nutrients by AMF and the consequent better growth, health and yield of plants are especially important for smallholder farmers who cannot afford fertilisers.

Water Use Efficiency (WUE) and Arbuscular Mycorrhizal Fungi (AMF)

Plant responses to drought vary depending on species, genotype, development stage, and drought severity (Bray, 1997). High-WUE plants produce more biomass per water unit lost (Heschel et al., 2002). However, data on WUE in indigenous crops like sweet potato and African ginger with AMF symbiosis remains limited (Anyia and Herzog, 2004; Neluheni et al., 2007). Indigenous crops exhibit substantial differences in WUE and stomatal conductance under low soil water conditions (Munjonji et al., 2018).

AMF enhances WUE by increasing stomatal conductance, aquaporin gene expression, and water absorption under drought conditions (Porcel et al., 2005; Ruíz-Sánchez et al., 2011). AMF-mediated processes, including hormone regulation and glomalin secretion, improve soil structure and water uptake efficiency (Gong et al., 2013). Mycorrhizal plants, such as grapevines and sweet potatoes, show improved photosynthesis, proline accumulation, and growth under drought stress (Valentine et al., 2006; Yooyongwech et al., 2016).

During drought stress, AMF colonisation enhances plant growth, nutrient accumulation, and physiological processes, such as photosynthesis and root architecture (Kapoor et al., 2013; Pavithra and Yapa, 2018). It activates drought-responsive genes, metabolic pathways, and hormonal signals, including abscisic acid (ABA), which modulates plant water regulation and stomatal behaviour (Doubková et al., 2013; Mohanta et al., 2017). AMF symbiosis is thus critical in boosting WUE and enhancing crop resilience to drought conditions.

Conclusions

The physiological and ecosystem effects caused by AMF range from an increase in the nutrient uptake efficiency of plants and improvements in plant stress resistance to improvements in soil structure and soil microbiology (Fester and Sawers, 2011; Gianinazzi et al., 2010). Furthermore, the close association of AMF with various other soil microorganisms suggests that many of the ecosystem effects apparently caused by AMF derive from complex interactions with other microorganisms within the mycorrhizosphere established by these fungi (Bonfante and Anca, 2009; Singh et al., 2008).

The global demand for AMF in the mycorrhiza-based industrial market was $268.8 million in 2019 and was projected to rise to $621.6 million by 2025 at an estimated compound annual growth rate of 14.8% (ReportLinker, 2020). However, commercial formulations of mycorrhizae are too expensive for many smallholder farmers and an alternative is needed to make this transformative biofertiliser accessible to these farmers. Studies have shown that significant increases in mycorrhizal colonisation resulted in yield increased yields averaging 23% (Lekberg and Koide, 2005). The ability of these farmers to produce mycorrhizal inoculum themselves can therefore lead to significant increases in yields which in turn will mean greater food security.

On-farm production of AMF

Introduction

Promoting local AMF production and educating farmers about bio-inoculants can reduce costs and enhance adoption. Smallholder farmers, who often lack access to fertilisers and advanced tools, are well-suited for AMF-based practices, making this an attractive, sustainable approach to improving agricultural productivity in SSA.

AMF symbiosis involves complex stages, including spore germination, root recognition, and arbuscule formation. Signals like plant-released strigolactones and fungal "myc factors" initiate and maintain the partnership, enhancing nutrient transfer (Giovannetti et al., 1994; Kosuta et al., 2003). These fungi, especially Glomeromycota species, are vital in arid regions for crop resilience and productivity (Bonfante and Anca, 2009).

Materials and methods

A trap pot system (within and outside of a greenhouse) was used to multiply mycorrhizal inoculum collected from undisturbed veld. Bahia grass was used as a trap plant and grown for three months after which inoculum was harvested. Inoculum was characterised by washing sporocarps from soil through wet sieving followed by sucrose density centrifugation. The number of sporocarps per 100 g soil was determined and morphologically classified.

Results

The trap pot method used to multiply the AMF for crude inoculum was effective, with 39 times increase in the number of sporocarps in the topsoil that was used to produce the inoculum. The vigorous growth by the grass in the greenhouse led to higher numbers of AMF sporocarps being produced in the pots in the greenhouse when compared to the trap trenches outside.

However, the trap trenches only had 30% less sporocarps produced than the pots in the greenhouse.

Discussion and conclusions

The trap trench system is a simple, low-cost production system for AMF crude inoculum that will be accessible to all farmers. Care should be taken to use starter soil from undisturbed or natural veld to increase the number and diversity of AFM starting materials. The trap trenches should be filled with soil where no agricultural crops have been planted to limit the chances of spreading soil-borne diseases. Trap plants should not be liable to become weeds and should not be quick to produce seed.

Evaluation of AMF and watering regimes on sweet potato cultivars

Introduction

In nutrient-poor ecosystems, AMF significantly influence plant productivity and community structure (Klironomos et al., 2000; Niemira et al., 1995). AMF symbiosis enhances plant traits such as shoot fresh weight, root dry weight, and tuber production, as shown in potatoes where AMF inoculation outperformed conventional fertilisers in yield and tuber size (Douds et al., 2007; McArthur and Knowles, 1993).

AMF's ability to improve crop growth and yield has been demonstrated across diverse environmental conditions. Commercial biostimulants containing AMF are increasingly used in agriculture, particularly where indigenous AMF levels are low (Miceli et al., 2016; Gianinazzi, 2014; Nzanza et al., 2012). The aim was to study the effect of on-farm produced mycorrhizal inoculum on two cultivars of sweet potato (Blesbok - white flesh and Bophelo - orange flesh).

Materials and methods

The study on sweet potato was conducted under a rain shelter and open field at the Agricultural Research Council - Vegetable Industrial and Medicinal Plants (ARC-VIMP; 25°59′S; 28°35′E; elevation 1160 m), Pretoria, South Africa. The experiment was conducted during the 2022/23 cropping seasons and repeated in the following season.

Plant growth parameters collected included chlorophyll content (using SPAD - Soil Plant Analysis Development), canopy temperature, photosynthetic efficiency as indicated by chlorophyll fluorescence (CF) was measured using a Handy Plant Efficiency Analyzer (PEA), leaf area index measured with Decagon Lp-80 Ceptometer. A root scanner was installed to investigate root and symbiosis development. During 2022/23 a hydroprobe (model 503DR Campbell Pacific Nuclear Inc., California, USA), was used to measure the soil moisture content.

Yield was determined as total and marketable yield. AMF in the soil from each treatment was quantified before and after planting and Biolog Ecoplates were used to determine microbial activity and diversity.

Results

White flesh sweet potato under 30% and 50% allowable water depletion level (ADL) showed longer vines than the other treatments. Plant height at low to moderate water stress resulted

in similar heights for both cultivars, while the severely stressed plants were stunted most. The chlorophyll content leaf readings on the treatment for both cultivars revealed lower values for 50% and 70% ADL.

During season one, the AMF inoculated WFSP had an increased yield of 10, 18 and 8.8 t ha^{-1} under 30, 50 and 70% ADL respectively, however in season two all these treatments had lower yields than the uninoculated controls. For the OFSP the yield increased with 8.7 and 10 t ha^{-1} in season one under 30 and 50% ADL respectively and with 12.7 and 2.5 t ha^{-1} in season two.

For OFSP, the well-watered treatment (30% ADL) showed significantly higher water use and fresh biomass yield when compared to other treatments. Water use significantly decreased with increased water stress levels (30-50% ADL) for both cultivars. However, a further increase in stress to 70% ADL had no significant effect on crop water use.

The total number of sporocarps in the soil increased in the rain shelter, which had a very low number to start with, but not in the open field which had higher numbers initially. There were no clear trends in microbial diversity and richness between the different treatments in the rain shelter or open field.

Discussion and conclusions

The integration of AMF inoculants is a practical strategy to enhance sweet potato resilience and productivity, particularly in water-limited environments. Expanding the use of AMF-based biostimulants offers a sustainable approach to improving crop performance.

Evaluation of AMF on nutritional quality of sweet potato

Introduction

Sweet potato (*Ipomoea batatas*) is an essential root crop valued for its role in food security, bioethanol production, and as a source of β-carotene and micronutrients (Laurie et al., 2012). It is commonly cultivated in rain-fed or low-precipitation regions, where water stress can significantly impair growth, physiological responses, and yield. Such stress reduces water potential, stomatal conductance, photosynthetic rate, and pigment levels in plants (Haimeirong and Kubota, 2003; van Heerden and Laurie, 2008; Yooyongwech et al., 2013).

Inoculating sweet potato with arbuscular mycorrhizal fungi (AMF) enhances tuber production, sugar content, β-carotene levels, and overall plant performance under water deficit conditions (Farmer et al., 2007; Saraswati et al., 2012; Tong et al., 2013). AMF form a symbiotic relationship with plant roots, functioning as an extended root system that boosts water and nutrient uptake, especially under limited irrigation (Bona et al., 2017; Miceli et al., 2016).

This study aimed to test the hypothesis that AMF inoculation promotes sweet potato and nutritional quality, predicting slight differences in nutritional quality performance among inoculated treatments.

Materials and methods

Leaves were collected from actively growing plants during the season and freeze dried. Fresh tubers were cut up and freeze dried after harvest. Freeze dried material were ground into a

fine powder using mortar and pestle and submitted to the analytical facilities at ARC-VIMP, ARC-NRE and ARC-AP (Irene) for analyses. Parameters measured included sugar, protein, β-carotene, vitamin C and mineral content.

Results

The total flavonoid content in the leaves of both sweet potato cultivars is unaffected by AMF and water stress levels. In both seasons' trials, white fleshed (Blesbok) sweet potato (WFSP) leaves treated with AMF had a lower total phenolic content than those treated without.

In the rain shelter trial under different levels of water stress, β-carotene and vitamin C levels of the WFSP leaves increased in the treatment with AMF did not show clear trends. For the OFSP there were no clear correlation between AMF inoculation and phenolic, flavonoid, β-carotene or vitamin C levels.

In the open field trial under different levels of inoculum applied, vitamin C levels for both WFSP and OFSP cultivars increased in treatments with AMF, regardless of the amount of inoculum applied, although WFSP 350 AMF only showed a slight increase. Similar to results from the leaf samples, no significant changes were observed for phenolic and flavonoid in the sweet potato tuber samples.

There were no clear trends in carbon, nitrogen, potassium, calcium, magnesium and phosphorous content between cultivars or treatments. Sodium content of the orange fleshed cultivar was significantly higher than that of the white fleshed cultivar. The lowest levels of iron, aluminium, manganese, zinc and boron was found in the AMF inoculated orange fleshed cultivar under 30% ADL.

Discussion and conclusions

There was no discernible pattern that would suggest that the nutritional value of sweet potatoes is significantly impacted by AMF inoculation. Total sugars and total non-structural carbohydrates in the leaves for treatments WFSP 150 and WFSP 250 increased the most as a result of AMF inoculation, whereas OFSP 150 showed a smaller increase in these compounds. The protein content of WFSP 150 AMF and all three OFSP AMF treatments appeared to be positively impacted by AMF inoculation.

Effect of AMF and watering regimes on African ginger

Introduction

African ginger (*Siphonochilus aethiopicus*), a medicinal plant native to Africa, is valued for its aromatic rhizomes and diverse health benefits, including anti-inflammatory and antimicrobial properties. It is traditionally used to treat colds, flu, respiratory issues, and menstrual discomfort. Additionally, it has culinary, cosmetic, and pharmaceutical applications. However, habitat loss and overharvesting threaten its survival, emphasizing the need for sustainable cultivation. The study examined the impact of AMF inoculation on its growth and yield in a greenhouse setting.

Materials and methods

The study was conducted in a greenhouse at the Agricultural Research Council - Vegetable Industrial and Medicinal Plants (ARC-VIMP; 25°59′S; 28°35′E; elevation 1160 m), Pretoria, South Africa during the 2023/24 season. Watering regimes and data collection were the same as for the sweet potato trial.

Results

Plant height did not show any difference based on AMF inoculation, however below ground biomass did. Fresh roots under all three watering regimes had a higher biomass when inoculated with AMF. The fresh rhizomes/tubers under 70% ADL showed a slight increase in weight.

When compared to the controls, the phenolic and flavonoid content of tubers inoculated with AMF under 50% and 70% water stress dropped, but the antioxidant activity of the AMF-inoculated treatment under 50% water stress dramatically increased. Mineral content did not differ between inoculated and uninoculated treatments. There was no increase in soil microbial activity in the AMF inoculated treatments.

Discussion and conclusions

AMF produced on-farm resulted in marginal increases in the yield of African ginger roots and rhizomes. AMF-inoculated treatments showed a promising increase in yield and antioxidant activity under 50% water stress, despite the fact that no discernible changes in aboveground growth were seen during the season.

Implementation of on-farm AMF production at smallholder farmer

Introduction

Soil microorganisms significantly influence crop yield and quality, ranging from harmful pathogens to beneficial growth promoters. Their role is especially critical in low-input agricultural systems (Franco et al., 2011). Rhizosphere and endophytic microorganisms are increasingly studied for their ability to enhance plant growth and health. The goal of soil-root interface studies is to manipulate these microorganisms to improve plant performance (Rovira, 1979).

On-farm production of AMF inoculum offers advantages over imported alternatives, such as cost savings, reduced transportation requirements, and adaptability to local conditions (Douds et al., 2006; Enkhtuya et al., 2000). Locally adapted species can outperform introduced strains and foster a taxonomically diverse inoculum, which enhances resilience to environmental changes and improves functionality (Smit et al., 2000; Hart and Reader, 2002; Adholeya et al., 2005). The aim was therefore to implement an on-farm mycorrhizal production system at a smallholder farmer and characterise the inoculum produced as well as evaluating its effect on crop production.

Materials and methods

A trap pot system was used to multiply the inoculum collected from undisturbed veld soil. Bahia grass was grown as trap plants for three months. Bell pepper seedlings were planted with 250 mL of produced inoculum. Control plants were planted without inoculum.

Chlorophyll content, measured by SPAD (Soil Plant Analysis Development), is a measurement used to assess the chlorophyll content in plant leaves and plant growth was measured with a tape measure. AMF in the soil was quantified after the trial.

Results

There was no statistical difference between chlorophyll content of inoculated and control plants, but AMF inoculated plants grew taller than the uninoculated plants. Unfortunately, the farmer did not keep separate records of yield from inoculated and uninoculated plants.

The average number of sporocarps recovered from soil in the inoculated treatments were 1 042 per 100g soil while that in the control was 726 per 100g soil. The initial number of sporocarps in the field before planting was 625 per 100g soil and the number in the inoculum was 1 242 per 100g soil.

Discussion and conclusions

Integrating AMF inoculants, particularly through on-farm production, provides a sustainable approach to enhancing agricultural systems. Producing diverse, locally adapted AMF strains ensures better plant health, improved soil properties, and resilience to environmental stresses.

Summary and general conclusions

The literature review focused on how AMF symbiosis is formed and how these symbioses influence water use efficiency and nutritional water production. It also investigated the use of AMF in agriculture and its effect on nutrition and crop yield. The use of AMF as biological control for diseases were also discussed and then the different types of AMF inoculum was investigated.

A simple and efficient method for on-farm production of AMF was tested and good results were obtained. This method produced a diverse inoculum that will be suitable for use with many crop plants.

AMF inoculation of WFSP and OFSP gave good results with increased yield in season one, however in the season two trial, the results differed considerably. Only the OFSP showed increased yield with AMF inoculation under all three watering regimes. Although clear differences between the soil microbial activity of inoculated and uninoculated was not seen, increased AMF numbers will assist in overall soil health.

AMF inoculation of African ginger showed an increase in fresh root mass in all treatments and a slight increase in tuber yield under severe (70%) water stress. A significant increase in antioxidant activity in the AMF inoculated treatment under 50% ADL was also noted.

The smallholder farmer successfully implemented on-farm production of AMF and was very enthusiastic to continue with the process. A video and an infographic were developed on the procedure for on-farm AMF production and is available for future training events.

Recommendations for future research

Inoculation of OFSP and WFSP with AMF under different watering regimes should be repeated. Roll out of the on-farm AMF production to multiple localities in South Africa is recommended. Further studies on the effect of AMF inoculation on disease suppression of common soil-borne pathogens, especially under drought stress conditions, should be conducted. Additional trials on other crops should be conducted. Training of farmers and extension officers should be conducted on the benefits of AMF and the on-farm production method.

Capacity building - training of farmers and students

One master's student (Ms Ndivhuwo Mulaudzi) and one work integrated learning student (Ms Charity Machevele) were trained during the course of this project. Farmer training was provided for approximately 60 farmers during the Climate Smart Agriculture event held 26-29 August 2024 at ARC-VIMP, Roodeplaat. An infographic and video about on-farm production of mycorrhizae was also produced. Ms Mulaudzi presented her work as a poster at two conferences, namely SASAT 2024 and Combined conference 2025.

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CHAPTER 1 INTRODUCTION AND BACKGROUND

The government, scientists, NGOs and industry has identified climate change as one of the major challenges that South Africa will face in the twenty-first century. Drought, exacerbated by climate change, is one of the primary factors currently limiting crop productivity, resulting in significant yield losses in many agricultural crop production areas. Mycorrhizal fungi can increase plant tolerance to abiotic stresses caused by climate change, reduce the risk of plant extinction, and allow time for plant dispersal and adaptation. Mycorrhizae comprises a broad range of mutualistic associations formed between plant roots and fungi. Although most plants can grow without these fungi, plants colonised with mycorrhizae generally grow much better than those without mycorrhizal symbionts. These symbionts are of extreme importance in environments with limited water and nutrients as the mycorrhizae can significantly increase water and nutrient uptake compared to plants without these associations. Although commercial formulations of mycorrhizae are available, they are generally too expensive for smallholder farmers to use. The project aimed to develop an on-farm system of mycorrhizal production that will use the locally adapted mycorrhizae to create mycorrhizal inoculum for smallholder farmers.

The outputs of this research will directly benefit smallholder farmers, especially in areas of limited water availability. Better yields with lower inputs are achievable for these farmers when they inoculate their crops with mycorrhizae. Inoculating crops with mycorrhizae can lower the crop water and fertiliser requirements, making farming more sustainable and profitable. This will help conserve the limited water resources in South Africa, while lowering the fertiliser inputs, which will help to protect the environment. This will support the National Water Resources Management Strategy, which recommends that water resources be used more effectively in all sectors, particularly in agriculture, which is the largest water user in South Africa.

Scope of study

This project proposed to develop a simple and efficient on-farm production system for Arbuscular mycorrhizal fungi (AMF). This will contribute to smallholder farmers' economic and social transformation by acquiring better farming knowledge, increased agricultural productivity and income generation. The research was conducted with the assistance of two female graduate students from previously disadvantaged backgrounds, who were be actively engaged in the various project activities, thus contributing to human capital development in assessing the water requirements and water use efficiency (WUE) and nutritional water productivity of crops in combination with AMF (see chapters 4-6 for results). Furthermore, the proposed research outcomes are expected to contribute to more efficient utilization of the available water resources, which is particularly important in South Africa, a water-scarce country. The climate projections for the majority of the country indicate a warming trend in the coming years and projected drying in many areas (Shongwe et al., 2009). The latter is of particular concern to the already water-scarce country. As a result, the South African government has recognized the need for implementation of water saving strategies in crop production to alleviate the increased pressure on available water resources for irrigation, which forms up to 60% of the total water use in South Africa. Smallholder farmers will benefit from

the generated knowledge through training and technology transfer. The governance can easily adopt the technology developed in this project to combat the impact of climate change through the accurate allocation of water to crops and improve crop resilience to drought using AMF.

Rationale

Crop production consumes 80-90% of the freshwater consumed by humans worldwide (Morison et al., 2008). Irrigated agriculture uses up to 62% of South Africa's (SA) water. Ironically, this sector wastes 35-45% of the water supplied (DWA, 2013), putting additional strain on a country with an annual rainfall of only 450-480 mm (Dennis and Dennis, 2012), well below the global average 860 mm. Roughly 43% of total rainfall falls on only 13% of the land area. Furthermore, only 9% becomes runoff that feeds water bodies (Dennis and Dennis, 2012). To meet SA's future economic development goals, water scarcity must be addressed. As a result of this effort, water-saving irrigation technologies and drought-tolerant cultivars have been developed. While indigenous or indigenized crops are important components of production systems due to their versatility, short growing season, and tolerance to marginal conditions, the focus has, however, been on commercial crops (Plucknett, 1983). Most smallholder farmers grow crops in marginal areas, and drought is one major abiotic stress exacerbating crop production and food security, especially in light of climate change in SA (Jovanovic and Stikic, 2012). Identification of mycorrhizal fungi that can increase plant tolerance to abiotic stresses and drought-tolerant genotypes will potentially enhance crop production and production efficiency by increasing the cultivated land and yields in marginal areas where the effect of climate change is expected to be more aggravated.

Water scarcity is currently one of the world's most pressing issues, and there is much uncertainty about the degree of water supply for future generations. (Jury and Vaux, 2005). In an era of scarcity, irrigation water management should be carried out as efficiently as possible, with the goal of saving water while maximizing yield (Fereres and Soriano, 2007). Increasing agricultural production to fulfil global food demands while guaranteeing the sustainable use of water resources is an essential goal for producers and academics, which might also be accomplished by optimizing irrigation systems (Rodriguez-Ramos et al., 2022). In arid and semi-arid environments, a viable and sustainable production approach considers optimum irrigation schedule to save water and enhance agricultural water use efficiency while preserving crop productivity. Excessive irrigation water consumption diminishes water productivity (WP) and raises input costs, lowering net returns (Kuscu and Turhan, 2022). Deficit irrigation (DI) is the application of less-than-ideal amounts of water (below crop evapotranspiration requirements) (Khapte et al., 2019). Although this irrigation strategy can save water, it may reduce production. DI, when used correctly, has successfully increased the efficiency of water use and WP of several crops without generating significant yield losses (Geerts and Raes, 2009). Nonetheless, a lack of water can be a substantial abiotic stressor in some crops, affecting the number of fruits per plant, fruit size, and production. Simultaneously, it may result in improved fruit quality and cost savings, and reducing water use may prevent soil leaching and groundwater pollution (Nangare et al., 2016; Zeng et al., 2009).

Mycorrhizal fungi are a group of mutualistic soil fungi that colonises the roots of most plants. The fungi are in a beneficial relationship with the plant as it supplies the plant with nutrients and increased water availability, while the plant supplies the fungus with carbon in the form of sugars (Brachmann and Parniske, 2006). Mycorrhizal fungi are of extreme importance in environments with limited water and nutrients as it enables the plants to access more water and nutrients than plants without mycorrhizal mutualists (Bitterlich et al., 2018; Douds and Millner, 1999). Mycorrhizae have also been credited with increasing a plant's disease resistance, improving a plant's ability to grow under drought conditions, and improving soil structure.

The two dominant types of mycorrhizal fungi are AMF and Ectomycorrhizal fungi. AMF forms symbioses with 80% of plant species, including most herbaceous and annual species, most arid and semi-arid woody species, and tropical hardwoods (Brachmann and Parniske, 2006). Ectomycorrhizal fungi colonise the roots of most temperate and boreal tree species, e.g. conifers, oaks and beech. They are much more specific in their choice of host plants, and they have not yet been successfully cultivated - they will not grow on the roots of crop plants such as maize, beans and onions for example. Very few plants do not form mycorrhizal associations at all, although most plants can grow without it. The plant families that do not form mycorrhizal associations include the cabbage and beet families; these are the Brassicaceae, Amaranthaceae, Caryophyllaceae and Chenopodiaceae.

AMF are the most important mycorrhizae in agricultural ecosystems since they colonize the majority of crop plants. The hyphae of the fungi act as an extension of a plant's root system and increase a plant's access to immobile nutrients including phosphorus (P), zinc (Zn) and copper (Cu). In plants that mycorrhizal fungi have infected, the fungus is the chief method of nutrient uptake, not the roots. While plant root hairs extend 1-2 mm into the soil, the mycorrhizae's hyphae explore a greater volume of soil and can extend up to 15 cm from the plant's roots. The fungi enter the cells of the roots where they form branched arbuscles within these cells, where the exchange of nutrients and carbon occurs.

While some standard agricultural practices, including frequent tillage and heavy P fertilization, negatively impact mycorrhizae, many sustainable farming practices can bolster native mycorrhizal fungal populations. Even soils that have been intensively managed for an extended period contain populations of mycorrhizae that can be augmented by using cover crops, developing a diverse crop rotation program, and growing crops that form a symbiosis with AMF.

Various commercial formulations of AMF exist, but these mostly consist of one or two different introduced species and are too expensive for smallholder farmers to use. The on-farm method of AMF production could be used to create an inoculum that is not only cheaper than single-species commercial inoculum, but also has the added benefit of containing a diverse group of locally adapted mycorrhizal fungi that can be used to boost a farm's native populations (Douds et al., 2005). Research has shown that using a multi-species inoculum with local isolates can be more efficient than commercial inoculum as indigenous AMF are more effective in promoting plant growth in their local soil than introduced species. Good starter soil can be

collected from any undisturbed area containing native vegetation in arid and semi-arid regions, while hedgerows, thickets and thriving perennial grasslands that have not been cultivated recently are good sources of AMF in temperate regions.

The Agricultural Research Council - Vegetable, Industrial and Medicinal Plants (ARC-VIMP) has been researching the use of AMF to improve drought tolerance of different vegetables and medicinal plants, and positive results were attained. This forms the basis of information to expand the research in testing the usefulness of endogenous AMF to enhance the drought tolerance of various crops, including sweet potato and African ginger.

Aims and objectives of the study

- Develop an on-farm AMF inoculum production system
- To characterise AMF produced by an on-farm system for species richness
- Test AMF produced by inoculating two crops and comparing growth, yield and disease incidence under different watering regimes
- To implement AMF inoculum production system on smallholder farms

Research approach and methods

The research was divided into different work packages to address the different aims. The four work packages were as follows;

Work package 1: Review of literature (Chapter 2)
The first task was to conduct a comprehensive literature review to compile existing knowledge and understand the current problems/challenges. The available literature on crop WUE, nutrient yield, AMF, and drought tolerance mechanisms will be gathered and critically analysed.

Work package 2: Development of an on-farm AMF production system (Chapter 3)
Collecting starter soil: Clear away about 0.5m2 of the vegetation underneath your target plant. Dig down to a depth of about 25 cm collecting the soil and as many fine roots as possible. Collect from under several different trees and shrubs. With stony soil, sieve it to get rid of large stones. Collecting soil from a production field should be avoided to reduce chances of introducing pathogens to the inoculum.

Production of inoculum: A ‘trap-pot’ system was used to multiply inoculum. Both pot and trench trap systems were evaluated. Several large (5 litre) plastic pots/basins were used for the pot system while a trench (100 cm x 50 cm to a depth of 50 cm) was dug into the ground and lined with a perforated plastic sack. The containers were filled with soil and compost mixture (5:1 ratio) and starter soil was added to the top 10 cm of each container and mixed. Four to five Bahia grass seedlings were be planted into each pot and up to ten in the trenches. Maintenance consisted of watering and weeding as needed. Plants were grown for three months for inoculum production.

Harvesting of inoculum: Ten days before using the inoculum watering was stopped and the bait plants cut down at the base of their stems. This killed the plants and tricked the fungus into producing reproductive spores. After 10 days, the inoculum was prepared by pulling up the roots of the bait plants which was be chopped into roughly 1 cm pieces and then mixed back into the soil from the trap-pot or trough. This mixture of roots and soil was the inoculum.

Characterisation of AMF: AMF spores from the soil samples (100 g) was be extracted by wet sieving with 500, 425, 250, 105, 45 and 38 µm mesh sizes, and decanting, followed by sucrose density gradient centrifugation. Spores, spore clusters and sporocarps obtained from the sieves were be studied under a stereo microscope. The best trap plot (pot / trough) and medium (soil, compost or soil/compost mixture) were determined by counting the number of spores from each treatment.

Work package 3: Determine the water requirements and water use efficiency of sweet potato and African ginger (Chapters 4, 5 and 6)

The trials were done at the ARC-VIMP (25°59′S; long. 28°35′E, elevation 1160 m). The field trial was planted in a field measuring 20 m x 15 m. Land preparation followed standard production practices, including primary tillage, followed by disking the soil to a fine tilth. Pre-plant fertiliser and top dressing were be broadcast and incorporated into the soil at a rate determined by soil analysis. Water was applied using a drip irrigation system outfitted with water flow meters and pressure gauges at manifold inlets.

Two trials were planted: the first trial was planted in a rain shelter, during rain, the rain shelter's rain sensor triggers an electric motor, and the shelter closes and covers the experimental trial. The trial was laid out in (3x2x2) factorial design (RCBD), replicated 3 times. Where there were 3 irrigation regimes (control - soil refilled to field capacity when 30% of available soil water (ASW) was depleted, irrigation treatment 2 - refilled to field capacity when 50% of ASW was depleted and irrigation treatment 3 - refilled to field capacity when 70% of ASW was depleted) two cultivars of sweet potato (Bophelo - orange flesh (OFSP) and Blesbok - white flesh (WFSP)) and 2 treatments namely with AMF and without AMF. The plots have 3 rows the spacing between plants is 30 cm, and between the rows is 60 cm. One row has 9 plants, the total number of plants per plot was 27 and Plot size is 4 m^2. The irrigation system was installed in the rain shelter, drip irrigation was used to provide 3 different levels of water provision to the plants. The sweet potato cuttings were obtained at arc VIMP sweet potato breeding scheme.

The second trial was planted in an open field and was laid out in a randomised complete block design where the was four treatments (150 AMF, 250 AMF, 350 AMF and Control without AMF), two sweet potato cultivars were planted (Bophelo - orange flesh (OFSP) and Blesbok - white flesh (WFSP)) replicated three times. Plots consisted of four rows with 10 plants each with 30 cm spacing between plants and 60 cm between rows. The field was irrigated as needed when there was no rain.

Harvesting was done manually 120-150 days after planting. Equal areas from the three mid-rows were harvested for analyses. The harvests were separated into shoots and roots, and fresh weights noted. The shoots were placed in labelled envelopes and oven dried at 40 °C until a constant weight is achieved and recorded. The tubers/roots will be gently washed and

cut into small pieces, placed into labelled envelopes and then oven dried at 50 °C until a constant weight was attained. The dry weights were recorded.

The following physical characteristics were evaluated:

Leaf Area Index (LAI): one-sided green leaf area per unit ground surface area, i.e. leaf area/ground area (m2/m2) will be measured using a ACCUPAR Ceptometer (LP-80 Decagon, USA) and readings will be done between 10 am and 2 pm.

Stem length: measured at the same time as the LAI measurements. At least three plants per replicate will be identified and stem lengths measured using a measuring tape non-destructively from the point of soil contact to the apical tip.

Nutrient content

Two medium storage roots were selected randomly from each plot. These were washed and then cut longitudinally into four quarters. Two opposite quarters were pureed in a food processor and then freeze dried. Zinc, Mg, N and Fe (mg 100 g^{-1}) were analysed at the ARC - Soil Climate and Water (ARC-SCW) laboratory and β-carotene (mg 100 g^{-1}) at the ARC-VIMP analytical laboratory.

Extraction of β-carotene from sweet potato was done using tetrahydrofuran methanol (1:1 vol/vol) according to the method of Biehler et al. (2010). Extracts were analysed using an HPLC-DAD (Shimadzu, Kyoto, Japan) at a wavelength of 450 nm. Iron and Zn contents were determined following a method recommended by the Association of Official Analytical Chemists (AOAC, 1990).

Work package 4: Setting up AMF production systems at smallholder farm (Chapter 7)

After the on-farm AMF propagation system has been tested and optimised, a smallholder farmer was identified to launch the pilot trial for smallholder farmers. An infographic and video were developed detailing the setup and use of the AMF. Researchers and technicians from the ARC-VIMP assisted farmers with setting up the system on their farm and monitoring the production and use of the inoculum. As AMF are generalist fungi, it can be inoculated on to any crops that can be colonised by mycorrhiza. After the successful proof of concept at the smallholder farms, the system can be implemented on a wider scale.

CHAPTER 2 LITERATURE REVIEW

Introduction

Water is necessary for all life on Earth. Water's availability – or lack thereof – can be both a driver and a hindrance to economic development, as it nurtures healthy ecosystems for food production and human and animal health, generates hydropower and supports industry, to name a few of its critical services for human well-being. Water availability is determined by the amount of physically available water and how it is stored, handled, and allocated to various consumers (UNESCO and UN-Water, 2020). Water scarcity is a reality in many nations and regions (Vörösmarty et al., 2010), and the future consequences of climate change will almost certainly exacerbate it. These climate change consequences, which are caused by the intensification and acceleration of the water cycle, might be direct or indirect. This causes increased rainfall and flooding in certain areas and more frequent and severe droughts in others, or perhaps both in the same area (IPCC, 2021).

Several countries, notably South Africa, suffer from severe water shortages due to a considerable gap between yearly precipitation and potential evapotranspiration. This shortage is exacerbated by climate change, which makes drought more severe in some parts of the world, as South Africa experienced in 2015. The high aridity in some areas significantly impacts the agricultural food crops grown in such countries, particularly for resource-poor households that rely on dryland crop production for their primary means of subsistence and revenue generation. South Africa is especially sensitive to climate change since farming relies heavily on rainfall and the rainy season length. Droughts and increased temperatures might have a major impact on food supplies. Drought has an impact beyond food shortages (Chaves et al., 2016); it negatively influences national economies and diminishes the country's capacity to export crops and produce foreign cash. Annual crop yields can be significantly increased if crop losses due to poor and erratic rainfall are reduced. Drought-tolerant crops have the potential to improve crop production efficiency by improving cultivation and yields in marginal locations.

Indigenous or indigenized crops are well suited for resource-poor households because they occupy smaller areas (home-grown gardens), mature in a shorter period (thus readily available), require low external agricultural production practises (grow naturally in "wild" or fallow fields, without the addition of fertiliser or irrigation, making them cheaper and easier to access), are easy to harvest on a daily basis, and do not require storage (Nyathi et al., 2016). However, very little is known about the best cultivation strategies for these crops, such as identifying the most drought-tolerant varieties/landraces for higher crop output and crop water use efficiency in areas with limited water resources (Nyathi et al., 2016). Drought tolerance screening in these highly nutritious crops will undoubtedly contribute to food security and improve the quality of life for those who rely on them.

Drought is the primary limiting factor for plant biomass, as well as its impact on species dispersion and ecosystem biodiversity, particularly in semiarid areas (Chaves et al., 2016). When water is scarce, plant species control their stomatal opening to reduce water loss; as a result, CO_2 assimilation reduces, and a chain reaction begins (Chaves et al., 2016; Galle et al., 2011). On the other hand, these metabolic alterations are affected by the degree and number of stressful situations.

According to research, certain species adapt their physiological reactions to stressful situations and can better endure future ones (Rivas et al., 2013). This process (hardening) implies that plants are more resistant to stress because of biochemical and/or epigenetic changes that occur after the initial exposure to environmental perturbation (Bruce et al., 2007).

Plants may associate with soil microbes to endure adverse conditions and the innate mechanisms of tolerance to various abiotic stressors (Abdul Rahman et al., 2021). Mycorrhizal fungi are a group of mutualistic soil fungi that colonises the roots of most plants. There are four main types of mycorrhizae recognised based on their morphological differentiation in root tissues and association with host plant lineages; these are arbuscular mycorrhizal fungi (AMF), ectomycorrhizae (E cm), ericoid mycorrhizae (ErM) and orchid mycorrhizae (OrM) (Brundrett and Tedersoo, 2018). More than 80% of terrestrial plants have symbiotic relationships with mycorrhizal fungi. AMF are the most common and widespread, developing symbiotic relationships with 72% of flowering plant species. The E cm and ErM are substantially less common, occurring at around 2% and 1.5%, respectively. Approximately 7% of plants has inconsistent AMF connections, while 8% of plants remain non-mycorrhizal (NM) (Brachmann and Parniske, 2006; Brundrett and Tedersoo, 2018). Very few plants are strictly NM and these include the cabbage and beet families namely the Brassicaceae, Amaranthaceae, Caryophyllaceae and Chenopodiaceae.

Several studies have shown that AMF symbiosis can reduce the negative consequences of water scarcity by increasing water intake (Augé, 2004), gas exchange and water usage efficiency (Frosi et al., 2016), as well as nutrient acquisition and translocation (Omirou et al., 2013). In return, the plant supplies the fungus with carbon in the form of sugars (Brachmann and Parniske, 2006). The effectiveness of AMF and plant association is proportional to the quantity of nutrients absorbed by the host per unit of carbon spent to support the symbiosis (Harris-Valle et al., 2017). Thus, leaf building cost, defined as the amount of glucose required to produce 1 g of dry leaf matter (Williams et al., 1987), can be one of the primary functional qualities used to measure the effectiveness of the symbiotic relationship. Symbiosis indirectly boosts photosynthesis to supply the plant's energy balance, altering the plant's carbon balance and nutrient utilization efficiency, particularly phosphorus (Smith and Smith, 2012). As a result, a positive energy balance linked with efficient nutrient usage is critical for competitive success and greater tolerance to environmental challenges (Grimoldi et al., 2006).

Mycorrhizal fungi are of extreme importance in environments with limited water and nutrients as it enables the plants to access more water and nutrients than plants without mycorrhizal mutualists (Bitterlich et al., 2018; Douds and Millner, 1999). Mycorrhizae have also been credited with increasing a plant’s disease resistance, improving a plant’s ability to grow under drought conditions, and improving soil structure.

Mycorrhizal fungi belong to the fungal phyla of Glomeromycota, Ascomycota, and Basidiomycota and around 6 000 species have been identified so far (Bonfante and Anca, 2009). AMF specifically belong to the phylum Glomeromycota which consists of four orders, 12 families, 41 genera and approximately 338 species (Redecker et al., 2013). However, most commercial AMF inoculants only include species belonging to genera *Glomus*, *Funneliformis*, *Rhizophagus* and *Septoglomus*. Among all the AMF species present in the products, *R. irregularis* and *F. mosseae* were the dominant species with *R. irregularis* being the most widespread, occurring in more than one-third of the products (Basiru et al., 2021).

How AMF symbiosis is formed

Several distinct stages are involved with the formation of the association between AMF and plants, these include spore germination, hyphal differentiation, appressorium formation, root penetration, intercellular growth and arbuscule formation (Giovannetti et al., 1994). AMF spores can germinate in the absence of roots, which gives it the ability to rapidly colonise suitable hosts when available.

Plant roots release “branching factors” (BFs) like strigolactones that induce morphological and cytological responses in the approaching fungal hyphae (Besserer et al., 2006; Buee et al., 2000). Among these, hyphal branching is the most prominent morphological change that can be attributed to fungus-plant recognition. In turn, fungal hyphae produce “myc factors” that lead to the transcriptional induction of symbiosis related genes in the host root (Kosuta et al., 2003). These pre-symbiotic recognition events are pre-requisite for formation of fungal appressoria structures on the root surface, invasion and colonisation of fungal hyphae inside the root cortex, and, finally, the formation of highly branched, tree-like fungal structures (arbuscules) inside plant cells. The surface increase associated with arbuscule formation is believed to aid nutrient exchange between the partners.

AMF have biochemical and physiological properties that differ from those of roots and can improve Phosphorus (P) availability. They acidify the rhizosphere by increasing proton outflow or increasing pCO_2 (Howeler et al., 1982). Mycorrhizal hyphae have a greater affinity for P, resulting in a lower Kinetics mycorrhiza (km) value, meaning they are better at taking up P from the soil solution. This is due to the fact that mycorrhizal fungi interconnect two different environments, namely the plant roots with the surrounding soil, and they extend hyphae centimetres into the soil resulting in a 10-fold increase in the effective root surface area (Mcnear, 2013; Rigou and Mignard, 1994).

In addition, mycorrhizal plants have increased photosynthetic capability (Boldt et al., 2011). AMF are known to be of major relevance due to their high capability to boost crop growth, yield, and quality through the acquisition of nutrients in less fertile soils, hence lowering the requirement for phosphate-based fertilisers (Roy-Bolduc and Hijri, 2011). Since research is focusing on increasing soil fertility for higher yields, the impact of microorganisms such as arbuscular mycorrhiza on sweet potato growth and yield should be considered.

The external hyphae of AMF can spread from the root surface to the soil beyond the P depletion zone and thus access a larger volume of un-depleted soil than the root alone. As a result, AMF is said to increase absorptive area (Smith and Read, 2008). Because the fungal hyphae have finer and thinner structures, they have better access to soil pores and may investigate larger soil volumes, resulting in more efficient mining for Pi sources. (Schnepf et al., 2011).

Water use efficiency and AMF

Plant responses to drought differ based on the species and genotype, cell type and subcellular compartments, age and stage of development, drought duration, and severity of water deficiency (Bray, 1997). As a result of their differences in water usage efficiency, different species' responses to the environment will differ. Plants with high water use efficiency are expected to have more biomass per unit of water lost during droughts than plants with low

water use efficiency (Heschel et al., 2002). Water use efficiency knowledge for various varieties/landraces of indigenous crops in conjunction with AMF, such as sweet potato (*Ipomoea batatas*) and African ginger (*Siphonochilus aethiopicus*), is currently minimal. Only a few studies have been undertaken to assess the water use efficiency of several species of indigenous vegetable crops such as cowpea, sweet potato, and amaranth (Anyia and Herzog, 2004; Neluheni et al., 2007). In terms of crop water usage efficiency under low soil water circumstances, these crops showed significant variances between types and stomatal conductance (Munjonji et al., 2018).

A study on the significance of the AMF symbiosis in the water interactions of the frankincense tree *Boswellia papyrifera* (Burseraceae-henceforth Boswellia) revealed that higher leaf area (Figure 2.1a) and better assimilation rate per unit leaf area were associated with mycorrhizal plants in the water pulse treatment (Figure 2.1c). Mycorrhizae or water pulse had little effect on respiration rate (Figure 2.1e). Transpiration rates were significantly higher in non-mycorrhizal plants with a consistent water supply and mycorrhizal plants under pulsed watering conditions (Figure 2.1b). Water use efficiency was higher in mycorrhizal plants than in non-mycorrhizal plants (Figure 2.1d). Stomatal conductance was higher in mycorrhizal plants than in non-mycorrhizal plants (Figure 2.1f) (Birhane et al., 2012).

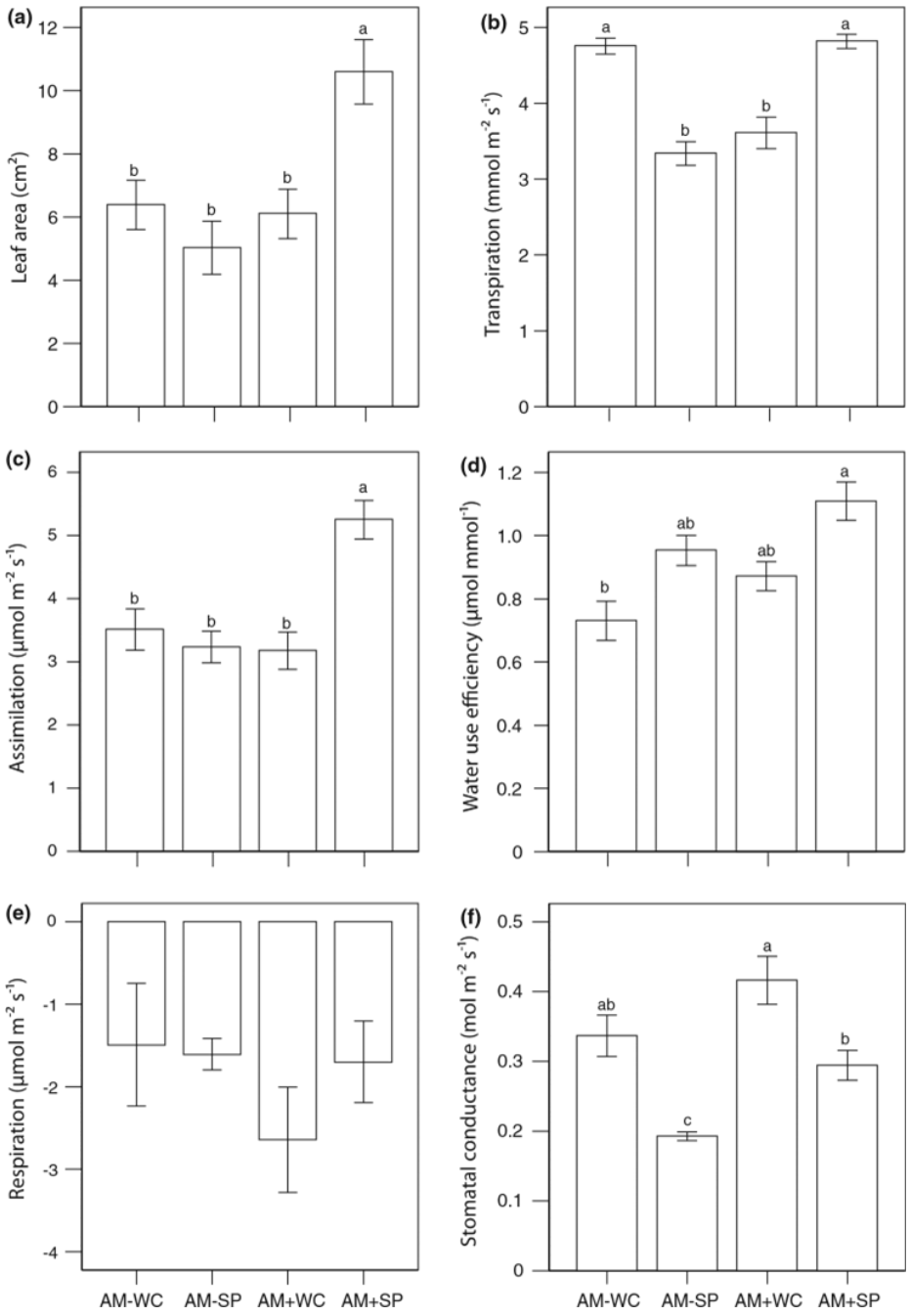


Figure 2.1 The effect of arbuscular mycorrhizae and water regime on 16-month-old Boswellia seedlings. Arbuscular mycorrhizal treatment (AM+) was compared with a control without inoculation (AM-), and water pulse every 2 weeks (SP) during the wet seasons was compared with a control with regular water supply during the wet season (WC).

Stomatal conductance is critical in the photosynthetic process. Drought stress reduces stomatal conductance significantly (Hassan et al., 2020), whereas AMF increases stomatal conductance and enhances plant water use efficiency (WUE) (Ruíz-Sánchez et al., 2011). AMF improves gene expression coding for aquaporins, and AMF-mediated increases in gene expression connected to aquaporins boost water absorption by plants and assure a better WUE under drought stress conditions (Porcel et al., 2005). AMF significantly enhanced

stomatal conductance and WUE in various plants such as *Poncirus trifoliata* and *Rosmarinus officinalis* under drought stress (Ruíz-Sánchez et al., 2011). AMF also influences the accumulation of several hormones, such as ABA, jasmonic acid (JA), and strigolactones, which sustain increased Leaf Relative Water Content (LRWC) and plant WUE under drought stress (Fernández-Lizarazo and Moreno-Fonseca, 2016).

Better water uptake and greater WUE in AMF associations are also associated with increased root activity and hydraulic conductivity (Avio et al., 2006). Furthermore, increasing the ABA level acts as an anti-transpirant, reducing water loss by stomatal closure and sustaining higher WUE under drought stress (Egamberdieva et al., 2018; Mohanta et al., 2017). AMF also secretes glomalin, which aids nutrient and water uptake and significantly increases WUE under drought stress (Gong et al., 2013). Similarly, AMF colonisation promotes root development and hydraulic characteristics, resulting in increased water uptake and WUE in plants exposed to drought stress (Ouledali et al., 2018). AMF hyphae also create a favourable soil network for improved nutrient and water uptake, significantly improving WUE under drought stress (Hamedani et al., 2022).

Augé (2001) reviewed more than 200 studies on 90 host species associated with 22 AMF species and found that in 75% of the cases mycorrhizal plants were observed to deplete the soil water more thoroughly than non-mycorrhizal plants. Similar results were seen in a study on the effect of AMF on tomato under drought conditions by Bitterlich et al. (2018), who found that inoculation with arbuscular mycorrhizae can result in an improvement in water availability and water transport within colonised substrates, leading to lower plant stress.

Yooyongwech et al. (2016) concluded that inoculation of AMF in sweet potato plants improved plant growth characteristics and enhanced water deficit tolerance via soluble sugars and free proline accumulation. The study demonstrated that phosphorus content in AMF inoculated sweet potato were significantly higher than in AMF un-inoculated plants. Leaf osmotic potential in plants without AMF-inoculation grown under water deficit conditions declined, leading to total chlorophyll degradation. In contrast, AMF-inoculated plants under water deficit conditions had an increase in photosynthetic pigments and increased net photosynthetic rate as well as growth. AMF inoculated plants consequently had significantly higher number of tubers per plant and tuber fresh weight under water deficit conditions.

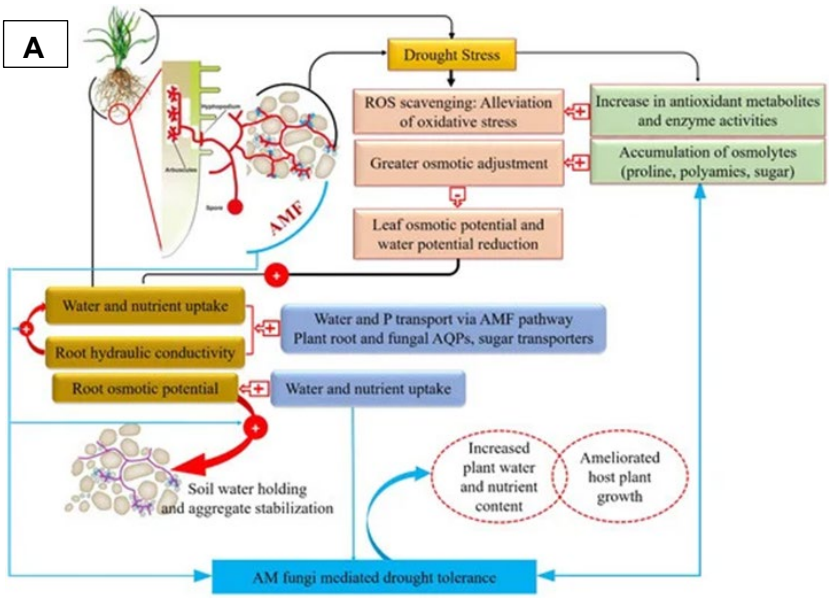
In grapevines it was found that AMF inoculated plants had better water use efficiency due to an increase in proline and higher investment in the photosynthetic capacity (Valentine et al., 2006). The increased photosynthetic response in AMF plants was associated with an increase in specific leaf mass and higher Rubisco activity and electron transport rates (Valentine et al., 2006).

Under drought stress, mycorrhizal interaction improves plant performance by enhancing plant growth (Pavithra and Yapa, 2018), WUE, and nutrient accumulation (Kapoor et al., 2013). AMF-colonisation enhances the development of broad hyphal networks and glomalin secretion throughout this process, which aids in water and nutrient uptake and hence improves soil structure (Figure 2.2**A** and **B**) (Doubková et al., 2019; Gong et al., 2013; Pagano, 2014). Furthermore, studies have shown that AMF uses extraradical hyphae to establish drought-adaptive strategies and influence plant mechanisms such as photosynthetic rate, root

hydraulic conductivity, and root architecture (Gholamhoseini et al., 2013; Lee et al., 2012; Ruiz-Lozano, 1995).

AMF-mediated responses involve diverse processes, such as stimulating drought-responsive genes and activating various metabolic pathways. Research has focused on the critical function of AMF symbiosis in drought relief by up- and down-regulating multiple biochemical and physiological pathways. AMF alter host plant water regulation by activating hormonal signalling or increasing osmolytes. AM symbiosis modulates stomatal conductance and other physiological features in response to drought stress using non-nutritional pathways such as ABA (Doubková et al., 2013).

The effect of mycorrhizae on *Cenchrus ciliaris* was investigated under two distinct water regimes (100 and 50% field capacity) by Khan et al. (2008). The study found that inoculating with mycorrhizae improved water use efficiency in all water regimes, as shown in **Table 2.1**. The water use efficiency without mycorrhizae was 1050 g of water at 100% field capacity and increased to 990, 961, and 915 g of water required by the plants following inoculation with *Gigaspora rosea*, *Glomus intraradices* + *Gigaspora rosea*, and *Glomus etunicatum* + *Glomus intraradices*, respectively. The WUE of *C. ciliaris* without mycorrhizae was 760 g of water at 50% field capacity, which increased to 704, 677, and 633 g of water required by plants injected with single and dual mycorrhizae inoculation, respectively.



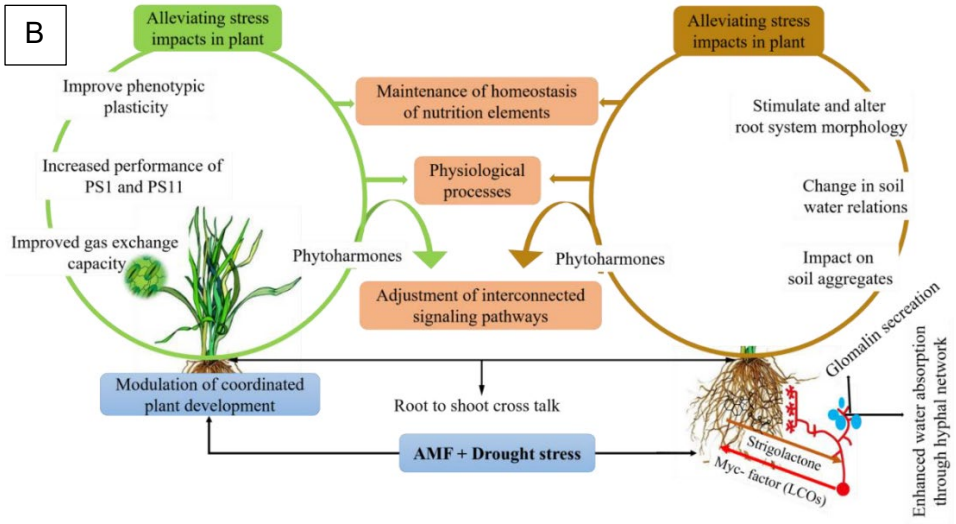


Figure 2.2 Arbuscular mycorrhizal fungi (AMF) symbiosis helps plants to maintain and regulate different processes in plants to cope with deleterious effects of drought stress, through either direct or indirect interaction, on plant growth performance (A). Schematic diagram showing a complex network of mechanisms mediated by AMF to alleviate drought stress symptoms in plants (B) (Bahadur et al., 2019).

Table 2.1 Water use efficiency (WUE) of mycorrhizae inoculated and uninoculated Cenchrus ciliaris under two water regimes (Khan et al., 2008).

Treatment	100% Field capacity	50% Field capacity
Control	1050[a]	760[e]
Gigaspora rosea	990[b]	704[f]
Glomus intraradices + *Gigaspora rosea*	961[c]	677[g]
Glomus etunicatum + *Glomus intraradices*	915[d]	633[h]

Any two names not sharing a letter differ significantly at a 0.05 probability level. LSD (0.05) for WUE = 10.19.

Nutritional water production and AMF

Crop water productivity is defined as the amount of water used per unit of total biomass or specified biomass (yield) (Steduto et al., 2005). There is a mismatch between food supply and nutritional needs due to a lack of nutritional concerns in crop production (Schönfeldt et al., 2017). Current food production must consider nutrition implications (Mabhaudhi et al., 2016). Nutrient density should rise as food production and productivity rise. Agriculture could boost food production and nutrition while using few resources. It is difficult to connect disciplines since there are no effective metrics for evaluating them. Nutritional water productivity (NWP) is important for examining the water-food-nutrition nexus (Renault and Wallender, 2000).

The beneficial effect of AMF application on relative water content and nutritional status in plants, as well as increased shoot accumulation of photoassimilates due to increased photosynthetic activity and improved stress tolerance in the presence of drought, may result in increased productivity in cultivated plants (**Figure 2.3**) (Posta and Hong Duc, 2020). Mycorrhizae can interact with the soil to supply nutrient requirements and respond more optimally to water and soil fertility (Syamsiah et al., 2014). Environmental factors such as soil

temperature, rainfall, and N content can influence mycorrhizae formation in soil (Torres-Arias et al., 2017). According to Lilleskov et al. (2002), rainfall influences the presence of mycorrhizae in the soil. Mycorrhizae are more active in low water conditions; therefore, using mycorrhizae in dryland cultivation is extremely appropriate due to mycorrhiza's ability to enlarge the plant's root area (Sukmasari et al., 2021). However, a gap exists in water-use efficiency and nutritional water productivity knowledge of staple food crops exposed to AMF.

AMF in agriculture - nutrition and crop yield

AMF are the most important mycorrhizae in agricultural ecosystems since they colonize the majority of crop plants. While some standard agricultural practices, including frequent tillage and heavy P fertilization, negatively impact mycorrhizae, many sustainable farming practices can bolster native mycorrhizal fungal populations. Even soils that have been intensively managed for an extended period contain populations of mycorrhizae that can be augmented by using cover crops, developing a diverse crop rotation program, and growing crops that form a symbiosis with AMF. Lekberg et al. (2008) showed that agricultural management practices differentially affect AMF abundance in low input cropping systems in the semi-arid tropics of Zimbabwe. They found that P fertilization, fallowing, and tillage did not significantly decrease AMF abundance. The most significant effects were instead seen when growing vigorous mycorrhizal plants prior to the dry season, in contrast to plants with lower biomass.

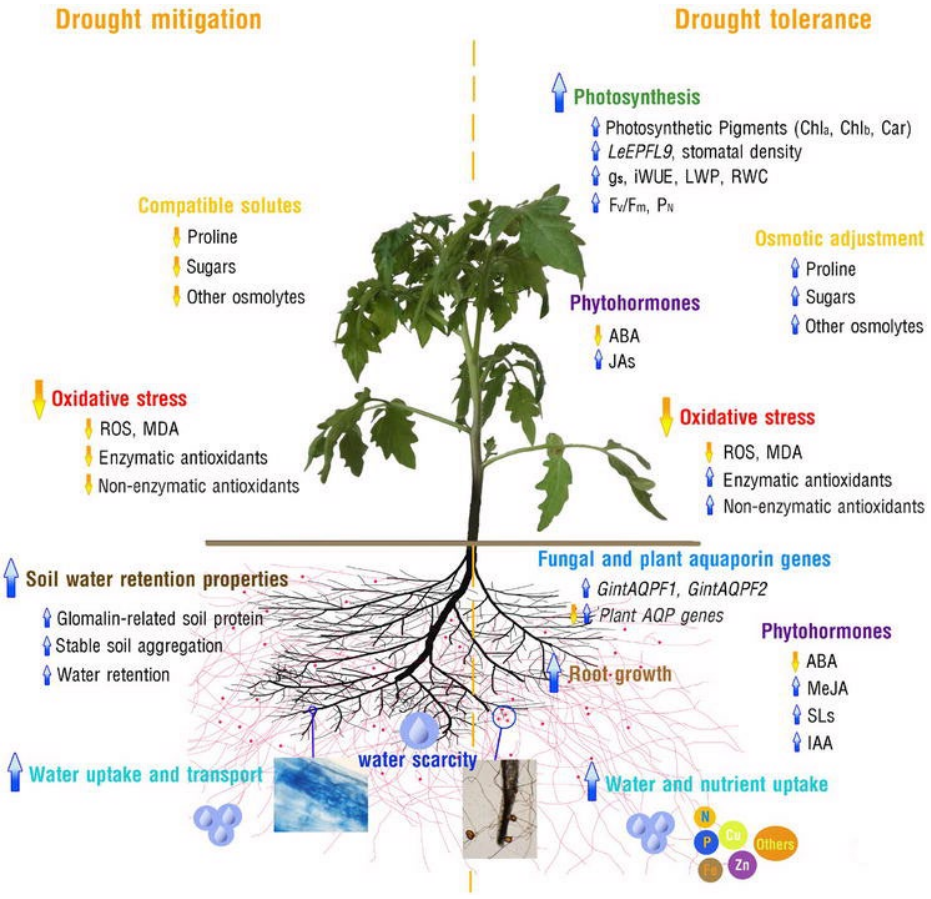


Figure 2.3 Strategies of mycorrhizal plants to cope with water scarcity: drought mitigation and drought tolerance (Posta and Hong Duc, 2020).

Figure 2.3 illustrates the multiple benefits/mechanisms that can be simultaneously induced by arbuscular mycorrhizal fungi in the host plant exposed to water deficit. The blue arrows show increase/up-regulation, whereas the orange arrows indicate decrease/down-regulation relative to control non-mycorrhizal plants. Italic words indicate genes. ABA, abscisic acid; AQP, aquaporin; Car, carotenoids; Chla, chlorophyll a; Chlb, chlorophyll b; Fv/Fm, the maximum quantum efficiency of PSII; gs, stomatal conductance; IAA, indole-3-acetic acid; iWUE, intrinsic water use efficiency; JAs, jasmonates; LWP, leaf water potential; MDA, malondialdehyde; MeJA, methyl jasmonate; PN, net photosynthesis rate; ROS, reactive oxygen species; RWC, relative water content; SLs, strigolactones (Posta and Hong Duc, 2020).

The hyphae of the mycorrhizal fungi act as an extension of a plant's root system and increase a plant's access to immobile nutrients, especially inorganic phosphate (Pi), but also zinc (Zn), copper (Cu), calcium (Ca), sulphur (S), manganese (Mn) and iron (Fe) (Harikumar and Potty, 2007; van der Heijden et al., 2006). Phosphate is taken up by AMF through active phosphate transporters that delivers the Pi to the plant (Harrison and van Buuren, 1995). In plants colonised by mycorrhizal fungi, the fungus and not the roots is the chief method of nutrient uptake. Plant root hairs extend 1-2 mm into the soil, the mycorrhizal hyphae explore a greater volume of soil and can extend up to 15 cm from the plant's roots (Smith and Read, 2008).

Beneficial rhizosphere microorganisms not only increase crop nutritional status, as previously stated but also crop quality. For example, AMF-colonised strawberries have higher amounts of secondary metabolites, leading to better antioxidant properties (Castellanos-Morales et al., 2010). AMF can improve crop nutritional quality by influencing and producing carotenoids and other volatile chemicals (Hart et al., 2015). AMF has been shown to improve tomato quality by Bona et al. (2017). In another study, Zeng et al. (2014) discovered that *Glomus diversiform* boosted the levels of sugars, organic acids, vitamin C, flavonoids, and minerals, resulting in improved citrus fruit quality. Mycorrhizal symbiosis promotes the accumulation of anthocyanin, chlorophyll, carotenoids, total soluble phenolics, tocopherols, and minerals (Baslam et al., 2011). AMF has been used in large-scale field production of maize (Sabia et al., 2015), yam (Lu et al., 2015), and potatoes (Hijri, 2016), demonstrating that AMF has a high potential for crop yield enhancement. AMF can also boost the manufacturing of beneficial phytochemicals in edible plants, allowing them to be used in a healthy food production chain (Rouphael et al., 2015; Sbrana et al., 2014).

According to Rouphael et al. (2015), abiotic stress reduction by AMF could occur through soil pH regulation, hence conserving its horticultural value. Furthermore, as mentioned below, AMF can play an important role in strengthening plant tolerance to harsh situations. The most notable trait is the acquisition of immobile nutrients beyond the range of plant roots by AMF via their hyphae, including macro- and micronutrients (Govindarajulu et al., 2005; Marschner and Dell,1994; Smith and Read, 2008). Plants with arbuscular mycorrhizae frequently have higher nutrient concentrations and improved growth and crop output (Hoeksema et al., 2010; Lehmann and Rillig, 2015; Pellegrino et al., 2015). AM fungal symbiosis outcomes are highly context-dependent (Hoeksema et al., 2010). Various biotic and abiotic factors influence the effect of AMF on the yield of their related hosts. The features of the host and the fungi themselves are the most important biotic variables. Crop plants respond differently to AMF

depending on their morphology (e.g., root characteristics: Yang et al., 2015), whereas AMF differ in the benefits they bring to the plant (Powell et al., 2009; Werner and Kiers, 2015).

AMF inoculation has received much attention because it enhances the water status of host plants in agroecosystems (Askari et al., 2019). Hijri (2016) unequivocally established the benefit of arbuscular mycorrhizal fungal inoculation on potato yield in a large-scale farming system. AMF are gaining popularity as natural bio-fertilisers due to their vital functions in increasing host plant nutrition and soil fertility (Karaca et al., 2013). AMF inoculation can promote plant growth by enhancing nutrient absorption, photosynthesis, and water stress tolerance (Ruíz-Sánchez et al., 2010). Meanwhile, AMF inoculum is an environmentally benign agronomic measure for increasing crop yield (Celebi et al., 2010), and it is seen as a potential option for assuring crop productivity and food security in rainfed agriculture (Rillig et al., 2016). Abiotic stressors such as drought, nitrogen imbalance, and temperature regimes, in particular, have reduced agricultural output by up to 70% (Kumar et al., 2020). Wu and Xia (2006) concluded that AMF had an important role in increasing crop output in rainfed agricultural systems by boosting the drought resilience of host plants.

AMF effect on crop yield

Leafy crops

Arbuscular mycorrhizal symbiosis show potential for increased agricultural production, including food and nutritional security (Tahjib-Ul-Arif et al., 2018). The use of AMF significantly improved plant mineral nutrition, particularly in terms of nitrogen and phosphorus uptake (Salvioli et al., 2012; Colella et al., 2014). Mycorrhizal symbioses benefit plant growth as well as plant protection, particularly against environmental stresses. Crop growth and productivity are primarily limited by a lack of essential nutrients, particularly phosphorus (Nagarathna et al., 2007). The AMF has been proposed to play a role in mediating the uptake of water during drought stress and of metals in contaminated ground (Farahani et al., 2008).

Leafy vegetables that lack natural AMF association, such as red amaranth and Indian spinach, can be inoculated with AMF, cow dung, and phosphorus to increase their nutrient content. The hexoses sugars from the host plant are accepted by AMF. Carbon transfer from plants to fungi can occur via arbuscular or intraradical hyphae. The host plants contribute significantly to the below-ground organic carbon pool by investing significant carbon in the mycorrhizal network. The primary benefit of mycorrhizae to plants has been attributed to increased nutrient uptake, particularly phosphorous (Tahjib-Ul-Arif et al., 2018).

Leguminous crops

Threats to food security will be exacerbated in a climate-stressed world, resulting in widespread hunger and malnutrition, particularly in the tropics, which include parts of Asia, Africa, Central America, and the Caribbean (Anttila-Hughes et al., 2021). Tropical latitudes have the most densely populated human populations (Rae, 2020), but tropical species are more sensitive to climate change than temperate species, making tropical crops, including tropical legumes, the most vulnerable to climate change (Perez et al., 2016). Soybean (*Glycine max*) and rice (*Oryza sativa*), two major crops that sustain global food security, have been

reported to face enormous biotic and abiotic challenges as the world accelerates toward a warmer, more variable climate in the future (Cheng et al., 2016).

By preventing certain stresses like pests and diseases, improving nutrient accessibility, and lowering the need for synthetic (or chemical) fertiliser, soil microorganisms provide novel opportunities to increase crop yields sustainably. According to numerous studies (Oliveira et al., 2022; Qin et al., 2021), AMF inoculation increases legumes' resistance to abiotic stresses like drought, heat, salinity, and extremely high temperatures.

Numerous recent studies have demonstrated how soil microorganisms, in particular AMF, can enhance soil fertility and aid in the growth of plants that are more resilient to climatic stresses. The tropical legume cowpea grew more quickly after receiving AMF inoculation, which improved the soil's mycorrhizal composition (Kavadia et al., 2021). Leguminosae (or Fabaceae) is a large flowering plant family that includes many agriculturally significant legumes, such as beans and peas. These plants coexist peacefully because of a natural symbiotic relationship with nitrogen-fixing root microorganisms (Reinprecht et al., 2020).

Root crops

Particularly in ecosystems with limited nutrients, soil microbes play a significant role in controlling plant productivity. The structure and productivity of the plant community can change in the presence of AMF (Klironomos et al., 2000; Niemira et al., 1995). The AMF symbionts could increase shoot fresh weight, root dry weight, and the number of tubers produced per potato plant (McArthur and Knowles, 1993), as well as stimulate leaf growth and expansion. In field tests, commercial inoculants containing AMF (*Glomus intraradices*) produced higher yields and larger tubers over two growing seasons than treatments using conventional chemical fertilisers (Douds et al., 2007).

AMF and disease incidence

Fungal disease incidence

Charcoal root rot is a soilborne infection caused by the fungus *Macrophomina phaseolina*. This pathogen infects over 500 plant species, including legumes like peanuts, soybeans, and chickpeas (Marquez et al., 2021). Multiple AMF species have been shown to reduce *M. phaseolina* symptoms in legumes. In chickpea plants, inoculation with individual AMF species *Glomus fasciculatum* (reclassified as *Rhizophagus fasciculatus*), *Glomus constrictum* (reclassified as *S. constrictum*), *G. intraradices* (reclassified as *R. intraradices*), *Gigaspora margarita*, or *Acaulospora* sp. were able to reduce root-rot severity and increase plant growth, chlorophyll content, and the number of pods compared to non-AMF plants (Siddiqui and Akhtar 2006; Siddiqui and Akhtar 2008).

The AMF species, *R. irregularis*, has been reported to upregulate disease resistance. Compared to non-AMF plants, soybean plants infected with *M. phaseolina* produce more lignin (Marquez et al., 2018). This action lessens the prevalence and severity of the disease, which improves plant growth and yield (Spagnoletti et al., 2020). Fungi are responsible for several wilt diseases in legumes, including Fusarium wilt in pigeon peas brought on by *Fusarium udum*

infection. Plants with the infection display chlorosis, drooping leaves and stems, and wilting (Pfenning et al., 2019). In comparison to non-AMF plants, inoculation of pigeon pea with AMF species *G. fasciculatum* (reclassified as *R. fasciculatus*) significantly reduced wilting severity and increased plant height, shoot dry weight, and phosphorous content (Siddiqui and Mahmood 1996). Inoculating potatoes with AMF inhibits the growth of the devastating pathogen *F. sambucinum*, reducing toxin production (Deja-Sikora et al., 2020).

Bacterial diseases

Mycorrhizal-induced resistance and immune responses like callose deposition, cell wall thickening, and production of ethylene, reactive oxygen species and antimicrobial compounds are all activated by AMF colonisation in host plants (Dowarah et al., 2021). According to some studies, numerous bacterial pathogens in tropical legumes exhibit disease symptoms that are lessened by AMF colonisation. For example, *Pseudomonas syringae* pv. *glycinea* (*Psg*) is a pathovar that causes bacterial blight in soybean plants by producing effector proteins that lower the host plant's immunity and cause lesions on soybean pods, chlorosis and necrosis in the leaves, and stem discolouration (Xin et al., 2018). When compared to non-AMF plants, the inoculation of soybean with the AMF species *Entrophospora infrequens* significantly reduced *Psg* plant colonisation (Malik et al., 2016). In comparison to non-AMF plants, inoculations of the AMF species *Glomus intraradices* (reclassified as *R. intraradices*), *Glomus versiforme* (reclassified as *Diversispora versiformis*), and *Gigaspora gigantea* reduced disease symptoms in the leaves. They also increased defence-related gene expression (Liu et al., 2007).

Ralstonia solanacearum causes bacterial wilt globally and is a significant soilborne plant disease that has a noteworthy impact on tomato production and causes substantial economic losses. AMF (*R. irregularis* MUCL 41833) can alleviate the symptoms of tomato bacterial wilt caused by *R. solanacearum* as well as Fusarium wilt (Chave et al., 2017). Research on the interactions between AMF and bacteria has shown that AMF can be useful in treating some bacterial illnesses and in helping to identify more effective AMF species for use against plant bacterial pathogens and in agricultural production. (Zhu et al., 2022)

Viral diseases

Viruses are obligate pathogens that multiply and spread in their hosts and can infect most organisms, including plants. Virus infections in plants frequently cause disease syndromes with symptoms such as dysplasia, necrosis, and chlorosis (Miozzi et al., 2019). AMF inoculation has long been recognized as a low-cost, long-term solution for controlling plant viruses, but the findings of studies examining the effects of AMF-plant virus interactions have been mixed. Few studies have shown that inoculating plants with AMF protects them from virus infection. Before and after virus infection, AMF (*R. irregularis*) vaccination increases tolerance to tomato bush stunting virus and cucumber mosaic virus (Khoshkhatti et al., 2020; Miozzi et al., 2020). Potato virus infection is controlled and mitigated by AMF (*G. irregulare*) inoculation (Deja-Sikora et al., 2020; Ismail et al., 2013). In AMF colonised tomato infected with Tomato Mosaic Virus, there was a notable decrease in the severity and incidence of the disease as well as the degree of viral accumulation under greenhouse conditions. Furthermore, AMF colonisation markedly improved the photosynthetic pigments, and flavonoid content (Aseel et al. 2019).

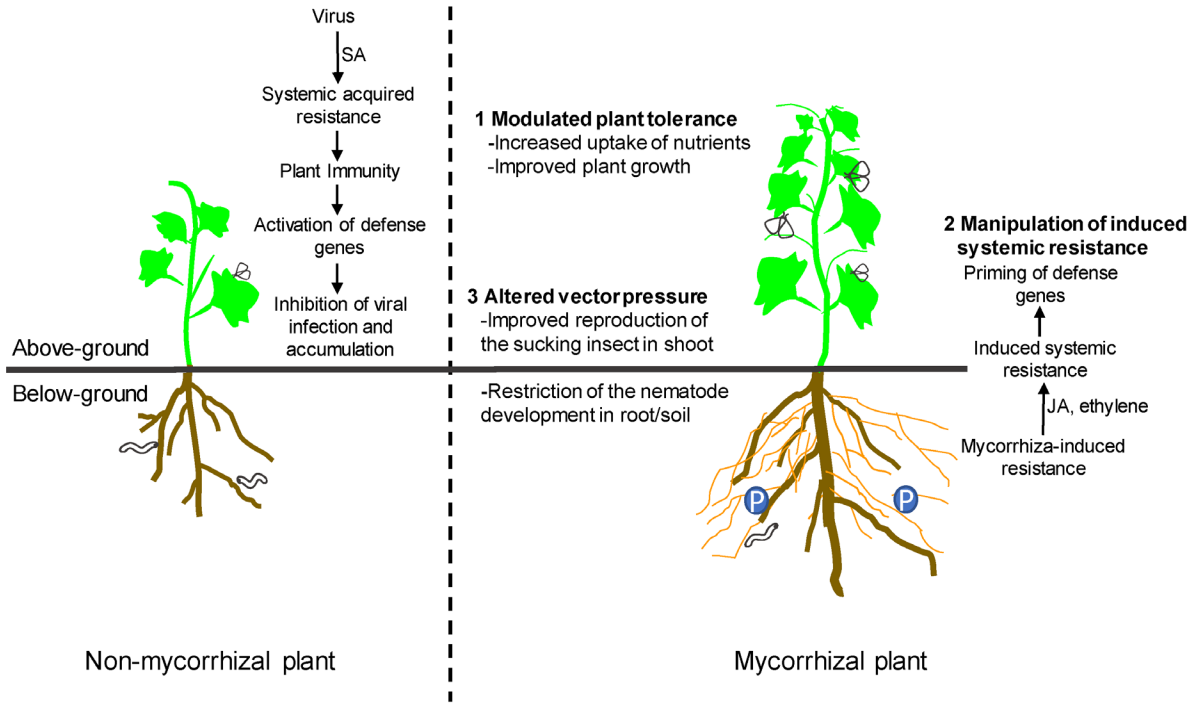


Figure 2.4 The underlying mechanisms of the impact of arbuscular mycorrhizal symbiosis on infection by viral pathogens, including modulated plant tolerance, the manipulation of induced systemic resistance, and altered vector pressure. SA: salicylic acid; JA: Jasmonic acid (Hao et al. 2019).

The grapevine fan leaf virus (GFLV) is transmitted by the ectoparasitic nematode *Xiphinema index*. AMF has been shown to have bioprotective effects against this nematode. This is due to local and systemic defence processes which are activated in the grapevine because of previously established mycorrhizal symbiosis. (Hao et al., 2018). Through the inhibition of nematode transmission, there is indirect mycorrhizal defence against the GFLV (Hao et al., 2018).

AMF as biocontrol

Biotic stress causes yield losses due to bacterial, viral, nematode phytopathogens, and herbivores (Dowarah et al., 2021). AMF protects host plants from various biotic stressors by functioning alone or in collaboration with other natural microbes (Dowarah et al., 2021). Many studies have also found that AMF promotes plant development and yield by increasing tolerance to biotic stress (Bernaola and Stout, 2020).

AMF have been demonstrated to protect plants against nematodes and soil borne pathogens by decreasing the inoculum levels and severity of disease (Caron 1989). These effects are due to several mechanisms including biochemical changes, biological mechanisms, nutritional changes and physical changes. The most obvious physical change conferred by mycorrhizae is the lignification of cell walls that prevent penetration by fungi like *Fusarium oxysporum* (Dehne et al., 1978). Other physical changes include a stronger vascular system that aids in

the flow of nutrients (Schoenbeck, 1979), and smaller syncytia with fewer cells that increase plant tolerance against cyst nematodes (Fassuliotis 1970).

Nutritional changes conferred by mycorrhizal symbioses include increased uptake of phosphate, Cu^{2+}, SO_4^{2-} and Zn^{2+} (Smith and Gianinazzi-Pearson, 1988). As susceptibility of plants to diseases are directly correlated with the plant's nutritional status (Walters and Bingham 2007), the increased nutritional status of mycorrhizal plants, make them more tolerant to diseases (Davis and Menge, 1980).

Various biochemical effects of AMF on plant diseases have been documented. These include increased exudation of amino acids like arginine that inhibited chlamydospore production of *Thielaviospsis basicola* (Baltruschat and Schönbeck, 1975) and higher amount of catechols that inhibited growth of *Sclerotium rolfsii* (Krishna and Bagyaraj, 1986). Root knot nematodes in tomato were also inhibited by increased exudation of serine and phenylalanine from AMF inoculated plants (Reddy, 1974).

Biological mechanisms that have been reported to contribute to disease control on AMF symbionts include the presence of higher numbers of beneficial soil organisms like actinomycetes (Secilia and Bagyaraj, 1987; Singh and Vyas, 2009) as well as direct inhibition of sporangium and zoospore production (Meyer and Linderman, 1986).

Production of AMF inoculum

Substrate-based inoculum

Soil as inoculum

Soil from the root zone of a plant hosting AMF can be used as inoculum (Habte and Osorio, 2001). As such, it is not seen as an inoculum production system in the sense of people managing the production, but it can be useful if nothing else is available. Soil inoculum is composed of soil, dried root fragments, and AMF spores, sporocarps, and fragments of hyphae, collected from undisturbed, natural veld. There are several disadvantages to using soil as inoculum as there may be weed seeds and pathogens in the soil, the mycorrhizal species composition and abundance is unknown, and the viability of these mycorrhizae are usually low (Habte and Osorio, 2001). Because the abundance and viability of the mycorrhizae is unknown, it is difficult to determine how much soil to add as inoculum to a growth medium or field. The main advantage to soil as inoculum is that it is very cheap. Spores extracted from soil can be used as inoculum, but these spores tend to have very low viability or can be dead. If the spores were collected from the root zone of an actively growing plant, and if the plant can be determined to be infected with AMF, then the spores might be reasonably viable (Habte and Osorio, 2001). If they are not, soil or root tissue from the site can be taken to start a "trap culture" to boost the number of viable spore propagules for isolation and further multiplication in what is called a crude inoculum.

Crude inoculum

Trap cultures for mycorrhizae are generally produced in topsoil, sand, or a mixture of both. The sand should be silica sand, not coral sand. Silica sand and sand-soil mixtures have the

advantage of drying more rapidly than topsoil alone once the inoculum production cycle is completed (Habte and Osorio, 2001). This is important to minimize the growth of other microorganisms in the inoculum during the drying process. Topsoil alone can be used for producing crude inoculum, although with certain soils poor drainage may be a problem. Removing roots from soil at the end of inoculum production is more difficult than from sand or sand-soil mixture. Compost and vermiculite mixtures can also be used, but the compost should be chosen carefully to ensure correct levels of phosphorous (P) and nitrogen (N) (Douds et al., 2008).

Environmental variables such as light intensity, soil and air temperature, and soil water status should be favourable for normal plant function. However, AMF development is favoured when the moisture content of the medium is slightly less than optimal for plant growth. Similarly, temperatures that is slightly higher than the optimum for host plant development favours AMF development (Habte and Osorio, 2001). Initiation and development of AMF activity depends on the host's supply of photosynthate and on its root exudations. Therefore, if these are reduced by conditions such as adverse conditions, shading or defoliation, AMF colonisation can be reduced (Ferguson and Menge, 1982; Graham et al., 1982).

Various containers can be used to hold solid matrixes during inoculum production, including plastic bags and pots made of concrete, clay, and plastic. The containers should have holes in the bottom to ensure adequate drainage. To minimize the amount of light reaching the medium, the containers should not be translucent. If clear material is used, it should be painted or enclosed by wrapping in an opaque material. The amount of starter inoculum to use will depend on its quality and for commercial production should adhere to several criteria. The starter inoculum must be highly infective, contain at least four infective propagules per gram, and be free of pathogenic microorganisms. The aim is to inoculate the inoculum-production medium at a rate of 500 infective AMF propagules per kilogram of medium. However, for on farm production, using field soil from your farm as starter inoculum has two advantages: it is free and the AM fungus community already in your field soil is adapted to your climate and soil conditions (Douds et al., 2005; Habte and Osorio, 2001).

Criteria for selecting host plants used for AMF inoculum production includes fast grow, adaptation to local conditions, good colonisation by AMF and production of large quantity of roots within a relatively short time (45-60 days). It should also be resistant to any pests and diseases common in the inoculum-production environment (Habte and Osorio, 2001).

To ensure maturation of the AMF spores in the inoculum, it is essential to grow the nurse plant in the inoculum-production medium for 12-14 weeks. The medium is then allowed to dry slowly by reducing the frequency of watering over a week and then withdrawing water completely for another week. If at the end of the last week the plant is dry, it is removed from the growth medium. The roots of the plant can be chopped into fragments 1 cm long and mixed with the medium, or they can be used separately as root inoculum. The moisture content of the medium at this time should be 5% or lower. If not, the crude inoculum must be spread on a clean surface in an environment with low humidity (RH ≤ 65%) and allowed to air-dry until the desired moisture content is reached (Habte and Osorio, 2001).

Root inoculum

Root inoculum has certain advantages over spore and crude inoculum in that it can colonize plant roots much faster and is much lighter than crude inoculum. Most importantly, they require much less time to produce than crude inoculum (Habde and Osorio, 2001). The basic principles mentioned previously to produce crude inoculum apply to root inoculum as well, except for the fact that the focus here is on the production of large quantities of roots heavily colonised by AMF, rather than on the production of mature spores. The lack of spore production in the system is why root inoculum can be produced in a much shorter time than crude inoculum. Sand or crushed basalt are suitable media for root inoculum production as it aids the ease of root removal and rapid drying at the end of the production period. However, this system generally yields less root mass under the nutrient regimes commonly used for inoculum production compared to media consisting of pure soil or soil-sand mixtures (Habde and Osorio, 2001).

Root inoculum can also be produced in a substrate free system. Some principles to adhere to are firstly that the trap plant used should be as different as possible from the plant species for which the inoculum is produced so that the possibility of spread of diseases and parasites through the inoculum to the target plant is minimized. Best results both in terms of root mass and AMF colonisation levels were observed if the starter inoculum contained 520 infective propagules per kilogram of medium (Habde and Osorio, 2001).

Substrate free inoculum

Techniques for producing AMF in non-solid media include flowing solution culture technique (Elmes and Mosse, 1984; Howeler et al., 1982; Mosse and Thompson, 1984), the flowing nutrient film technique, the stationary solution technique (Parvathi et al., 1984), and the aeroponic technique. Hydroponic and aeroponic systems require constant monitoring and adjustment of the nutrient solutions involved.

For the flowing solution culture technique, plants are supported in a structure that allows their roots to be bathed by a continuously flowing solution of dilute nutrients. Plants can be pre-colonised by AMF prior to being placed in the system, or they can be inoculated after being placed in the system. In the flowing nutrient film technique, roots of plants are bathed with a thin film of flowing nutrient solution. However, sparse sporulation of AMF was reported by Mosse and Thompson (1984) using this technique. The stationary solution culture technique is similar to the flowing solution culture technique except that there is no flow, and the solution is continuously aerated (Crush and Hay, 1981) These techniques are hydroponic techniques for producing inoculum. They are useful for producing limited quantities of clean root inoculum, but the spore production in these systems is not optimal.

In the aeroponic technique of inoculum production, plant roots are continuously exposed to a nutrient solution mist in a closed chamber. This technique has proven useful in producing clean root inoculum and spores as the highly aerated rooting environment of aeroponic culture stimulates rapid and abundant sporulation of the AMF (Hung et al., 1988; Sylvia and Hubbell, 1986). De Santana et al. (2014) tested sweet potato plants inoculated with *Claroideoglomus etunicatum* and *Glomus clarum* in an aeroponic system and in a soil and sand mixture. The density of glomerospores of AMF per gram of roots in the aeroponic system was much higher than per gram of soil in pots. Similar results were found by Sylvia and Hubbell (1986) who

tested colonisation of *Paspalum notatum* roots by *Glomus mosseae* and *G. intraradices*. The aeroponic inoculum production is effective in producing high quality inoculum, however the infrastructure is expensive, and more technical knowledge is required than for soil-based production systems. The nutrient solution for aeroponic cultivation should consist of modified Hoagland's nutrient solution (Sylvia and Hubbell, 1986).

Nutrient management

Managing the chemical composition of the medium in which the AMF interact with their host can be more problematic than managing the physical environment for inoculum production. Because AMF directly influence the uptake of only those nutrients whose movement toward the root surface is limited by diffusion, nutrients not limited by diffusion must be supplied in the medium in sufficient amounts for normal host growth (Habde and Osorio, 2001). The supply of immobile nutrients, particularly phosphorus (P), and the supply of nitrogen (N) must be carefully monitored, because these nutrients appear to regulate the formation of the arbuscular mycorrhizal association. High concentrations of P are also known to suppress AMF colonisation of roots (Habte and Manjunath, 1987; Jasper et al., 1979; Menge et al., 1978)

Plant species highly dependent on AMF can grow in soils with solution P concentrations of 0.02-0.2 mg/L or higher and still sustain high levels of mycorrhizal colonisation on their roots. However, these P concentrations will significantly limit AMF colonisation in species that are only moderately to marginally dependent on AMF, and these species must therefore be grown at a soil P concentration lower than 0.02 mg L^{-1} (Manjunath and Habte, 1988).

Nitrogen requirements should also be managed carefully. At high concentrations, N is believed to inhibit root colonisation by mycorrhiza. Nitrogen in the form of ammonia at levels higher than 200 mg/kg can be very problematic (Aziz and Habte, 1989; Chambers et al., 1980). Habte and Osorio (2001) have shown that N concentration of 80-120 mg L^{-1} is adequate for inoculum production purposes.

Douds et al. (2008) found good results for mycorrhizal inoculum production when using a compost mixture that is high in N, low in P and with moderate K levels (yard clippings and dairy manure). This compost was used for on-farm inoculum production in a 1:2 or 1:4 dilution of compost and vermiculite. When using microbial compost with high P, low N and moderately high K, dilution ratios had to be much higher at 1:19 and 1:49.

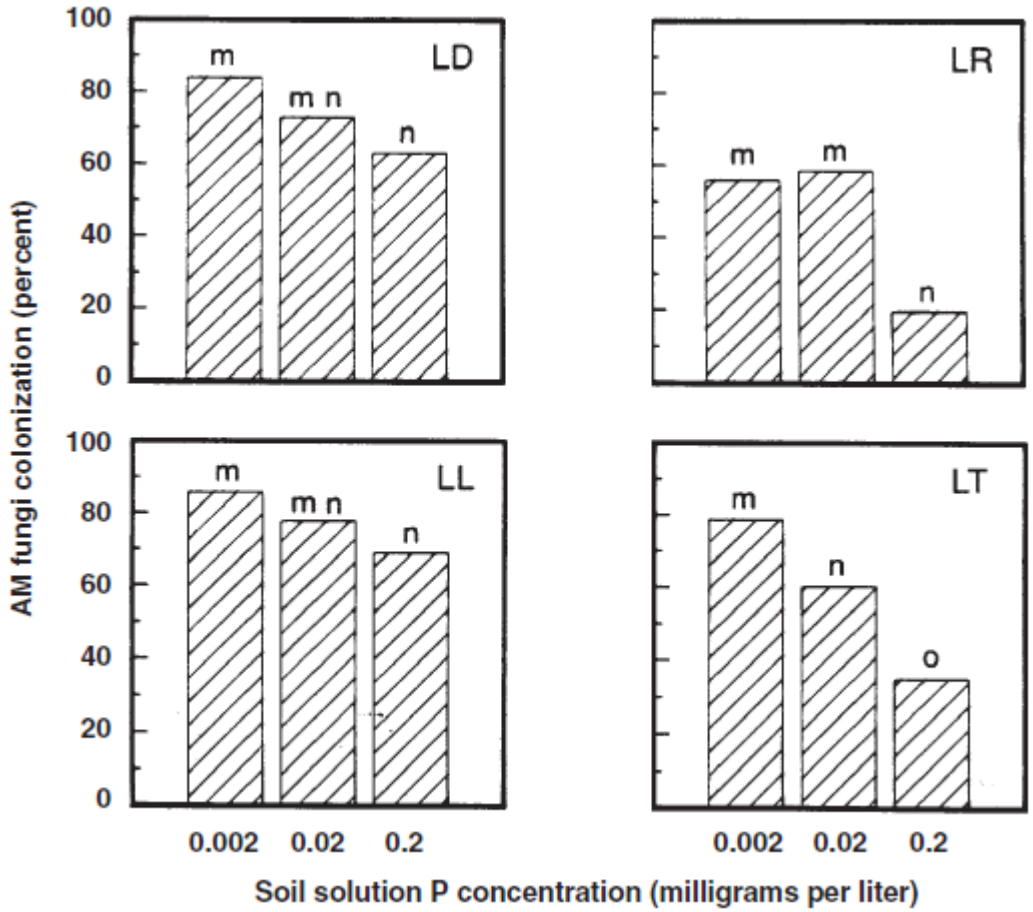


Figure 2.5 Sensitivity of AMF colonisation to soil solution P concentration in four Leucaena species. Means followed by the same letter within a Leucaena species are not significantly different from each other at the 5% level (Manjunath and Habte, 1988).

Inoculum storage

Both root and crude inoculum must be dried to a moisture content of less than 5% before they are stored. It is recommended that inoculum be stored in closed plastic containers in a dehumidified room at 22 °C. The inoculum should be dried as rapidly as possible to minimize growth of other microorganisms (Habde and Osorio, 2001). Crude inoculum can be dried at room or greenhouse temperature by spreading it thinly on a clean surface in a clean, non-humid environment (RH 65% or lower). High-quality crude inoculum can be stored for up to two years at 22 °C with minimal loss in viability. Air-dried cultures of this kind can be packaged in plastic bags and stored at 5 °C for at least four years (Ferguson and Woodhead, 1982). Root inoculum is best dried in a forced-air oven at 60 °C (Habte and Byappanhalli, 1998). Root inoculum dried under greenhouse conditions has a very short shelf life compared to oven-dried material, and even when dried in the oven has a shelf life of less than 100 days at 22 °C. It is possible to extend the shelf life of root inoculum through cold storage (Douds and Schenck, 1990). Storage is not a great issue for on-farm production of inoculum in the tropics since the inoculum are intended for use upon completion of the production cycle (Douds et al., 2005).

Application of inoculum

The application method and amount of inoculum applied depends on the type of inoculum and type of planting material (Habte and Osorio, 2001). Mixing inoculum thoroughly with the soil is the most straightforward method of applying inoculum in the field as well as in the greenhouse, but it is effective only when large amounts of inoculum are applied. This approach is better with crude inoculum than it is with root inoculum, because root fragments do not readily disperse in soil. Inoculum can be placed at various depths (up to 5 cm) from the surface of the soil as a layer or applied in bands near the seed row (generally 5 cm below and 5 cm to the side of it). Any type of inoculum can be placed close to seedling roots at the time of transplanting. For example, spores can be pipetted directly onto roots either at the time of transplanting or to roots of an established plant after making a hole adjacent to the roots.

Crude inoculum and root inoculum can also be applied to established plants by placing inoculum in holes bored into the soil where roots are likely to be contacted. Before planting, seedling roots can be inoculated by dipping them in a viscous medium (1% methyl cellulose or 10-20% gum arabic) containing AMF propagules, usually spores. If a crude inoculum contains four to eight infective propagules per gram, application of 50 g kg^{-1} soil usually produces rapid initiation of AMF colonisation of target plants with a minimal lag period. Root inoculum is generally more effective in stimulating plant growth in quantities substantially lower than are normal for crude inoculum.

Conclusions

The physiological and ecosystem effects caused by AMF range from an increase in the nutrient uptake efficiency of plants and improvements in plant stress resistance to improvements in soil structure and soil microbiology (Fester and Sawers, 2011; Gianinazzi et al., 2010). Furthermore, the close association of AMF with various other soil microorganisms suggests that many of the ecosystem effects apparently caused by AMF derive from complex interactions with other microorganisms within the mycorrhizosphere established by these fungi (Singh et al., 2008; Bonfante and Anca, 2009).

The global demand for AMF in the mycorrhiza-based industrial market was $268.8 million in 2019 and was projected to rise to $621.6 million by 2025 at an estimated compound annual growth rate of 14.8% (ReportLinker, 2020). However, commercial formulations of mycorrhizae are too expensive for many smallholder farmers and an alternative is needed to make this transformative biofertiliser accessible to these farmers. Studies have shown that significant increases in mycorrhizal colonisation resulted in yield increased yields averaging 23% (Lekberg and Koide, 2005). The ability of these farmers to produce mycorrhizal inoculum themselves can therefore lead to significant increases in yields which in turn will mean greater food security.

CHAPTER 3 DEVELOPMENT OF AN ON-FARM AMF INOCULUM PRODUCTION SYSTEM

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts, which means that they require the presence of actively growing plants during their reproduction. This makes production of large amounts of inoculum of AMF problematic (Habte and Osorio, 2001). The formulation and storage of the produced inoculum can also be problematic, as we are dealing with living organisms. Selling AMF inoculum on the market means to provide mycorrhizal inoculum (spores, mycelia and colonised roots) to users growing different crops in different environments. From these inoculum formulations, the AMF must colonize the roots of the host plants. The resulting symbiosis should increase the host's fitness in comparison to non-mycorrhizal plants (Feldmann and Schneider, 2020).

Several production systems have been developed for the large-scale production of AMF, each with its own advantages and disadvantages, as discussed in Chapter 2. Inoculum production can be either substate based (soil, crude and root inoculum) or substrate free (hydroponic and aeroponic systems) and there are advantages and disadvantages to each system.

Substrate based production systems often consist of nursery plots with soil or containers with different substrates, in which inoculated plants are cultured in open field or nursery beds (Sieverding et al., 1991). Advantages of these systems are that they are low cost, simple (low technology input), adapted for local use and enable the mass production of a single or a combination of AMF species. The disadvantages are that the produced inoculum has limited applications (less suitable for molecular and other laboratory studies), the systems are easily contaminated (Akhtar and Abdullah, 2014), and it is not well adapted for the development of an industrial activity (Feldmann and Grotkass, 2002; Feldmann and Idczak, 1994).

The main advantage of a substrate free systems is that the root pieces can be directly used as inoculum. However, the liquid nutrient solutions are highly prone to the growth and development of algal contaminants and the spore production rates could also be affected by lack of a carrier substrate (Akhtar and Abdullah, 2014).

The aim of this work package was to test the feasibility of an on-farm system for production of crude inoculum of locally adapted mycorrhizae and evaluate the success of the system.

Materials and Methods

Starter soil (veld soil) was collected from an undisturbed area (**Figure 3.1**) on the ARC-VIMP farm north of Pretoria (25°35'06.8"S, 28°20'35.8"E, elevation 1155.27 m). The vegetation was cleared away and digging down to a depth of about 25 cm, the soil and as many fine roots as possible were collected. Stony soil was sieved to get rid of large stones.

Figure 3.1 Clearing away vegetation in the undisturbed veld to collect starter soil.

A ‘trap-pot’ system was used to multiply inoculum. Both pot (inside a greenhouse) and trench trap systems (outside) were evaluated. Several large (5 litres) plastic pots/basins were used for the pot system, while three trenches (150 cm long x 50 cm wide x 50 cm deep) were dug into the ground and lined with a plastic sheet. The plastic was perforated to allow for drainage. The containers were filled with a mixture of red topsoil (purchased) and compost at a ratio of 5:1 and the starter soil were added at approximately 5% v/v to each container and mixed with the top 15 cm of soil. Four to five Bahia grass (*Paspalum notatum*) seedlings were planted into each pot and up to 30 were planted in the trenches (**Figure 3.2**). Maintenance consisted of watering and weeding as needed. Plants were grown for at least three months for inoculum production. Inoculum production was done in the winter months in Gauteng, which necessitated watering for the outside trap trenches as well, as there was no rain.

Figure 3.2 Trap trench lined with plastic, filled with red topsoil and compost mixture.

Ten days before harvesting the inoculum, watering was stopped. This killed the plants and tricked the fungus into producing reproductive spores. After 10 days, the inoculum was prepared by cutting off the tops of the grass and pulling up the roots of the bait plants, which were chopped into roughly 1 cm pieces and then mixed back into the soil from the trap-pot or trench (Figure **3.3**). This mixture of roots and soil served as the inoculum (Final inoculum).

Figure 3.3 Inoculum from trench and pots being mixed.

Approximately 1 kg of soil from the trap trenches as well as the trap pots, were collected for counting of sporocarps. Soil was also collected from the ARC-VIMP rain shelter and open field sites where the trials with the produced AMF were to be conducted (ARC-VIMP, 25°59′S; 28°35′E, elevation 1160 m). Soil was collected from the rain shelter and open field with a soil auger to a depth of 30 cm from five locations throughout each field. The five soil samples were mixed and representative samples from the rain shelter and open field, respectively, were taken for analyses. AMF spores from the soil samples (100 g) were extracted by wet sieving with 500, 425, 250, 106, 45 and 38 µm mesh sizes (**Figure 3.4**). The soil from each of the sieves was washed into separate Falcon tubes, centrifuged for 3 min, and then the water was poured off. This was followed by sucrose density centrifugation with 50% sucrose; whereafter sporocarps would be in the sucrose fraction. Sporocarps were washed under running water on a 38 µm sieve to remove all sugar, whereafter it was washed into 50 mL Falcon tubes. The total volumes were then adjusted to 30 mL. Fractions from the 500 and 425 µm were combined as well as the 45 and 38 µm sieves, leaving a total of four tubes from each sample. Sporocarps obtained from the sieves were counted using a stereo microscope at 8x magnification.

Figure 3.4 Test sieves of different sizes were used for wet sieving of AMF.

Ten mL of the collected spore suspension was pipetted onto the De Grisse Counting dish (**Figure 3.5**), and spores, spore clusters and sporocarps were grouped into morphological groups and counted under a stereo microscope. A Discovery V.8 stereo microscope with an Axiocam camera and Zen software was used to capture images. Families were identified following the descriptions of the International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (https://invam.ku.edu/).

Figure 3.5 De Grisse Counting dish used to count sporocarps.

Results and Discussion

The Bahia grass in the greenhouse (**Figure 3.6A**) grew more vigorously and larger than those outside (**Figure 3.6B**), although the root systems of the two sets of plants were comparable.

Figure 3.6 Bahia grass (*Paspalum notatum*) grown in trap pots in the greenhouse (A) and in the trap trench outside (B).

Table 3.1 gives the number of sporocarps recovered by wet sieving from each of the veld soil, topsoil (used in trap pots and pits), rain shelter and open field where the trials were planted, as well as the trap trenches and pots after three months and finally the inoculum produced.

The veld soil, which was collected to serve as starter inoculum, had the highest number of sporocarps, followed by the greenhouse trap pots and then the trap trenches. The lowest numbers of sporocarps were recovered from the red topsoil that was purchased for use in greenhouse trials.

Table 3.1 The number of sporocarps recovered from 100g of soil.

Source	Number of sporocarps
Veld soil (starter soil)	4 644
Topsoil	78
Rain shelter	558
Open field	1 635
Trap trench	2 133
Trap pot	3 054
Final inoculum (trap pot + trench)	2 623

Observed sporocarps (**Figure 3.7**) could be broadly classified into 13 morpho-groups based on colour and size. Based on morphological identification, most of these isolates belonged to the Glomeraceae family, but some Gigasporaceae were also present.

The highest diversity of AMF was found in the veld soil that was used to inoculate the trap pots and trap trenches, with 14 different morpho-groups present (**Table 3.2**). All of these groups were present in comparable ratios in the inoculum produced. The lowest diversity was found in the topsoil, with only five morpho groups, followed by that of the rain shelter, with seven morpho groups. The open field had slightly more diverse, with nine different morpho-groups.

The trap pots in the greenhouse and trap trenches outside together had all of the same morpho-groups as the veld soil; however, the different systems enriched different groups of AMF. The outside pits had 35% of the white (<100 µm) sporocarps, which is more than double the amount of the next largest groups, namely the yellow and reddish-brown sporocarps at 9 and 12%, respectively. In the greenhouse, there were almost equal amounts of the white and yellow (<100 µm) sporocarps, with 27 and 31%, respectively. The groups that were present in low numbers in the veld soil were not all present in both the trap pots and pits.

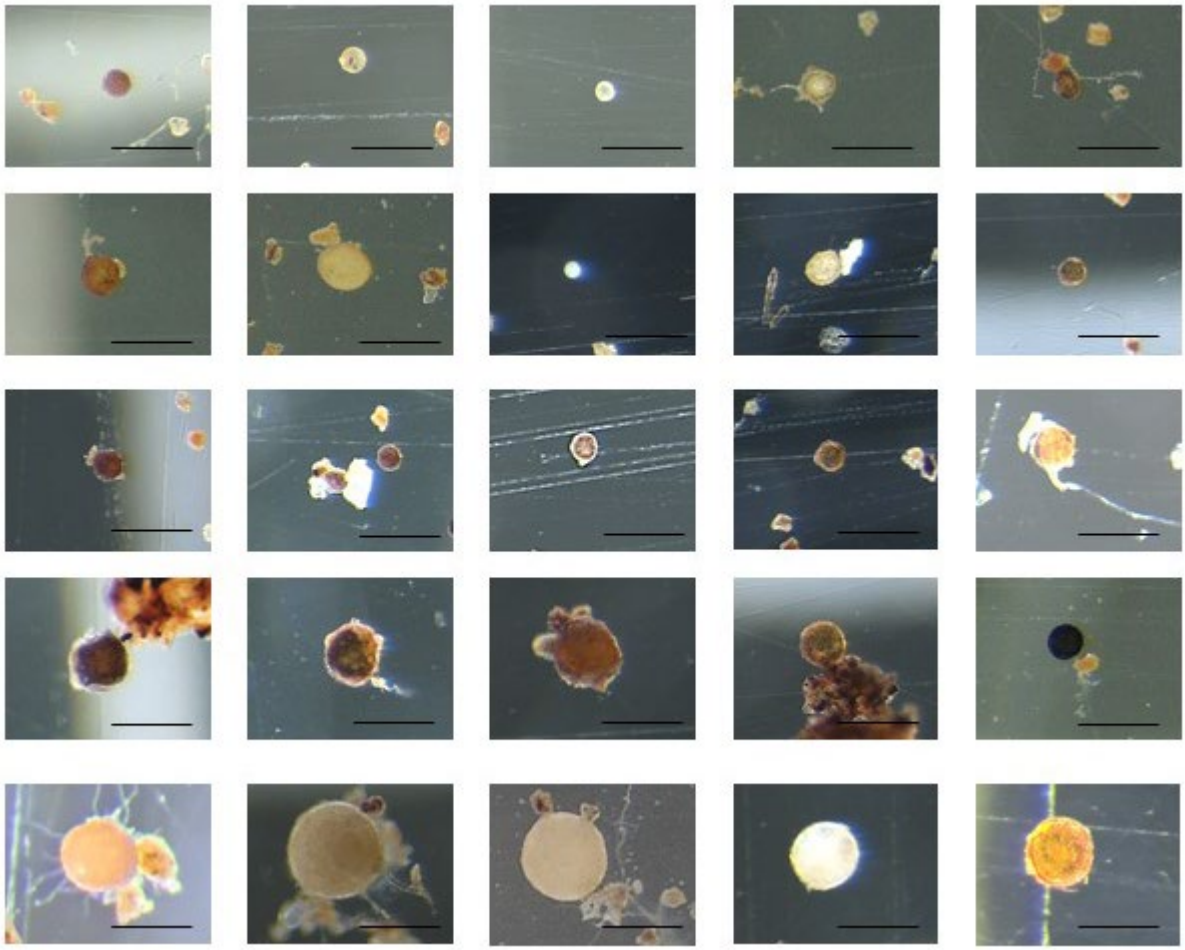


Figure 3.7 Diversity of AMF sporocarps was observed, ranging from white to yellow, brown and black. Sizes vary from 50 µm to more than 200 µm. Scale bars are 200 µm.

Table 3.2 Morphological groups of AMF and percentage of sporocarps in each group from different sources.

Morpho-group	Veld soil	Topsoil	Trap pot (Greenhouse)	Trap trench (outside)	Inoculum	Rain shelter	Open field
White >100 µm	2		5	10	1	4	3
White <100 µm	26	20	27	35	36	18	49
Cream ~ 100 µm	1			3	5		
Yellow >100 µm	3		3	7	6	5	1
Yellow <100 µm	29	33	31	9	10	20	24
Black <100 µm	21	27	15	8	9	38	16
Black >100 µm	2		2	2	2	8	1
Reddish-brown <100 µm	5	7	5	12	9	9	2
Reddish-brown >100 µm	1		1		3		
Yellow-brown	1			6	5		
Brown ~ 100 µm	2	13	6	6	3		2
Grayish-brown ~ 100 µm	1		0.5	2	4		
Light brown	2		4		7		2
Green-yellow ~ 100 µm	1		0.5				
Number of morpho-groups	**14**	**5**	**12**	**11**	**13**	**7**	**9**

The trap pot method used to multiply the AMF for crude inoculum was effective, with a 39-fold increase in the number of sporocarps in the topsoil that was used to produce the inoculum. The vigorous growth by the grass in the greenhouse led to higher numbers of AMF sporocarps being produced in the pots in the greenhouse when compared to the trap trenches outside. However, the trap trenches only had 30 % less sporocarps produced than the pots in the greenhouse.

Commercially produced inoculants contain more than 100 propagules per gram (Basiru et al., 2021) compared to the on-farm produced inoculum with 26 propagules per gram. Although some products with multiple AMF species have higher propagule concentrations, this is not a rule, as many inoculants with multiple AMF species were found to have fewer propagules than single-species inoculants (Basiru et al., 2021). Crude inoculum produced on-farm therefore has much lower numbers of propagules but are produced at a fraction of the cost of commercial inoculum.

The inoculum produced was used in field trials that were planted in a rain shelter and open field at Roodeplaat, which are reported in Chapters 4, 5 and 6. This soil in the rain shelter is especially relatively poor in naturally occurring AMF with eight times less sporocarps than the undisturbed veld soil (starter soil). The low number of AMF is probably due to the intensive land use with no return to natural veld under the rain shelter. The number of AMF sporocarps in the open field is almost three times higher than in the rain shelter. This may be because the open field is not as intensively used as the rain shelter and had been covered with weeds in the previous season.

Intensively farmed fields generally have the lowest numbers of AMF. Douds et al. (1995) found that farming systems and tillage regimes had significant effects on the AMF populations, with low input methods leading to the highest numbers of AMF. Reduced tillage, especially no-till, leaves the extraradical mycelial network in the soil intact. This promotes rapid colonisation of a new crop and enhances early-season mycorrhiza-mediated P uptake (McGonigle and Miller, 1993). Other factors that will increase AMF activity and survival are crop rotations, cover crops, and phosphorus management (Douds and Johnson, 2003).

Crude inoculum should not be stored long-term but rather used in the same season (Douds et al., 2005). However, the general rules for storage of AMF inoculum are to keep it in a cool, dry environment (Daft et al., 1987). The compost-based inoculum generation approach in temperate areas makes use of the AM fungi's inherent capacity to survive the winter and is intended for use right away (Douds et al., 2005).

The trap trench system is a simple, low-cost production system for AMF crude inoculum that will be accessible to all farmers. Care should be taken to use starter soil from undisturbed or natural veld to increase the number and diversity of AFM starting materials. The trap trenches should be filled with soil where no agricultural crops have been planted to limit the chances of spreading soil-borne diseases. Trap plants should not be liable to become weeds and should not be quick to produce seed.

The trap pot system (both in pots and trenches), used to multiply the AMF for inoculum successfully trapped the diversity of AMF present in the natural veld soil. Either system will provide adequate amounts and diversity of AMF for smallholder farmers. Species level

identification of AMF is difficult based on morphology alone; however, most morphological groups could be classified in the Glomeraceae family, with some Gigasporaceae also present.

The Glomeraceae family includes abundant genera such as *Glomus*, *Rhizophagus*, *Funneliformis* and *Septoglomus*, which have been reported in all continents and are the genera found most often in commercial inoculants (Basiru et al., 2021). Members of the Glomeraceae have shown different levels of effectiveness in colonizing host roots and performing under various field conditions. For instance, under different management strategies, species of *Glomus* and *Rhizophagus* have reportedly outperformed other genera, such as *Gigaspora* and *Scutellospora*. (Veresoglou et al., 2011). The diversity of AMF present in the produced inoculum should therefore enhance the chances of successful colonisation of crop plants under different conditions.

The roots of the trap plants were cut into approximately 10 mm pieces and mixed back into the inoculum soil. These roots are colonised by the AMF and serve to increase the number of propagules that are available to colonise the newly inoculated plants. The number of sporocarps present in the inoculum is therefore an underestimation of the total number of AMF propagules available, meaning that this method is an effective means of on-farm AMF production.

Conclusions

Although there are many different methods for producing AMF inoculum, the only method that is suitable for low cost on-farm production is to produce crude inoculum. The use of on-farm soil as a starter culture has the added benefits that the indigenous AMF will be more effective in promoting plant growth in the local soil and climate than introduced species (Douds et al., 2005; Sreenivasa and Bagyaraj, 1989). Another benefit it that *Gigaspora* spp., which is normally present in healthy soils are usually absent from commercial inoculants. These species are good producers of glomalin, which aids in soil aggregation, thereby improving soil structure (Miller and Jastrow, 2000).

Significant quantities of a taxonomically diverse inoculum can be produced using materials readily available to farmers. This technique saves the associated costs of processing and shipping, which are included in the price of commercially available inoculum. These factors, along with proven crop production improvements, point to the possibility of higher financial returns for farmers using AMF and the accompanying environmental benefits derived from reduced use of pesticides and fertilisers.

CHAPTER 4 THE EFFECT OF AMF AND WATER REGIMES ON GROWTH OF SWEET POTATO CULTIVARS

Introduction

Sweet potato, (*Ipomoea batatas* (L.) Lam.), a root crop that stores carbohydrates, is a significant source of food, bioethanol, β-carotene and micronutrients (Laurie et al., 2012). It is primarily grown in rain-fed or low-precipitation locations (Gomes et al., 2005). It is well established that sweet potato plants grown under water deficit stress exhibit decreased growth and yield, as well as impairment in various physiological responses such as low water potential, pigment degradation, chlorophyll fluorescence, CO_2 assimilation limitation, low stomatal conductance and decrease in net photosynthetic rate (Haimeirong and Kubota, 2003; van Heerden and Laurie, 2008; Yooyongwech et al., 2013). Previously, research has shown that inoculating sweet potato with arbuscular mycorrhizal fungus (AMF) increases shape, sugar and carotene content, and tuber production (Farmer et al., 2007; Saraswati et al., 2012; Tong et al., 2013).

AMF have been demonstrated to have a positive effect on crop growth and production under a variety of environmental situations by building intimate symbiotic interactions with plants (Redecker et al., 2000). AMF can be thought of as an extension of the root system, increasing the host's water and nutrient uptake capacity (Bona et al., 2017). Thus, the presence of functionally suitable AMF is critical for improving the plant performance of vegetable crops, especially in the case of shortage irrigation (Miceli et al., 2016). Many commercial microbial biostimulants containing AMF are readily available on the market, and their use in agricultural fields is expanding (Gianinazzi, 2014). Several experiments conducted in various environmental conditions and on a variety of crops have demonstrated that when indigenous fungi are absent or present in low levels in the rhizosphere, soil inoculation with AMF can be a viable and rewarding way to benefit plant growth and yield (Miceli et al., 2016; Nzanza et al., 2012).

The objective of this study was to determine the effect of on-farm produced mycorrhizal inoculum on orange flesh (Bophelo) and white flesh (Blesbok) sweet potatoes. The trials were conducted in a rain shelter to evaluate the effect of different irrigation levels in combination with AMF as well as in the open field to evaluate different amounts of inoculum applied.

Materials and Methods

Study site and treatment details

The study was conducted under a rain shelter and open field at the Agricultural Research Council - Vegetable Industrial and Medicinal Plants (ARC-VIMP 25°59′S; long. 28°35′E, elevation 1160 m), Pretoria, South Africa. The experiment was conducted during the 2022/23 season and repeated in the 2023/24. Before setting up the experiment, soil samples were collected at a depth of 0-30 and 30-60 cm to determine the study site's chemical and physical properties (**Table 4.1**). The soil at the experimental site is a sandy clay loam of the Hutton form (Soil Classification Working Group 1991), with a 20 to 22% clay content. During the experimental period, weather data was obtained from an automatic weather station using an automatic weather station (Campbell Inc., Nebraska, USA) installed close to the experimental

field. **Figure 4.1** illustrates the daily average air temperatures (maximum and minimum), relative humidity (maximum and minimum), rainfall and evapotranspiration for the 2022/23 and 2023/24 seasons. Recommended nitrogen, phosphorus and potassium fertilisers were applied based on the soil nutrient status (**Table 4.1**). Fertiliser was applied according to soil analysis results done at the Agricultural Research Council - Natural Resources and Engineering (ARC-NRE), Pretoria, South Africa (**Table 4.1**).

Table 4.1 Physical and chemical characteristics of the soil at the experimental site.

Trial period	Soil Sample depth (cm)	Soil texture (%)			P*	K	Ca	Mg	Na	pH
		Sand	Silt	Clay	mg/kg					H2O
2022/2023	0-30	74	4	22	91.1	94.0	830	287	102	7.52
	30-60	78	2	20	63.6	79.0	744	253	113	7.42
2023/2024	0-30	86.0	4.0	10.0	54.73	76	698	254	48.3	7.38
	30-60	82	6.0	12.0	42.36	68.5	661	226	43.7	7.26

*P-Bray 1

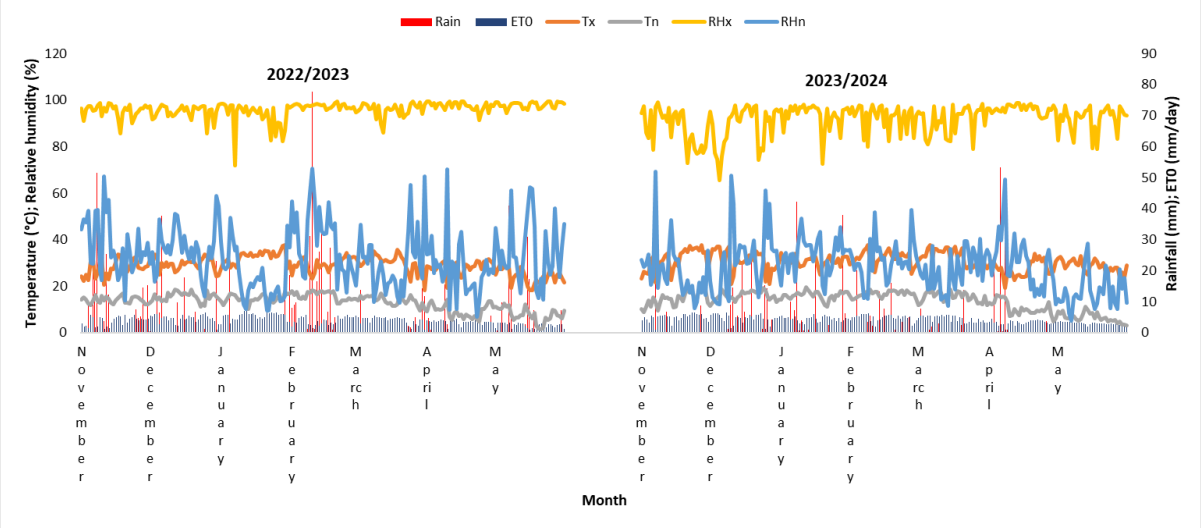


Figure 4.1 Weather conditions at ARC-VIMP experimental site during the 2022/23 and 2023/24 seasons.

Two trials were planted: the first trial was planted in a rain shelter, where during a rainfall event, a rain sensor triggers an electric motor, which closes and the shelter's roof and covers the experiment. The trial was laid out in a 3 by 2 by 2 factorial design (RCBD), replicated 3 times. Where there were three irrigation regimes (irrigation 1 - soil refilled to field capacity when 30% of available soil water (ASW) was depleted, irrigation treatment 2 - refilled to field capacity when 50% of ASW was depleted; and irrigation treatment 3 - refilled to field capacity when 70% of ASW was depleted). Two cultivars of sweet potato (orange flesh – Bophelo (OFSP) and white flesh – Blesbok (WFSP)) were planted with two treatments namely, with AMF and without AMF. The plots had 3 rows where the spacing between plants was 30 cm,

and between the rows was 60 cm. Each row had 9 plants, with the total number of plants per plot being 27 and the plot size 4 m^2. The drip irrigation system was installed in the rain shelter, which was used to provide 3 different levels of water provision to the plants. The sweet potato cuttings were obtained from the ARC-VIMP sweet potato breeding scheme.

Soil water content was monitored using a calibrated neutron probe (503DR CPN Hydro Probe neutron water meter (Campbell Pacific Nuclear, California, USA) at the experimental site. Access tubes were placed in each plot, and measurements were taken at 0.2-meter intervals down to 0.8 meters. Irrigation scheduling was based on the depletion of available soil water (ASW), which is the difference between field capacity and permanent wilting point. While measurements were taken to 0.8 meters, irrigation was specifically managed based on the ASW depletion within the sweet potato's effective root zone, estimated at 0.4 meters. The percentage of ASW depletion was calculated using a formula by Panda et al. (2003).

$$Depletion\ of\ available\ soil\ water = 100\frac{1}{n}\sum_{1}^{n}\left(\frac{FC_i-\theta_i}{FC_i-PWP_i}\right)$$ [Equation 1]

Where *n* is the number of layers of the actual rooting depth used in the soil water content measurement,
FCi is the soil water content at field capacity for the i^{th} layer, *q*i is the soil water content in the i^{th} layer, and
PWPi is the soil water content at a permanent wilting point on a volume basis.

Crop water use or evapotranspiration (ET) was determined by using the soil water balance of equation (Tesfaye et al. 2006), as follows:

$$ET = I + P - D - R - \Delta S$$ [Equation 2]

where, ET refers to crop water use/crop evapotranspiration (mm), I represents irrigation (mm), P represents precipitation (mm), D refers to drainage (mm), R is the surface runoff (mm), and ΔS is the change in soil water storage between planting and harvest (mm). Runoff and drainage were assumed to be negligible, as irrigation was carefully managed to prevent over-irrigation or runoff, and rain was excluded by the rain shelter.

The second trial was planted in an open field and was laid out in a RCDB where the was four treatments (150 mL AMF, 250 mL AMF, 350 mL AMF and control without AMF), two sweet potato cultivars were planted (as above) replicated three times. Plots consisted of four rows with 10 plants each with 30 cm spacing between plants and 60 cm between rows. The field was irrigated as needed when there was no rain to limit water stress.

Application of AMF in the field

AMF was applied on the planting day (**Figure 4.2**) by opening holes using the rake handle and sweet potato cutting inserted into the hole. Then 250 mL of AMF inoculum was poured into

the hole, which was then closed using soil. In the open field, 150 mL, 250 mL and 350 mL was measured with a measuring cup

Figure 4.2 AMF inoculum applied with sweet potato cutting.

Equipment Used for Data Collection

Neutron probe

A Hydroprobe (Model 503DR CPN Campbell Pacific nuclear Inc., California, USA), was used to measure the soil moisture content during the first season, as per recommendations by Mulovhedzi (2017). A total of 40 access tubes of 1.2m were installed in the rain shelter using a soil auger. The calibration was done in representative soil on the site. Measurements were taken on a wet spot and a dry spot. The dry spot was prepared by leaving the soil to dry out for five months. The wet spot was prepared by saturating the soil, to reach field capacity (FC), the entire profile was soaked and allowed to drain for 48 hours. The gravimetric approach was utilized to determine the soil water content (SWC) from wet (FC) and dry (PWP) spot several times, while simultaneously obtaining counts with the Hydroprobe at 0.2 m intervals of a 1 m soil profile. Five neutron probe access tubes were installed to a depth of 1 m, in the field. twice a week, the sensor was lowered through the access tubes to measure the amount of water in the soil. *Soil Plant Analysis Development (SPAD) meter*

A SPAD was used to estimate the chlorophyll content in plant leaves, which provides an indication of the plant's health and photosynthetic activity. Chlorophyll is a pigment essential for photosynthesis, and its concentration can give insights into the plant's ability to produce energy through sunlight absorption. Three plants were selected per plot and SPAD readings were taken for each plant (**Figure 4.3**).

Figure 4.3 Chlorophyll content measurement being taken in sweet potato field.

Infrared Thermometer:

An infrared thermometer (**Figure 4.4**) was used to measure the temperature of plant surfaces without making physical contact. These devices detect the infrared radiation emitted by the plant and provide instantaneous temperature readings. Three plants per plot were measured to assess the temperature of leaves and stems. The soil surfaces temperature was also measured.

Figure 4.4 Infrared thermometer taking temperature reading.

Handy Plant Efficiency Analyzer (PEA) chlorophyll fluorometer

Photosynthetic efficiency as indicated by chlorophyll fluorescence (CF) was measured using a PEA chlorophyll fluorometer (Hansatech Instruments, U.K.) (**Figure 4.5**). The measurement of CF was done weekly. Three plants per plot were initially dark adapted for 30 minutes before CF measurements were taken and single measurement were taken per plant. Values of Fv/Fm (the measurement of quantum yield potential of photosynthesis, or maximal photochemical efficiency of PSII) were recorded and used for analysis.

Figure 4.5 Handy PEA clip taking readings.

Ceptometer

A Ceptometer was used to measure the leaf area index, photosynthetic active radiation and fractional interception. One reading was taken from above (**Figure 4.6**) and one reading from below the plant at the rain shelter and at the field one reading from above was taken and two readings from below the plants. Data was collected weekly with a Decagon LP-80 Ceptometer, on rainy and cloudy days data were not collected.

Figure 4.6 Ceptometer used to take reading above plants.

Root Scanner

A 1.1 m long transparent acrylic tube was installed vertically at a 45° angle, with 0.1 m protruding above the soil surface as per recommendations by Iversen et al. (2011). To guarantee a snug fit, the acrylic tubes were inserted between plants inside a row by excavating a hole with a matching diameter auger. One acrylic tube was installed in each treatment, however due to the shortage of acrylic tubes, only 8 acrylic tubes were installed. The bottom apertures were closed to prevent water from entering the tubes. Similarly, when not in use, the upper openings of the tubes were closed to prevent water and debris from entering. Every 0.2 m up to 0.8 m deep, a probe was placed into each tube to assess root growth. By scanning 360° of the interior of the acrylic tube with a portable laptop and a scanner (CI-600 Root Scanner, CID Bio-Science, Camas, WA, USA), take and store root photographs with a 300

dpi resolution. When the crop reached maturity, the root scanner was used to collect non-destructive root samples (**Figure 4.7**).

Figure 4.7 Photo from root scanner showing sweet potato root development and AMF symbiotic relationships.

Quantification of AMF

Approximately 1 kg of soil (composite samples from the three replicates) from each of the 12 treatments in the rain shelter and eight treatments in the open field, were collected after harvest for counting of sporocarps. AMF spores from the soil samples (100 g) were extracted and characterised as described in Chapter 3.

Analyses of soil microbial composition

Soil samples were suspended in saline buffer and homogenized. 150 µL of the aqueous suspension was inoculated on 96 well Biolog microplates which were then incubated at 26 °C for one week. The rate of colour change (pattern development) in each well was recorded and the optical density (OD) of each well was read on Biolog MicroLog™ 3E or MicroStation™ Systems every 24 hours for seven days until the readings became constant. When the OD

reading started to show consistency over the duration of incubation, the last reading was taken for statistical analysis. Before analysis, each plate's reading was normalized by subtracting the blank well (control) values from each plate well. Total carbon source utilization (microbial response) was quantified by the average well colour development (AWCD) in each well as and data was statistically analysed. Similarly, the specific utilization of all carbon sources for the group substrates (carbohydrates, amino acids, amines, polymers, and carboxylic acids was determined. 1.7. Using an OD of 0.25 as a threshold for positive response, the Shannon Weaver index (H) value i.e. the richness and evenness of response of the microbial communities was calculated.

Results and Discussion

The amounts of water used by each treatment of two sweet potato cultivars during the 2022/23 growing season for the rain shelter trial are presented in **Table 4.2**. The data showed significant differences among water treatments, but the sweet potato cultivars did not significantly differ in water use for the same treatments within a specific season. The results are clear that, for OFSP, the well-watered treatment (30% ADL) showed significantly higher water use and fresh biomass yield when compared to other treatments. Water use significantly decreased with increased water stress levels (30-50% ADL) for both cultivars. However, a further increase in stress to 70% ADL had no significant effect on crop water use. As expected, when the irrigation interval became longer (higher ADL percentage), the topsoil layer dried out more, the proportion of water that was taken up from the deeper soil layers (0.4-0.80 m) increased, and overall less water in total was taken up (Zhang et al., 2004).

Table 4.2 Total fresh biomass yield (tuber) and water use of two sweet potato cultivars subjected to different irrigation regimes during the 2022/23 and 2023/24 growing seasons.

Cultivar	AMF / Control	MAD (%)	Season	Fresh tuber biomass yield (t ha^{-1})	Seasonal crop water use (mm)	Crop water productivity (t ha^{-1} mm^{-1})
Orange flesh	AMF	30	**1**	53.94^{a}	505.87	0.11
		50		46.72^{a}	455.84	0.10
		70		17.44^{b}	253.05	0.07
	C	30		45.28^{a}	445.84	0.10
		50		36.78^{a}	386.96	0.10
		70		21.33^{b}	279.99	0.08
White flesh	AMF	30		37.67^{a}	289.78	0.13
		50		41.00^{a}	307.77	0.13
		70		28.72^{b}	241.52	0.12
	C	30		27.39^{b}	234.32	0.12
		50		22.83^{b}	209.74	0.11
		70		19.89^{b}	193.85	0.10
Orange flesh	AMF	30	**2**	43.86^{a}	323.18	0.14
		50		23.24^{b}	211.95	0.11
		70		22.74^{b}	209.26	0.11
	C	30		31.17^{a}	254.71	0.12

Cultivar	AMF / Control	MAD (%)	Season	Fresh tuber biomass yield (t ha^{-1})	Seasonal crop water use (mm)	Crop water productivity (t ha^{-1} mm^{-1})
		50		20.72^{b}	198.34	0.10
		70		18.56^{b}	186.68	0.10
White flesh	AMF	30		14.98^{b}	235.96	0.06
		50		15.31^{b}	238.27	0.06
		70		14.39^{b}	231.88	0.06
	C	30		28.87^{a}	332.16	0.09
		50		13.80^{b}	227.81	0.06
		70		14.14^{b}	230.19	0.06

Values in a column with the same letter are not significantly different at $p<0.05$.

The cultivars showed differences in crop water productivity, as indicated in **Table 4.2**. Crop water productivity of OFSP was higher under 30% ADL. However, water stress levels did not influence the water productivity of WFSP. In addition, WFSP's crop water productivity was higher than OFSP. Similar findings were reported by Mulovhedzi (2017), where the water productivity of WFSP under full irrigation, supplemental irrigation and rainfed treatments were higher than the water productivity of OFSP under the same treatments. This was attributed to WFSP's ability to produce higher yields per unit of water used, indicating more efficient water utilization compared to OFSP.

Plant growth parameters

The results showed differences between the treatments in the vine length during both cropping seasons in response to the different irrigation regimes for both cultivars (**Figure 4.8** and **Figure 4.9**). WFSP under 30% and 50% ADL showed longer vines than the other treatments. Plant height at low to moderate water stress resulted in similar heights for both cultivars, while the severely stressed plants were stunted the most. Mofokeng et al. (2015) also reported that the plant height of *Pelargonium sidoides* DC was reduced under severely stressed conditions compared to the well-watered treatments. Mabhaudhi et al. (2011) reported similar results for Bambara groundnut. The AMF inoculation did not lead to differences in vine length for highly water stressed (70% ADL) treatments.

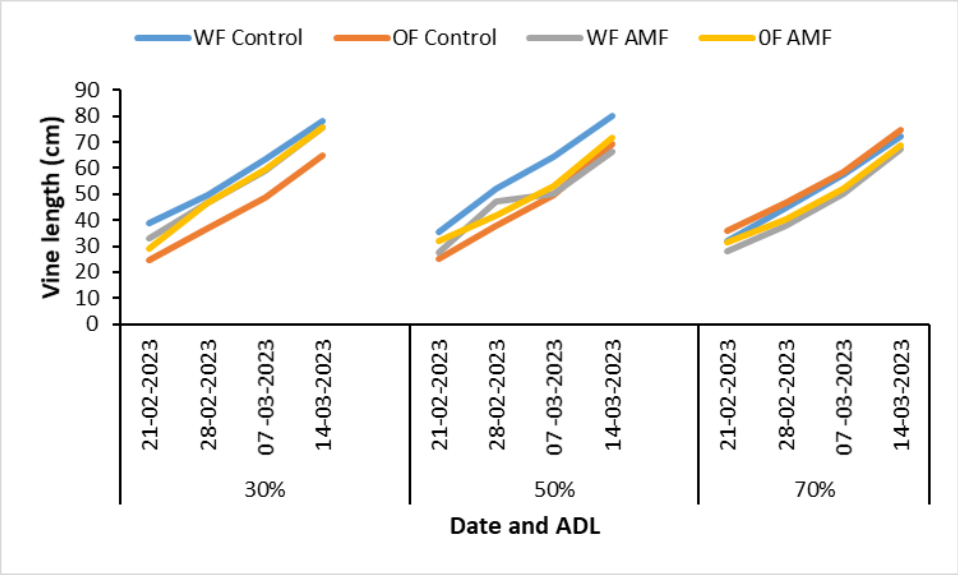


Figure 4.8 Vine length (cm) of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) during 2022/23 season.

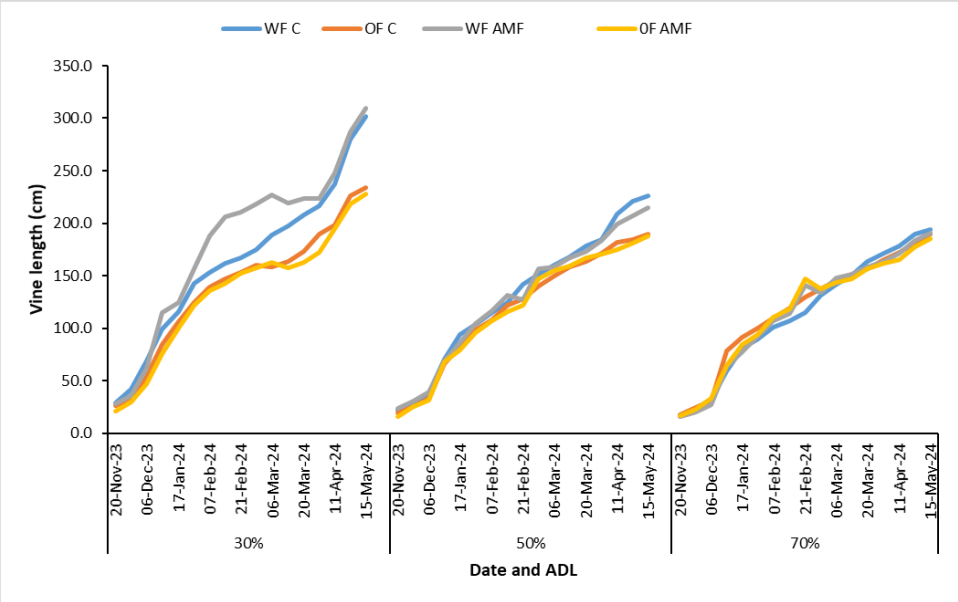


Figure 4.9 Vine length (cm) of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) for the 2023/24 season.

The chlorophyll content leaf readings (**Figure 4.10** and **Figure 4.11**) on the treatment for both cultivars revealed lower values for 50% and 70% ADL. The results indicated that using a SPAD 502 meter, it should be possible to detect species differences in chlorophyll content per unit leaf area at different treatments. Previous research has found a substantial association between SPAD readings and N fertiliser rate at various harvesting times (Debaeke et al., 2006). According to Cartelat et al. (2005), chlorophyll readings can effectively detect abiotic stress, such as N shortages in growing crops. However, the chlorophyll content cannot be

used to anticipate the amount of nitrogen to apply to a crop during the growing season. In both dryland and irrigated pumpkins, a positive connection was seen between chlorophyll content and leaf N levels (Swiader and Moore, 2002). According to Argenta et al. (2004), a portable chlorophyll meter is a tool for instantaneously assessing plant N status. Swiader and Moore (2002) also commented on the chlorophyll meter's potential use as an N management tool for determining plant N condition. However, depending on plant species and growth conditions, chlorophyll content levels in response to N requirements may differ.

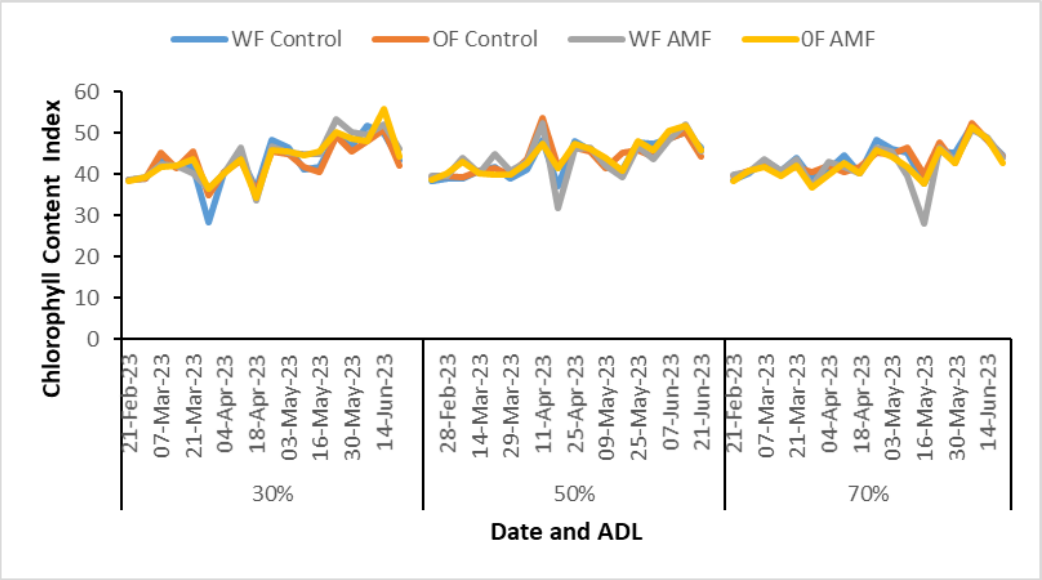


Figure 4.10 Chlorophyll content reading of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) for the 2022/23 season.

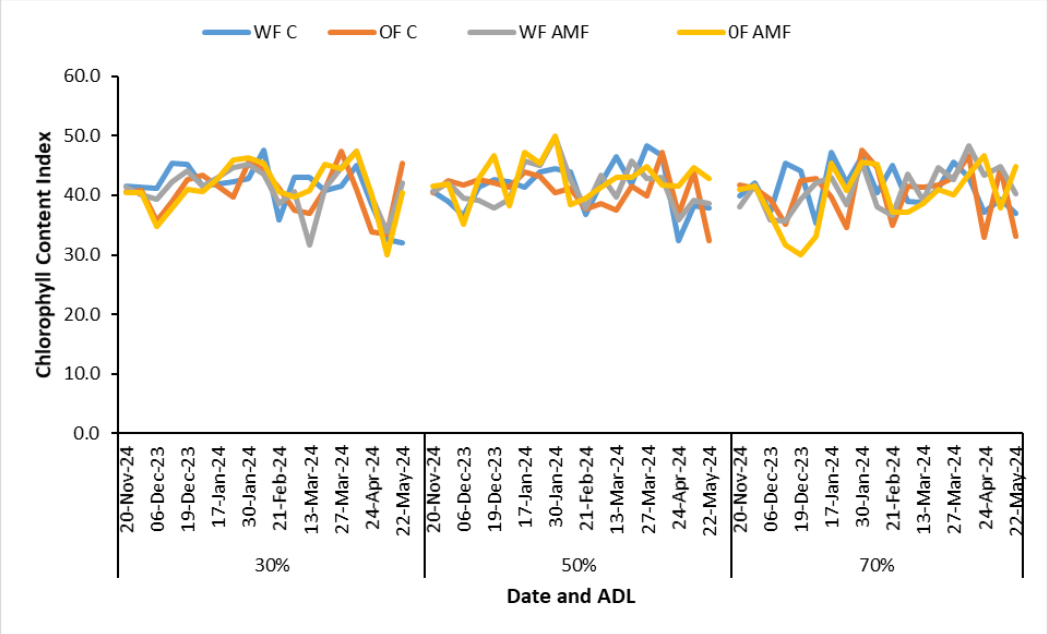


Figure 4.11 Chlorophyll content of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) for the 2023/24 season.

Plant physiological activity can be influenced by air temperature, leaf temperature, and even media and nutrient solution temperature. Furthermore, comparing plant temperature to air temperature can indicate whether or not a plant is stressed (Niu et al., 2019). Both cultivars showed a response in canopy temperatures with moisture stress levels. The canopy temperature of both cultivars was lower in the well-watered (30% ADL) treatment compared to the moderate (50% ADL) and severe water-stressed (70% ADL) treatments (**Figure 4.12** and **Figure 4.13).**

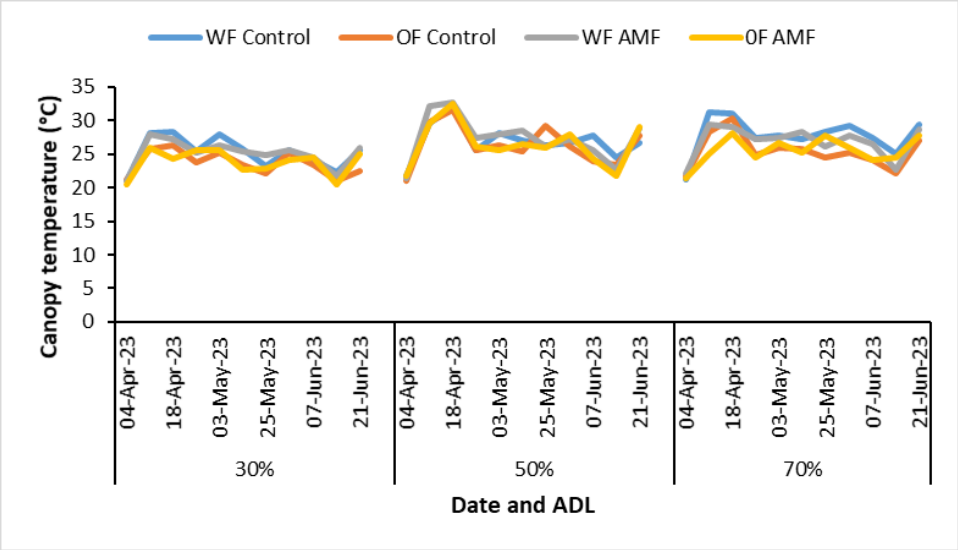


Figure 4.12 Canopy temperature of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) for the 2023 season.

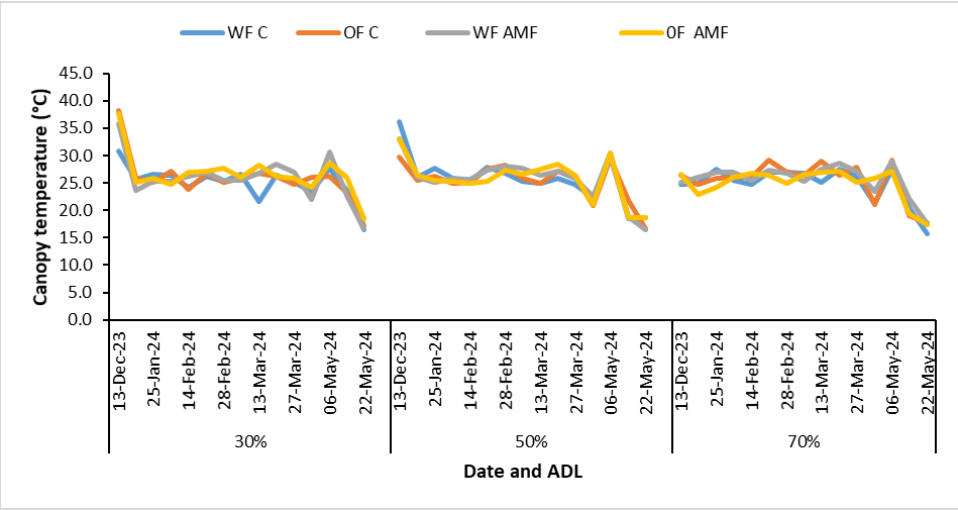


Figure 4.13 Canopy temperature of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) for the 2023/24 season.

Yield

The fresh sweet potato yield differed significantly ($p < 0.05$) between cultivars and irrigation regimes (**Figure 4.14** and **Figure 4.15**). The highest fresh sweet potato yield was obtained for both cultivars in well-watered circumstances (30% ADL). When compared to stress treatments, well-watered plants produced more. This was attributable to the 30% ADL treatments having greater values for growth characteristics such as plant height, LAI, and SPAD. However, stress treatments lowered transpiration, resulting in poorer photosynthesis and lower storage organ production. During the both seasons, the OF AMF treatment under 30 % ADL showed an increase in yield over the control from 32 to 38 t ha^{-1} in 2022/23 and 31 to 42 t ha^{-1} in 2023/24. Under 50% ADL the WF AMF treatment had an increase of ten t ha^{-1} in both seasons. AMF inoculation under 70% ADL were not beneficial to yield. Tubers were unmarketable mainly due to mechanical damage from animal predation, harvest, insects and all tubers that were less than 100g. No significant diseases were recorded during the trials.

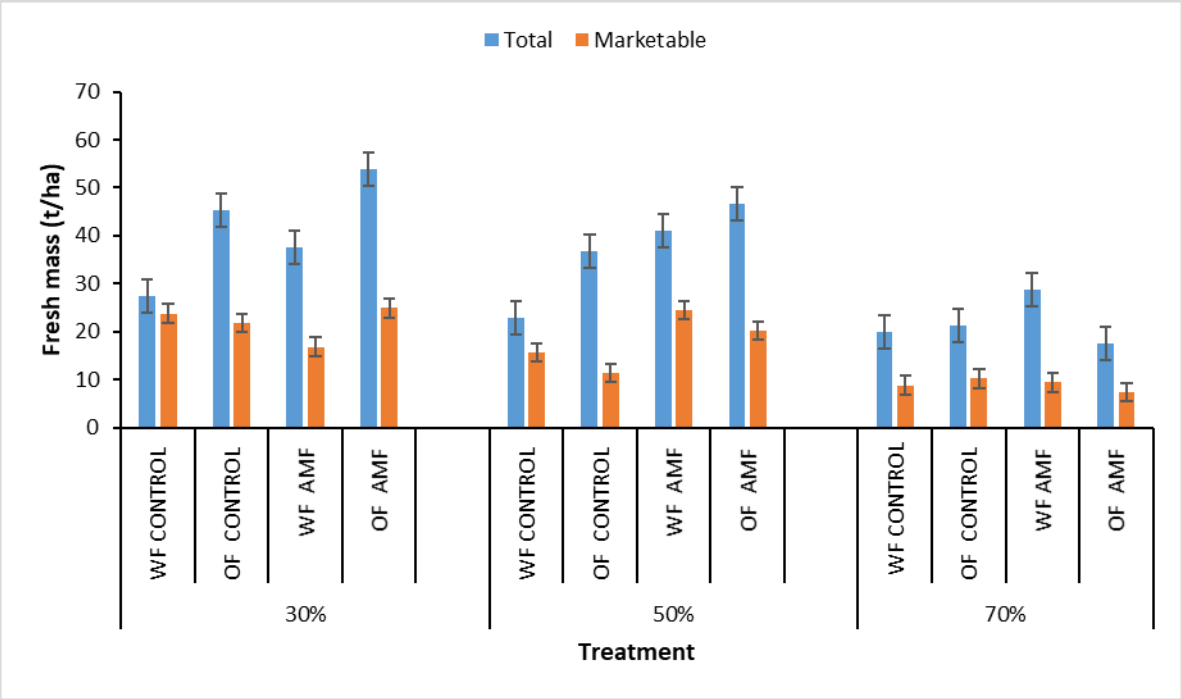


Figure 4.14 Total fresh and marketable yield (tubers) of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) for 2022/23 season.

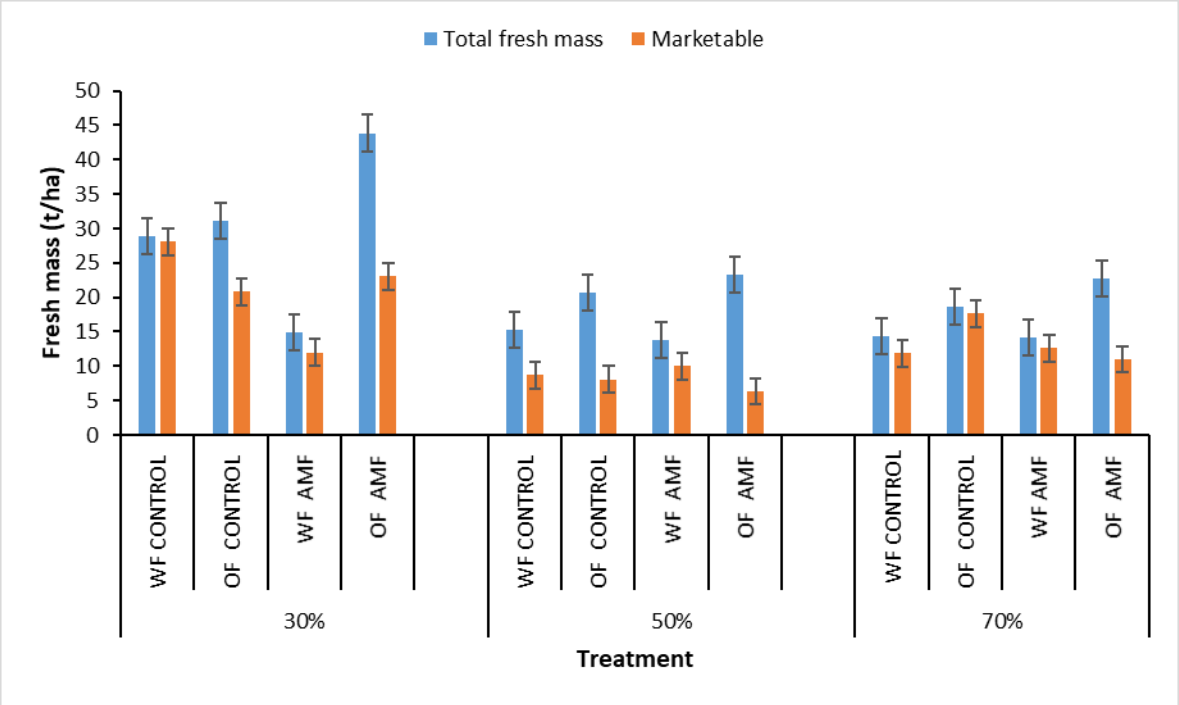


Figure 4.15 Total fresh and marketable yield (tubers) of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) for 2023/24 season.

Results of fresh tuber mass between the different seasons differed significantly for WFSP with increased yield in for AMF inoculated treatments in all watering regimes in season one and decreases in season two. Under 30% ADL there was a 10 t ha^{-1} increase in yield in the AMF over control in season one and a 13 t ha^{-1} decrease in season two. Under 50% ADL season one had 18 t ha^{-1} increase and 1 t ha^{-1} decrease in season two. For the OFSP both seasons showed an increase in yield for AMF under 30 and 50% ADL. Under 30% ADL season one and two had 8.7 t ha^{-1} and 13 t ha^{-1} increase in yield for respectively, while 50% ADL showed 10 and 2.5 t ha^{-1} increase for the two seasons respectively.

This highlights the significance of efficient irrigation management to maximize output at any crop evapotranspiration level (Bekele and Tilahun, 2007). Under water-stressed conditions, the effect of water depletion level on cultivars revealed that maximum available depletion of less than 50% resulted in no variations in fresh tuber production. WFSP output was only lowered by about 10%, whereas even mild stress of 50% ADL reduced yield by half. Other drought-sensitive crops, such as potatoes, have shown similar effects (Onder et al., 2005; Kirda, 2002).

Similarly, as water stress levels increased, the commercial yield of both cultivars decreased. Earlier experiments on onion found that well-watered treatments yielded higher yields than stressed treatments (Mulu and Alamirew, 2012). Peanut yield was reduced by 80% when accessible soil water was depleted, according to Reddy and Reddy (1993). A study of several irrigation regimes on potato development and yield found that total fresh and marketable tuber output rose with increasing irrigation (Kirda, 2002).

AMF composition in soil after harvest

The total number of sporocarps recovered from soil in the rain shelter after harvest is presented in **Table 4.3**.

Table 4.3 Mycorrhizal sporocarps recovered from rain shelter after harvest.

Treatment	No. of sporocarps 2022/23	No. of sporocarps 2023/24
WFSP 30% ADL Control	648	654
WFSP 30% ADL AMF	1371	858
OFSP 30% ADL Control	570	1002
OFSP 30% ADL AMF	588	2310
WFSP 50% ADL Control	876	969
WFSP 50% ADL AMF	816	2472
OFSP 50% ADL Control	882	563
OFSP 50% ADL AMF	1644	1254
WFSP 70% ADL Control	828	876
WFSP 70% ADL AMF	1299	1005
OFSP 70% ADL Control	486	698
OFSP 70% ADL AMF	1650	1704

The total number of sporocarps present in soil before the trial was 558 sporocarps per 100 g soil. During the first trial (2022/23), the number sporocarps had increased after the treatments with AMF especially in WFSP 30% ADL AMF, WFSP 70% AMF, OFSP 50% ADL AMF and OFSP 70% ADL AMF. There is no clear indication on whether either of the cultivars were better colonised than the other. In the 2023/24 growing season, the largest increase in sporocarps were for OFSP 30% ADL control, which almost doubled in number and OFSP 30% ADL AMF, which increased fourfold. The other marked increase was for the number of sporocarps in the WFSP 50% ADL AMF treatment. Most of the other treatments maintained about the same number of sporocarps as in the first season.

The total number of sporocarps recovered from soil after harvest in the open field is presented in Table 4.4. The initial number of sporocarps in the open field before the first trial was 1635. There was not a significant change in the levels detected between the different treatments and it can therefore be assumed that the natural inoculum played as big a role in colonisation of the crop as the inoculum. The number of sporocarps declined for most of the treatments in the second trial. It is known that tilling of fields has a negative impact on AMF numbers in the soil and the planting of the same field for a second consecutive season will negatively affect the natural communities in the soil. The crude AMF inoculum used to inoculate the trial contained approximately 2600 sporocarps per 100 g soil, which may not be sufficient.

Table 4.4 Mycorrhizal sporocarps recovered from open field after harvest.

Treatment	No. of sporocarps 2022/23	No. of sporocarps 2023/24
WF Blesbok Control	2394	1719
WF Blesbok 150	2706	1548
WF Blesbok 250	1674	1392
WF Blesbok 350	2106	1186
OF Bophelo Control	1206	990
OF Bophelo 150	2394	1101
OF Bophelo 250	1275	1950
OF Bophelo 350	2370	1248

Soil microbial composition at end of season

Carbon source utilization pattern of the soil microbial communities was determined from the average well colour development (AWCD) of the Biolog microplates. The highest C-source utilization by the microbial communities for 2022/23 season was recorded for soil samples OF control irrigation 2 (50% ADL), OF AMF irrigation 3 (70% ADL) and WF control irrigation 2 (**Figure 4.16**). For the 2023/24 season the highest numbers were recorded for WF AMF irrigation 2, WF Control irrigation 2 and WF AMF irrigation 2 (**Figure 4.17**). There was no conclusive evidence of benefit to the soil microbial composition with AMF inoculation.

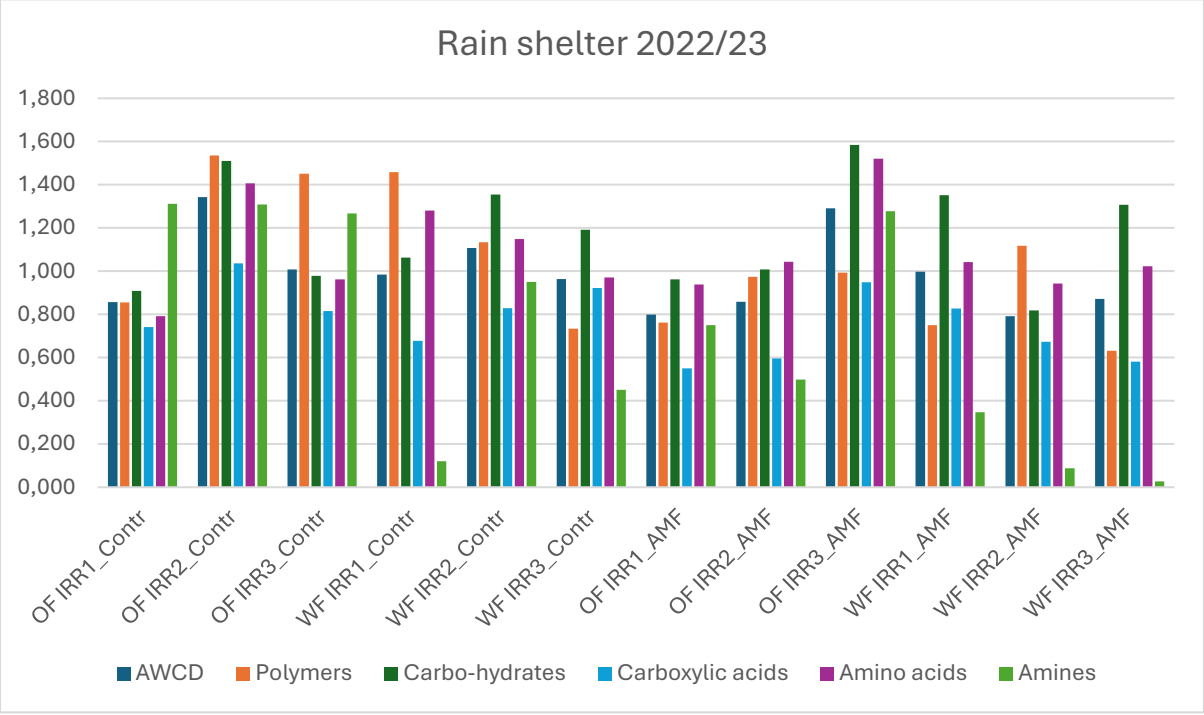


Figure 4.16 AWCD values that show the extent of utilization of five major groups of carbon sources by microbial communities of soil samples collected from the rain shelter at the end of the 2022/23 season.

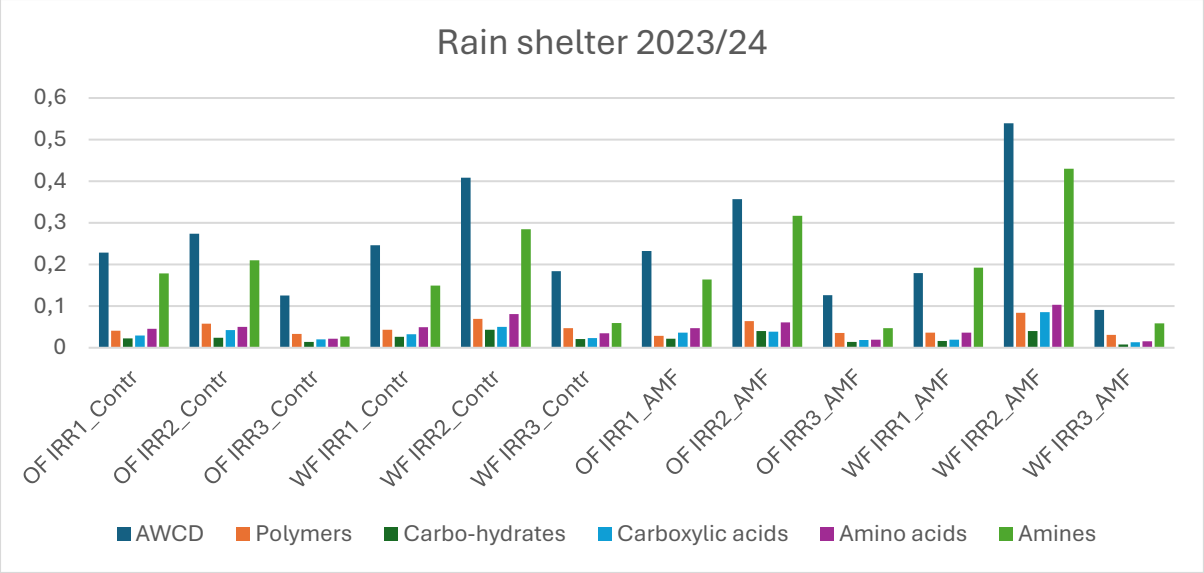


Figure 4.17 AWCD values that show the extent of utilization of five major groups of carbon sources by microbial communities of soil samples collected from the rain shelter at the end of the 2023/24 season.

The Shannon Weaver Diversity Index (H') indicates the ability of the microbial community to degrade more or fewer types of carbon sources (**Figure 4.18**). During the 2022/23 season the highest diversity was observed for samples OF control irrigation 2, OF AMF irrigation 3 and WF control irrigation 2. During the 2023/24 season, the highest diversity was observed for the controls of OF irrigation1, WF irrigation 1 and WF irrigation 2.

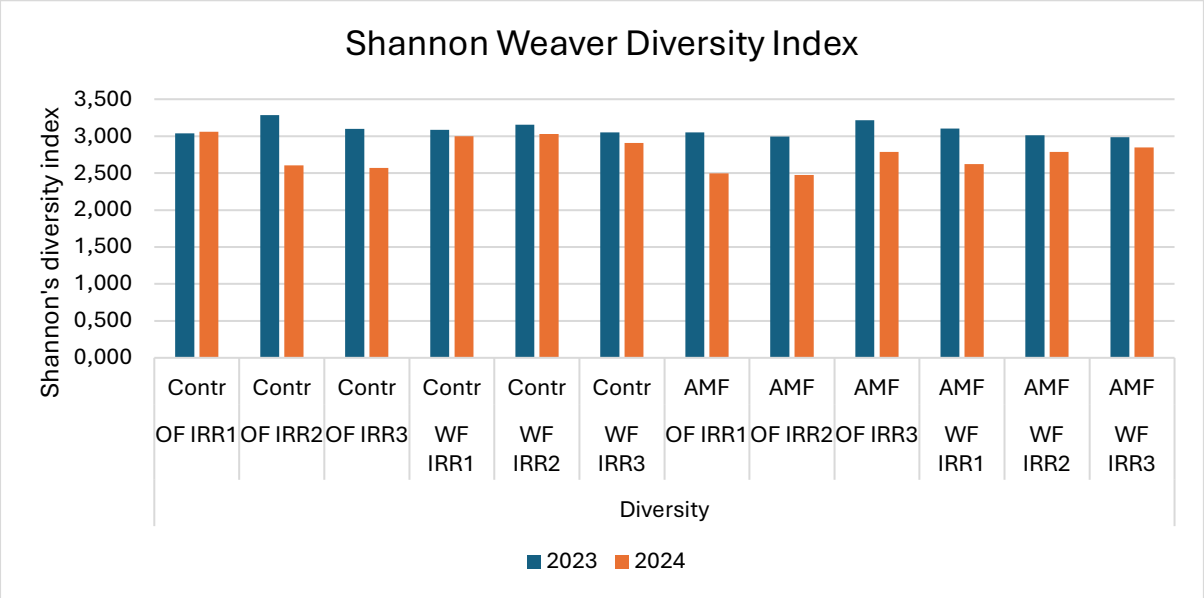


Figure 4.18 Shannon Weaver diversity index of soil samples collected from the rain shelter after both seasons.

Statistically significant richness (S) (i.e. total number of carbon sources utilized at OD values ≥ 0.25) out of the 31 carbon sources was observed for samples OF control irrigation 2, WF Control irrigation 2 and OF AMF Irrigation 3 with the highest carbon source utilization for

2022/23 season **(Figure 4.19**). During the 2023/24 season the highest carbon source utilization was found for WF AMF irrigation 2, WF Control irrigation 1, WF control irrigation 2 and OF control irrigation 2.

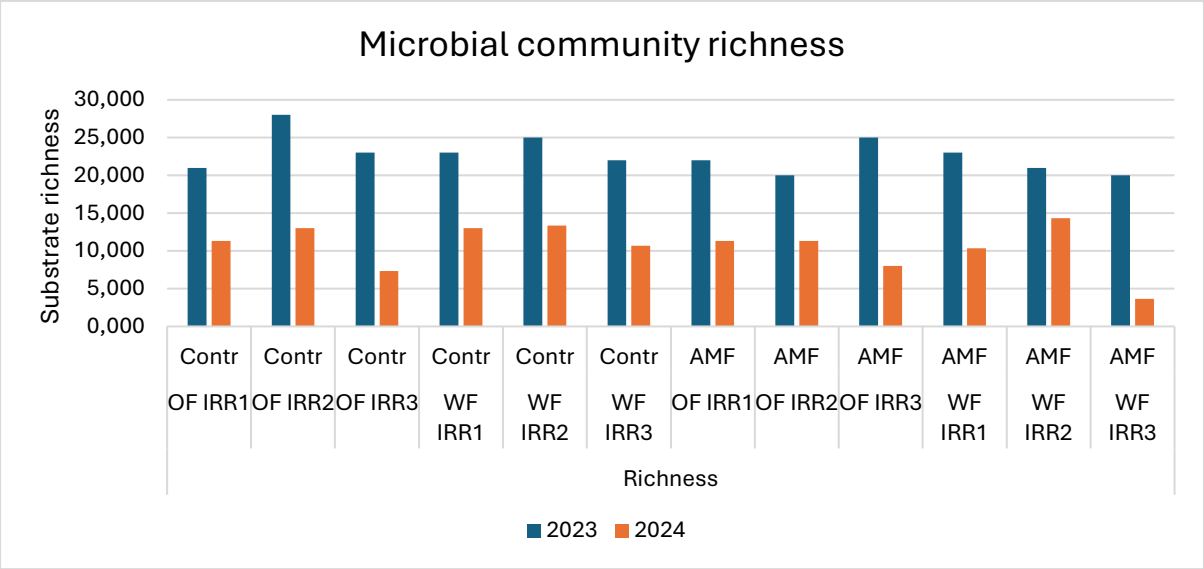


Figure 4.19 Richness of microbial composition of soil samples collected from the rain shelter after both seasons.

The highest C-source utilization by the microbial communities in the open field for 2022/23 season was recorded for soil samples Bophelo 350 AMF, Blesbok 250 AMF and Blesbok 150 AMF (**Figure 4.20**). During the 2023/24 season the highest value was again recorded for Bophelo 350 AMF, with Bophelo control and Bophelo 250 also significant (**Figure 4.21**).

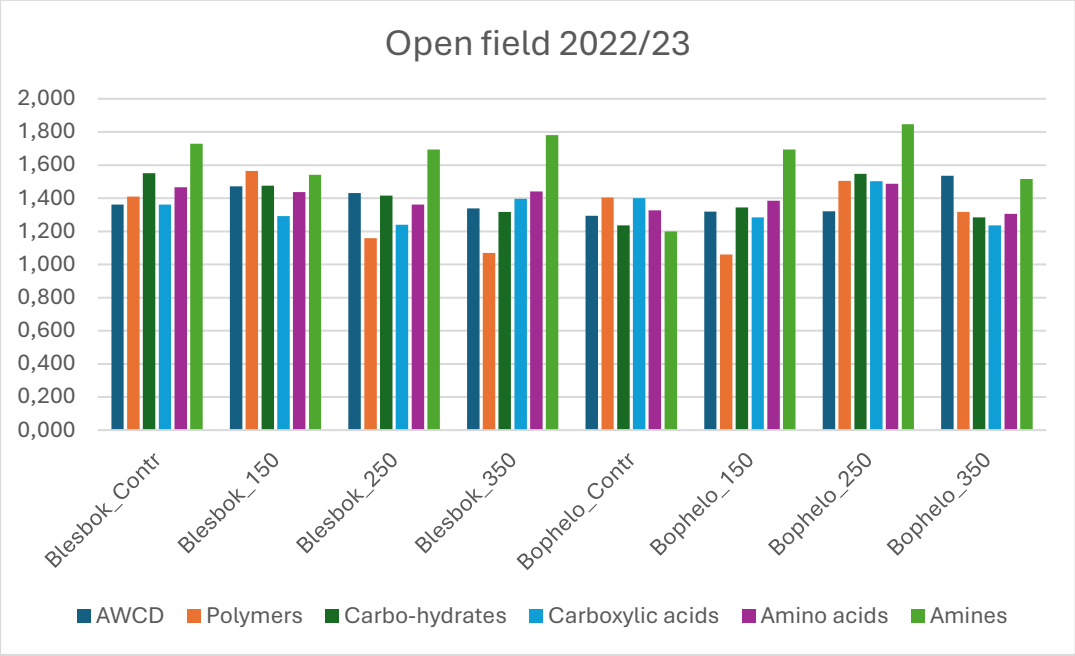


Figure 4.20 AWCD values that show the extent of utilization of five major groups of carbon sources by microbial communities of soil samples collected from the open field after 2022/23 season.

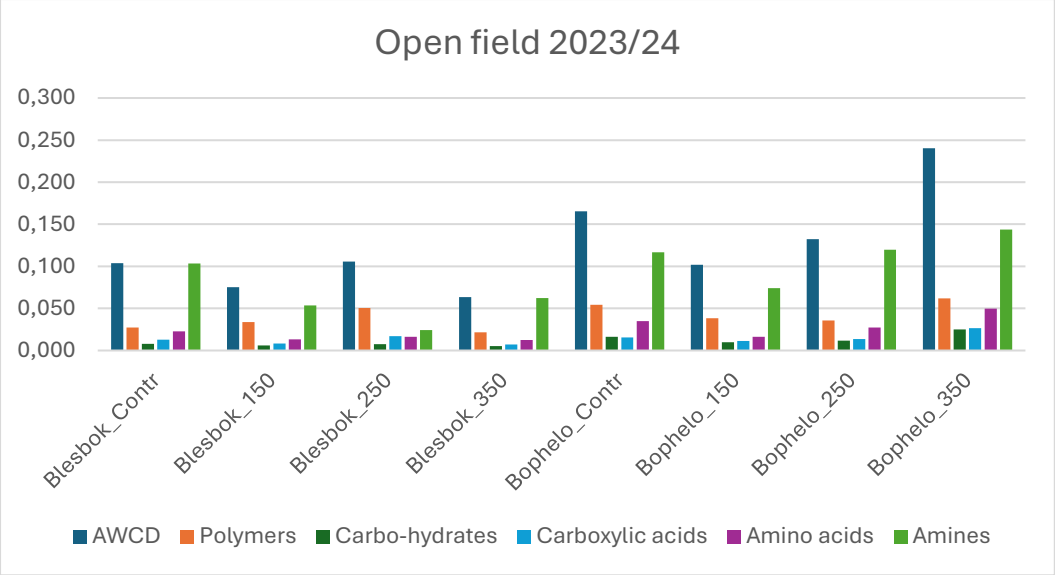


Figure 4.21 AWCD values that show the extent of utilization of five major groups of carbon sources by microbial communities of soil samples collected from the open field after 2023/24 season.

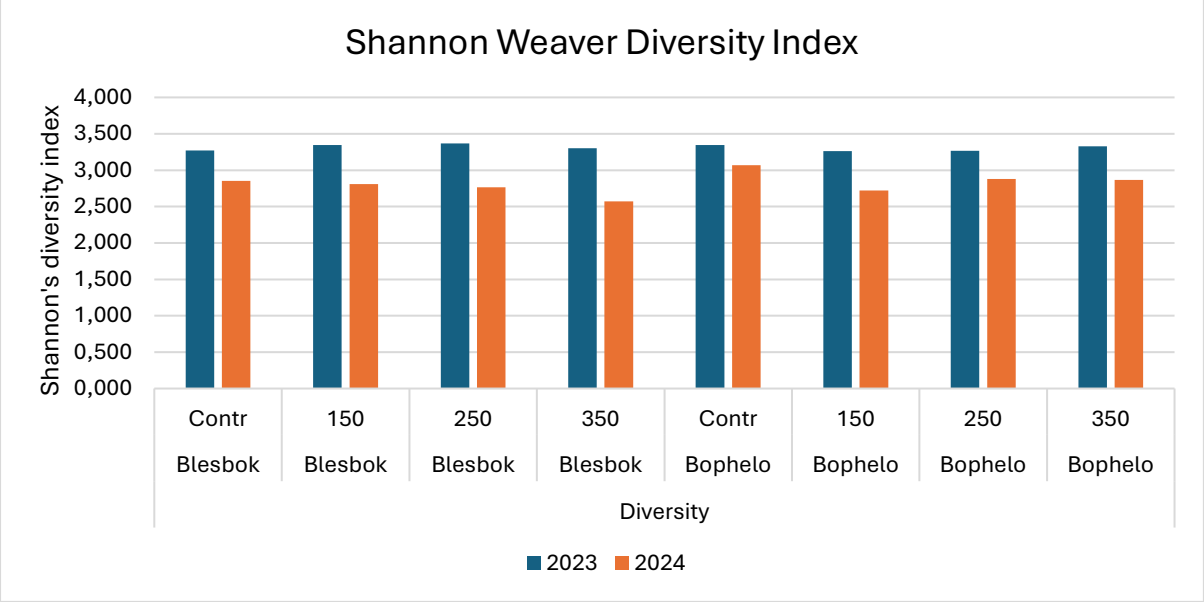


Figure 4.22 Shannon Weaver diversity index of soil samples collected from the open field trial after both seasons.

Significant differences in Shannon Weaver Diversity Index (H') (i.e. the ability of the microbial community to degrade more or fewer types of carbon sources at a threshold ODi value ≥ 0.25) was not observed during the 2022/23 trial (**Figure 4.22**). During the 2023/24 season the diversity was highest for Bophelo Control followed by Blesbok control Bophelo 250 and Blesbok 150 (**Figure 4.22**).

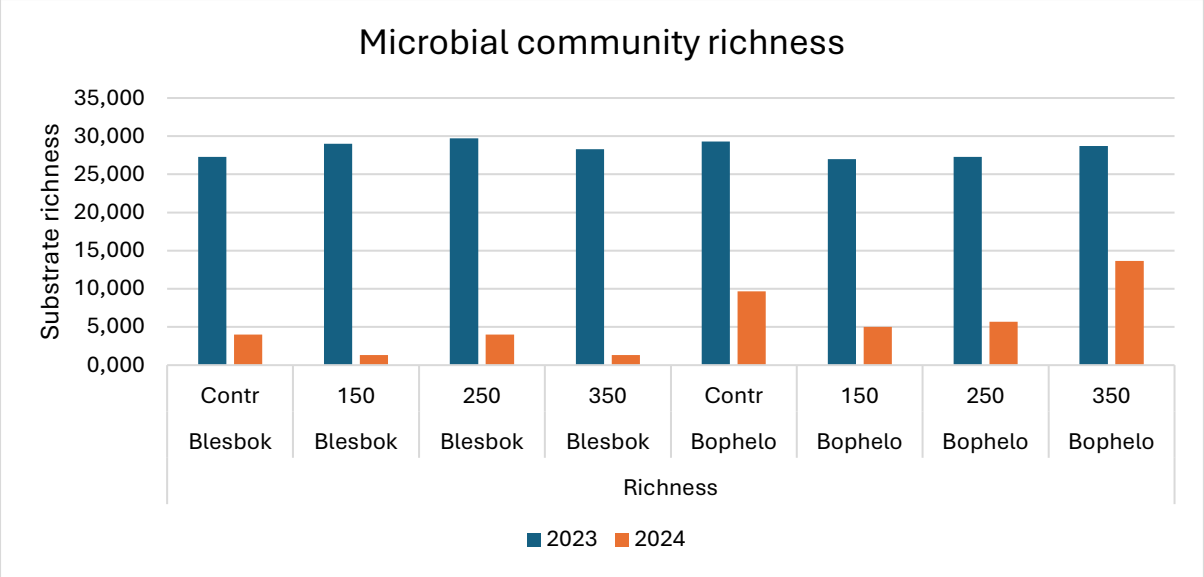


Figure 4.23 Richness of microbial composition of soil samples collected from the open field after both seasons.

Richness (S) (**Figure 4.23**) is the total number of carbon sources utilized at OD values ≥ 0.25) out of the 31 carbon sources tested. The highest richness was observed for samples Blesbok 250AMF, Blesbok 150 AMF, Bophelo control and Bophelo 350AMF during the 2022/23 season. During the 2023/24 season the highest richness was observed for Bophelo 350 followed by Bophelo Control and Bophelo 250.

Conclusions

The large differences in yield between seasons one and two suggest that the study should be repeated to determine which set of data is more representative. Based on only the first season's data, both WFSP and OFSP had increased yield with AMF inoculation under 30 and 50% ADL and WFSP under 70% ADL also showed increased yield. However, when both season's results are considered, only OFSP had consistently higher yields with AMF inoculation.

There were no clear differences observed in above ground parameters measured during the growing season or the soil microbial diversity. In general, the microbial diversity did not show any benefit from AMF inoculation. However, increasing soil microbial diversity and consequently soil health, takes time. Different strategies, including crop rotation, inoculation with AMF and other beneficial organism and incorporation of organic material in soil are needed. These strategies should be followed for several years before appreciable differences will be clear.

CHAPTER 5 THE CONTRIBUTION OF AMF TO NUTRITIONAL QUALITY CONTENT OF SWEET POTATO

Introduction

Sweet potato [*Ipomoea batatas* (L.) Lam] is an important staple food crop in many parts of the tropics. Globally, it is ranked as the seventh most important food crop after wheat, rice, maize, Irish potato, barley, and cassava (Mwanga et al., 2001). It is a substantial source of carbohydrates and β-carotene (FAO, 2002). In Africa, sweet potato is the second most important root crop after Irish potato, primarily produced in East African countries around Lake Victoria. It is a staple food crop in southern Africa, where it is among the priority food security crops (Aritua and Gibson, 2002; Kapinga et al., 2007).

The productivity of sweet potato is constrained by poor soil fertility, particularly low levels of potassium (K), phosphorus (P), nitrogen (N), sulphur (S), and micronutrients (copper, zinc, iron, manganese, molybdenum, boron, chlorine, and nickel) (Bourke, 2005, 2009; Bailey et al., 2008; Kirchlof, 2009; Taraken et al., 2010; Uwah et al., 2013). The estimated nutrient removal by sweet potato from the soil is 100, 90, and 200 kg ha^{-1} of N, P, and K, respectively, which can result in 20-40 t ha^{-1} of marketable roots, depending on cultivar and management (Traynor, 2005). In highly nutrient-depleted soils of sub-Saharan Africa (SSA), balanced nutrition of N, P, and K is required to enhance crop yield (O'Sullivan et al., 1997). In southern Africa, sweet potato tuber yields on smallholder farms average 4.5 t ha^{-1} (International Potato Centre [CIP], 2006), which is only 10% of the attainable yield (45-48 t ha^{-1}) of the cultivar Namulonge Sweet Potato 11 (NASPOT 11; Mwanga et al., 2011) and the potential yield (45 t ha^{-1}) in SSA (CIP, 2006). NASPOT 11 has a long-elliptic storage root shape when grown in light soils, has a high dry matter content (~34%), which translates into increased yield and good to excellent consumer acceptance, depending on growth conditions.

Possible interventions to alleviate soil fertility limitations include the application of inorganic fertilisers, organic fertilisers, and biofertilisers. Biofertilisers are increasingly being integrated into soil fertility management programs in Asia and Africa (Vanlauwe et al., 2010). The intensified use of biofertilisers such as AMF is emerging as an environmentally friendly alternative soil fertility management practice with the potential to increase and sustain crop yields more cheaply compared to continuous application of inorganic fertilisers alone (Sharma et al., 2013). Introducing AMF to soils that already contain AMF could be beneficial, as the carrying capacity of some agricultural soils may be low, yet high spore densities are required to increase the volume of hyphae in the soil and the percentage of roots colonised (Rosendahl, 2008). Several benefits of AMF have been reported, including improved plant survival and acclimatization, increased growth and nutrient uptake (especially P, Zn, Mn, Mg, Cu, K, and N), and increased crop yields (Kapulnik et al., 2010; Ortas, 2010; Kavoo-Mwangi et al., 2013), as well as improved water use efficiency (Augé, 2001). These benefits have been reported for crops such as olive (Porras-Soriano et al., 2010), banana (Kavoo-Mwangi et al., 2013, 2014), turmeric (Radhika and Rodrigues, 2010), cassava (Ceballos et al., 2013), and sweet potato (Abdel-Razzak et al., 2013). Abdel-Razak et al. (2013) reported that integrating AMF inoculum with superphosphate fertiliser under the recommended P level enhanced root productivity and quality compared to when the treatments were applied singly. However, despite the Abuja Declaration of 2006 to increase inorganic fertiliser use to at least 50 kg of nutrients per hectare,

little impact has been realized in most parts of SSA due to financial constraints faced by smallholder farmers.

Moreover, a significant proportion of applied P fertiliser is not available to plants due to strong fixation of P on iron (Fe), manganese (Mn), and aluminium (Al) oxides (Cardoso and Kuyper, 2006) in most tropical soils. Therefore, we hypothesized that the introduced AMF would improve P availability through the solubilization of P present in the soils and moisture mobilization, leading to increased growth and yield of sweet potato. This prompted the investigation into how to reduce inorganic P fertiliser use by integrating N and K with biofertiliser to increase sweet potato yields.

Recently, the impact of AMF symbiosis on N uptake has been studied. AMF play a direct or indirect role in N-cycling processes in the soil. Changes in soil aggregation and aeration impact denitrification processes and reduce leaching of inorganic N. There is a strong correlation between leaf chlorophyll content and photosynthesis, with N playing a crucial role in chlorophyll formation. A direct relationship between N content, CO_2 assimilation rate, chlorophyll content, and rubisco activity has been reported (Coskun et al., 2017; Valkov et al., 2020). Moreover, the presence of AMF in soil affects pH and N availability. AMF shift the microbial community in the rhizosphere and surrounding bulk soil using both fungal and AM-mediated root exudates. AMF preferentially uptake N in the form of NH_4^+ and transfer it to the cytoplasm, which is translocated into the intraradical hyphae via the vacuole; NH_4^+ is then released into the apoplastic compartment (Jin et al., 2005; Govindarajulu et al., 2005). In non-colonised plants, reduction of NO_3 mainly occurs in the leaves, whereas in AMF-colonised plants, reduction of NO_3 occurs in the roots (Kaldorf et al., 1998). Furthermore, the activities of glutamine synthetase and glutamate synthase are significantly higher in plants colonised by AMF than in non-colonised plants (Field and Mooney, 1983; Pilbeam, 2018). It is still unclear how plants take up the ammonium released by AMF (Balestrini et al., 2020). In the roots of mycorrhizal plants, high amounts of several amino acids are transferred to the colonised host. AMF symbiosis can modify the physiology and environment of the host, enhancing nutrient uptake without direct phosphorus aid. This may lead to changes in the composition of the soil microbial community (Elliott et al., 2020). Increasing nutrient use efficiency and tolerance to abiotic stresses is a key role of AMF in agriculture (Carillo et al., 2020). AMF contribute to crop productivity and mitigate negative factors in agriculture.

This study aimed to test the hypothesis that AMF inoculation promotes sweet potato and nutritional quality, predicting slight differences in nutritional quality performance among inoculated treatments.

Materials and Methods

Leaves were collected from actively growing plants during the season and freeze dried. Fresh tubers were cut up and freeze dried after harvest. Freeze dried material were ground into a fine powder using mortar and pestle and submitted to the analytical facilities at ARC-VIMP, ARC-NRE and ARC-AP (Irene) for analyses.

Parameters measured

Sugar and Protein, and Polyphenol Concentration

For the determination of sugar content, 0.1 g of plant material was homogenized with 8 mL of ethanol (80%) and centrifuged for 10 min at 5000 rpm. To 0.2 mL of supernatant, 200 µL of phenol (5%) and 1 mL H_2SO_4 were added and stirred. After cooling, the absorbance at 485 nm was read (Dubois et al., 1956). Soluble proteins in the fruits as fruit quality marker were measured following the method of Bradford (1976). Briefly, 5 mL of Bradford reagent was supplemented with 0.1 mL of the ethanolic extract. After homogenization, the reaction mixture was placed for 30 min at 30 °C. Then, the absorbance was read at 595 nm. Polyphenol content was measured according to the method described by Yamamoto et al. (1977) with slight modifications. Plant material (1 g fresh weight) were ground in 8 mL of methanol (80%). To ensure a maximum extraction, two supplementary extractions were made by washing the residues with methanol (80%). The mixture of the filtrate and additional filtration was centrifuged at 1000 g for 5 min. Then, 0.2 mL of the supernatant was supplemented with 0.4 mL of Folin-Denis reagent and distilled water in a total reaction mixture of 3 mL. After 3 min, 1 mL of saturated aqueous Na_2CO_3 solution was added at ambient temperature for 1 h to complete the reaction. After that, the absorbance was determined at 765 nm using gallic acid as standard.

Quantification of vitamin C content

Vitamin C content was determined using a method described by Odriozola-Serrano et al. (2007) with slight modifications. Plant material (0.2 g) was extracted using 10 mL of 4.5% metaphosphoric acid. The mixture was sonicated in an ultrasonic bath containing ice-cold water for 30 min before filtration. The prepared samples were then analysed using a Shimadzu HPLC (LC2030C 3D, Shimadzu Corporation, Kyoto, Japan) equipped with a C18 Luna column (150 £ 4.6 mm, 5m) at 25 °C. Water: acetonitrile: formic acid (99: 0.9: 0.1 v/v/v) was used as the mobile phase at a flow rate of 1 ml/min in isocratic mode, with an injection volume of 20 mL and detection wavelength of 245 nm. Ascorbic acid prepared at different concentrations was used as a standard for the preparation of a calibration curve. The vitamin C content was expressed as mg/100 g dry weight (DW) of sample.

Quantification of β-carotene content

β-carotene content was determined using a method described by Biehler et al. (2010) with slight modifications. The procedure was carried out in the dark to avoid the effect of light on *β*-carotene. Methanol (5 mL) was added to 0.2 g sample and vortexed for 10 s, followed by the addition of 15 mL hexane: acetone (1:1 v/v). The mixture was vortexed for 10 s before sonicating in an ultrasonic bath containing ice-cold water for 15 min. Saturated sodium chloride solution (5 mL) was added to the mixture, vortexed for 10 s and centrifuged at 2000 rpm for 2 min. The collected supernatant was filtered through a 0.45 mm syringe filter before analysis using a Shimadzu HPLC (LC2030C 3D, Shimadzu Corporation, Kyoto, Japan) equipped with a C18 Luna column (150 £ 4.6 mm, 5m) at 35 °C. Acetonitrile: dichloromethane: methanol (70:20:10, v/v/v) was used as a mobile phase at a flow rate of 1 ml/min in isocratic mode, with an injection volume and detection wavelength of 20 mL and 450 nm, respectively. Identification ion and quantification of β-carotene was achieved by plotting a Calibration curve using a β-carotene standard. β-carotene content was expressed as mg/100 g DW of sample.

Quantification of mineral elements

Quantification of mineral elements was done according to the method described by Ang and Lee (2005) with slight modifications. An amount of 0.5 g of sample was digested with 10 mL of 1:3 (v/v) nitric acid: hydrochloric acid. The mixture was boiled over a hotplate at 95 °C until the sample was dissolved. The mineral elements in the digested samples were quantified using inductively coupled plasma atomic emission spectroscopy (ICPE-9820, Shimadzu Corporation, Kyoto, Japan).

Results and Discussion

The total phenolics, tannins and flavonoid content of orange flesh sweet potato (OFSP) and white flesh sweet potato (WFSP) cultivars, as influenced by AMF application and water stress levels in the rain shelter trial, is indicated in **Table 5.1** for 2022/23.

Table 5.1 Total phenolic, total flavonoid and condensed tannins content (average of 3 samples) in sweet potato leaf samples collected from the rain shelter for 2022/23 season

Treatment	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CE/g)	Condensed tannins (mg cyanidin chloride/g sample)
WF Irr1 Control	20.44	4.15	1.43
WF Irr2 Control	17.05	5.23	3.96
WF Irr3 Control	15.57	4.73	0.55
WF Irr1 AMF	16.94	3.33	0.20
WF Irr2 AMF	16.79	2.88	2.84
WF Irr3 AMF	13.89	3.19	0.17
OF Irr1 Control	14.31	2.42	0.28
OF Irr2 Control	14.45	3.37	2.67
OF Irr3 Control	12.47	2.44	1.30
OF Irr1 AMF	13.33	2.92	0.40
OF Irr2 AMF	9.80	3.18	0.56
OF Irr3 AMF	13.56	3.87	0.06

In **Table 5.2** the total phenolic, flavonoid, β-carotene and vitamin C content of leaf samples are given for OFSP and WFSP cultivars for 2023/24 season in the rain shelter. The results revealed that AMF and water stress levels have no impact on the total flavonoid content in the leaves of either sweet potato cultivars. The total phenolic content of WFSP (Blesbok) leaves were reduced in the treatments with AMF compared to those without in both season's trials.

Table 5.2 Total phenolic, flavonoid, β-carotene and vitamin C (average of 3 samples) in sweet potato leaf samples collected from the rain shelter for 2023/24 season.

Treatment	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CE/g)	B-carotene	Vitamin C
WF 30% Control	17.76	8.24	2.07	5.75
WF 50% Control	15.92	5.93	2.73	6.77
WF 70% Control	10.77	7.29	1.04	6.6
WF 30% AMF	12.79	6.43	4.59	5.83
WF 50% AMF	16.66	7.2	2.06	6.55
WF 70% AMF	9.78	4.71	1.72	6.54
OF 30% Control	15.96	5.48	7.16	9.34
OF 50% Control	17.3	7.28	8.33	15.77
OF 70% Control	15.1	5.57	6.17	9.14
OF 30% AMF	11.95	8.45	4.56	17.84
OF 50% AMF	11.71	5.61	7.25	12.32
OF 70% AMF	12.72	4.85	7.57	17.47

During the 2023/24 trial, total phenolic, flavonoid and vitamin C content for WFSP tubers under 50% and 70% ADL increased for treatments inoculated with AMF. For the OFSP the total phenolic and flavonoid content increased for 30% ADL, but not for 50% and 70% ADL treatments. There was a significant increase in the vitamin C content of the OFSP for 70% ADL (**Table 5.3**). Although inconsistent, there is a general trend of increased nutritional levels of sweet potato tubers under water stress when it is inoculated with AMF.

Table 5.3 Total phenolic, flavonoid, β-carotene and vitamin C (average of 3 samples) in sweet potato tuber samples collected from the rain shelter for 2023/24 season.

Treatment	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CE/g)	β-carotene (mg/100 g)	Vitamin C (mg/100 g)
WF 30% Control	4.702	0.656	0.41	25.82
WF 50% Control	4.773	0.491	0.41	24.37
WF 70% Control	5.029	0.489	0.51	23.31
WF 30% AMF	5.611	0.628	0.52	24.79
WF 50% AMF	6.064	0.717	0.47	27.75
WF 70% AMF	7.739	1.500	0.43	29.28
OF 30% Control	7.365	1.466	19.26	44.81
OF 50% Control	7.204	1.124	12.40	43.07
OF 70% Control	8.009	2.860	12.68	36.50
OF 30% AMF	11.746	2.681	12.24	44.62
OF 50% AMF	7.853	1.473	9.46	46.87
OF 70% AMF	6.035	1.229	13.23	55.45

β-carotene and vitamin C levels of the WFSP leaves increased in the treatment with AMF did not show clear trends (**Table 5.4**). For the OFSP there were no clear correlation between AMF inoculation and phenolic, flavonoid, β-carotene or vitamin C levels.

Table 5.4 Total phenolic, flavonoid, β-carotene and vitamin C (average of 3 samples) in sweet potato leaf samples collected from the open field for 2023/24 season.

	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CE/g)	β-carotene (mg/100 g)	Vitamin C (mg/100 g)
WFSP Control	15.89	10.17	1.22	11.32
WFSP 150	13.36	8.98	2.28	15.71
WFSP 250	17.75	10.2	2.11	14.84
WFSP 350	14.07	6.21	3.12	13.63
OFSP Control	15.24	9.64	2.37	14.36
OFSP 150	17.22	8.58	2.65	4.81
OFSP 250	18.28	12.48	3.28	26.7
OFSP 350	13.56	7.4	2.92	18.06

Vitamin C levels for both WFSP and OFSP cultivars increased in treatments with AMF, regardless of the amount of inoculum applied, although WFSP 350 AMF only showed a slight increase (**Table 5.5**). Similar to results from the leaf samples, no significant changes were observed for phenolic and flavonoid in the sweet potato tuber samples.

Table 5.5 Total phenolic, flavonoid, β-carotene and vitamin C (average of 3 samples) in sweet potato tuber samples collected from the open field for 2023/24 season.

	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CE/g)	β-carotene (mg/100 g)	Vitamin C (mg/100 g)
WFSP Control	5.454	1.031	0.48	39.11
WFSP 150 AMF	6.896	1.893	0.5	64.76
WFSP 250 AMF	6.883	1.393	0.56	54.74
WFSP 350 AMF	7.318	1.342	0.43	41.38
OFSP Control	6.926	1.735	6.65	52.95
OFSP 150 AMF	8.122	1.785	9.05	85.56
OFSP 250 AMF	6.815	1.741	6.06	59.88
OFSP 350 AMF	7.326	1.439	6.76	73.13

Sweet potato leaves and tuber samples were collected from the rain shelter and open field during the 2023/24 season and submitted for analyses of the amino acids, protein content, starch, total sugars and total non-structural carbohydrates. The results for leaf samples are presented in **Table 5.6** and **Table 5.7** and for tuber samples in **Table 5.10** and **Table 5.11**. Results for mineral content for leaf samples collected from the rain shelter and open field are presented in **Table 5.8** and **Table 5.9** respectively.

There was a marked increase in the total sugars and total non-structural carbohydrates in the leaves for treatments WFSP 150 and WFSP 250 and a smaller increase of these substances in OFSP 150 (**Table 5.7**). However, there was a marked decrease in the same sugars and carbohydrates in OFSP 250 and OFSP 350 leaves. The largest protein increases were observed in WFSP 150, OFSP 250 and OFSP 350 leaves. Further investigation is needed to find correlations between the treatments and results.

Mineral content of leaves from the rain shelter (**Table 5.8**) showed that the control of OFSP under 70% ADL had the highest carbon and nitrogen content. There were no clear trends in carbon, nitrogen, potassium, calcium, magnesium and phosphorous content between cultivars or treatments. Sodium content of the OFSP was significantly higher than that of the WFSP. The lowest levels of iron, aluminium, manganese, zinc and boron was found in the AMF inoculated OFSP under 30% ADL.

Calcium and sodium content of WFSP leaves in the open field (**Table 5.9**) where lower than that of the OFSP. No other clear trends were observed either between cultivars or treatments, although the nitrogen content of the WFSP was slightly higher than that of the OFSP.

Tuber samples (**Table 5.10**) of OFSP showed little change in amino acid levels between AMF inoculation and control samples, with most AMF treatments leading to slightly lower amino acid levels. The WFSP had slightly increased amino acid levels for 30% ADL and 50% ADL, but lower levels for 70% ADL although protein levels for all three AMF irrigation treatments were higher than that of the uninoculated control.

Amino acid content of WFSP samples from the open field were similar for inoculated and control treatments (**Table 5.11**). There was a slight increase in the protein content for WFSP 150 AMF and all three OFSP AMF treatments.

Table 5.6 Amino acid, protein, starch and total non-structural carbohydrates of sweet potato leaf samples from rain shelter in g/100g for 2023/24 season.

	OF 30% Con	OF 30% AMF	OF 50% Con	OF 50% AMF	OF 70% Con	OF 70% AMF	WF 30% Con	WF 30% AMF	WF 50% Con	WF 50% AMF	WF 70% Con	WF 70% AMF
Arginine	0.85	1.07	0.87	0.72	0.92	0.9	1.12	1.33	1.19	1.24	1.35	1.38
Serine	0.78	0.9	1.41	0.67	0.91	0.89	0.96	0.86	0.86	0.91	0.96	1.01
Aspartic Acid	2.01	2.5	2.19	1.75	2.13	2.05	2.56	2.99	2.52	2.59	2.92	3.18
Glutamic acid	1.81	2.17	1.74	1.7	1.96	1.86	2.2	2.43	2.24	2.32	2.51	2.61
Glycine	0.81	0.96	1.07	0.71	0.93	0.88	1.02	0.97	1.01	1.06	1.11	1.13
Threonine	0.68	0.84	0.77	0.61	0.74	0.73	0.85	0.81	0.82	0.86	0.92	0.93
Alanine	0.76	0.94	0.86	0.67	0.84	0.81	0.95	0.92	0.95	1	1.08	1.07
Tyrosine	0.72	0.92	0.55	0.58	0.72	0.66	0.7	0.79	0.67	0.88	0.93	0.76
Proline	0.79	0.99	0.81	0.69	0.88	0.85	1.01	0.99	1.01	1.09	1.23	1.15
HO-Proline	0.07	0.08	0.06	0.07	0.08	0.08	0.09	0.08	0.08	0.08	0.1	0.09
Methionine	0.23	0.33	0.27	0.29	0.16	0.28	0.26	0.24	0.19	0.29	0.3	0.2
Valine	1.05	1.23	1.02	0.83	1.1	1.09	1.21	1.11	1.24	1.31	1.35	1.35
Phenylalanine	0.84	1	0.82	0.69	0.87	0.85	0.99	0.96	1	1.06	1.09	1.09
Leucine	1.13	1.38	1.11	1	1.26	1.18	1.39	1.37	1.43	1.51	1.59	1.56
Isoleucine	0.77	0.93	0.75	0.62	0.8	0.8	0.89	0.82	0.91	0.97	0.99	0.96
Histidine	0.78	0.75	-	0.48	1.24	0.91	0.85	0.63	0.61	0.85	0.61	0.75
Lysine	1	1.25	-	0.85	1.1	1.01	1.08	1.19	1.14	1.31	1.37	1.26
*Protein	16.78	19.81	20.1	14.66	18.35	17.51	20.12	20.26	20.09	21.81	22.54	22.56
Starch	0.2	0	0	4.2	0.2	0	0.6	0	0.7	0	0	0

Table 5.7 Amino acid, protein, starch and total non-structural carbohydrates of sweet potato leaf samples from the open field in g/100g for 2023/24 season.

	WFSP Control	WFSP 150	WFSP 250	WFSP 350	OFSP Control	OFSP 150	OFSP 250	OFSP 350
Arginine	0.94	1.03	0.85	1.13	0.66	0.59	0.73	0.57
Serine	0.74	0.78	0.68	0.91	0.53	0.5	0.6	0.49
Aspartic Acid	2.16	2.13	1.76	1.05	1.02	1.28	1.71	1.14
Glutamic acid	2.2	2.16	1.86	2.27	1.35	1.27	1.74	1.23
Glycine	0.79	0.85	0.73	0.96	0.59	0.53	0.66	0.52
Threonine	0.67	0.72	0.63	0.74	0.45	0.44	0.55	0.43
Alanine	0.76	0.82	0.7	0.88	0.54	0.49	0.6	0.48
Tyrosine	0.68	0.7	0.56	0.42	0.26	0.38	0.47	0.39
Proline	0.84	0.86	0.73	0.92	0.53	0.5	0.64	0.48
HO-Proline	0.06	0.07	0.06	0.07	0.05	0.05	0.05	0.05
Methionine	0.42	0.39	0.29	0.29	0.23	0.22	0.14	0.2
Valine	0.96	1.01	0.82	1.05	0.64	0.58	0.84	0.6
Phenylalanine	0.75	0.81	0.66	0.85	0.52	0.47	0.61	0.47
Leucine	1.1	1.2	1	1.23	0.75	0.69	0.89	0.69
Isoleucine	0.69	0.72	0.59	0.75	0.47	0.43	0.6	0.44
Histidine	0.58	0.48	0.35	0.55	0.22	0.24	0.35	0.26
Lysine	1.02	1.05	0.89	1.05	0.61	0.61	0.78	0.61
*Protein	16.77	17.49	14.53	16.48	10.43	10.5	13.1	10.81
Total sugars	24.8	29.51	27.24	24.96	32.4	33.27	28.88	31.21
Total non-structural carbohydrates	24.8	29.66	27.86	24.96	36.93	37.5	29.56	34.62

Table 5.8 Mineral content of sweet potato leaf samples from rain shelter in 2023/24 season.

	WFSP Control	WFSP 150 AMF	WFSP 250 AMF	WFSP 350 AMF	OFSP Control	OFSP 150 AMF	OFSP 250 AMF	OFSP 350 AMF
Total C%	43.10	42.50	42.60	41.20	40.50	42.30	41.40	40.80
Tot. N%	3.17	2.90	2.99	2.47	2.53	2.00	2.06	2.03
K%	2.82	2.93	2.44	3.24	1.69	2.14	2.01	1.84
Ca%	0.63	0.64	0.65	0.54	1.03	0.92	1.04	1.06
Mg%	0.55	0.54	0.52	0.48	0.53	0.50	0.59	0.53
P%	0.53	0.44	0.40	0.57	0.43	0.42	0.49	0.45
Na (mg/kg)	209.00	262.00	314.00	227.00	1625.00	1492.00	1818.00	1728.00
Fe (mg/kg)	648.00	657.00	618.00	539.00	643.00	609.00	512.00	516.00
Al (mg/kg)	610.00	630.00	552.00	521.00	661.00	656.00	535.00	525.00
Mn (mg/kg)	64.90	68.30	75.00	57.50	80.00	64.50	77.10	80.10
Zn (mg/kg)	22.40	20.50	18.50	22.50	16.10	20.10	21.60	16.30
B (mg/kg)	72.20	75.10	68.80	67.40	76.60	74.80	79.60	67.50
Cu (mg/kg)	9.84	9.01	7.33	8.71	6.92	7.37	8.76	8.71

Table 5.9 Mineral content of sweet potato leaf samples from open field in 2023/24 season.

	WFSP Control	WFSP 150 AMF	WFSP 250 AMF	WFSP 350 AMF	OFSP Control	OFSP 150 AMF	OFSP 250 AMF	OFSP 350 AMF
Total C%	43.10	42.50	42.60	41.20	40.50	42.30	41.40	40.80
Tot. N%	3.17	2.90	2.99	2.47	2.53	2.00	2.06	2.03
K%	2.82	2.93	2.44	3.24	1.69	2.14	2.01	1.84
Ca%	0.63	0.64	0.65	0.54	1.03	0.92	1.04	1.06
Mg%	0.55	0.54	0.52	0.48	0.53	0.50	0.59	0.53
P%	0.53	0.44	0.40	0.57	0.43	0.42	0.49	0.45
Na (mg/kg)	209.00	262.00	314.00	227.00	1625.00	1492.00	1818.00	1728.00
Fe (mg/kg)	648.00	657.00	618.00	539.00	643.00	609.00	512.00	516.00
Al (mg/kg)	610.00	630.00	552.00	521.00	661.00	656.00	535.00	525.00
Mn (mg/kg)	64.90	68.30	75.00	57.50	80.00	64.50	77.10	80.10
Zn (mg/kg)	22.40	20.50	18.50	22.50	16.10	20.10	21.60	16.30
B (mg/kg)	72.20	75.10	68.80	67.40	76.60	74.80	79.60	67.50
Cu (mg/kg)	9.84	9.01	7.33	8.71	6.92	7.37	8.76	8.71

Table 5.10 Amino acid, protein, starch and total non-structural carbohydrates of sweet potato tuber samples from rain shelter in g/100g for 2023/24 season.

	OF 30% Con	OF 30% AMF	OF 50% Con	OF 50% AMF	OF 70% Con	OF 70% AMF	WF 30% Con	WF 30% AMF	WF 50% Con	WF 50% AMF	WF 70% Con	WF 70% AMF
Arginine	0.339	0.23	0.311	0.3	0.205	0.222	0.192	0.207	0.186	0.215	0.32	0.198
Serine	0.52	0.29	0.388	0.348	0.255	0.238	0.24	0.307	0.271	0.269	0.447	0.288
Aspartic Acid	1.678	1.04	1.377	1.349	0.76	0.773	0.922	1.264	0.783	0.954	1.634	0.811
Glutamic acid	0.727	0.52	0.699	0.61	0.415	0.484	0.452	0.479	0.43	0.489	0.77	0.529
Glycine	0.311	0.18	0.283	0.271	0.19	0.181	0.185	0.21	0.183	0.185	0.288	0.197
Threonine	0.33	0.17	0.238	0.237	0.149	0.167	0.141	0.166	0.176	0.135	0.201	0.15
Alanine	0.31	0.19	0.294	0.266	0.19	0.185	0.195	0.234	0.173	0.213	0.333	0.198
Tyrosine	0.19	0.2	0.247	0.23	0.211	0.143	0.173	0.222	0.191	0.19	0.225	0.18
Proline	0.301	0.21	0.365	0.278	0.237	0.211	0.222	0.226	0.204	0.205	0.345	0.224
HO-Proline	0.026	0.01	0.016	0.01	0.019	0.016	0.012	0.017	0.012	0.014	0.029	0.023
Methionine	0.172	0.06	0.09	0.121	0.199	0.085	0.11	0.159	0.051	0.311	0.616	0.108
Valine	0.408	0.24	0.418	0.366	0.251	0.255	0.249	0.293	0.269	0.258	0.358	0.278
Phenylalanine	0.341	0.21	0.329	0.291	0.203	0.224	0.202	0.249	0.234	0.237	0.324	0.231
Leucine	0.38	0.23	0.387	0.331	0.24	0.251	0.22	0.262	0.223	0.252	0.38	0.236
Isoleucine	0.276	0.17	0.299	0.267	0.183	0.196	0.169	0.194	0.171	0.193	0.278	0.176
Histidine	0.235	0.26	0.513	0.562	0.647	0.542	0.524	0.627	0.218	0.241	0.396	0.292
Lysine	0.374	0.25	0.372	0.34	0.304	0.349	0.213	0.272	0.224	0.266	0.332	0.258
*Protein	7	4.67	6.88	6.22	4.54	3.78	4.49	5.37	4.05	4.88	2.67	4.4

Table 5.11 Amino acid, protein, starch and total non-structural carbohydrates of sweet potato tuber samples from the open field in g/100g for 2023/24 season.

	WFSP control	WFSP 150 AMF	WFSP 250 AMF	WFSP 350 AMF	OFSP control	OFSP 150 AMF	OFSP 250 AMF	OFSP 350 AMF
Arginine	0.111	0.098	0.112	0.084	0.083	0.163	0.129	0.122
Serine	0.151	0.246	0.137	0.105	0.113	0.218	0.187	0.197
Aspartic Acid	0.299	0.36	0.268	0.206	0.173	0.317	0.05	0.359
Glutamic acid	0.244	0.238	0.259	0.207	0.262	0.312	0.117	0.293
Glycine	0.105	0.146	0.104	0.079	0.079	0.15	0.138	0.127
Threonine	0.08	0.086	0.082	0.06	0.058	0.107	0.078	0.094
Alanine	0.095	0.113	0.094	0.066	0.075	0.133	0.074	0.115
Tyrosine	0.096	0.083	0.1	0.055	0.074	0.097	0.03	0.075
Proline	0.08	0.134	0.12	0.089	0.066	0.184	0.11	0.143
HO-Proline	0.011	0.01	0.011	0.009	0.008	0.015	0.008	0.008
Methionine	0.101	0.059	0.129	0.085	0.178	0.148	0.117	0.036
Valine	0.132	0.143	0.117	0.102	0.094	0.183	0.115	0.144
Phenylalanine	0.116	0.104	0.112	0.08	0.081	0.134	0.109	0.123
Leucine	0.138	0.13	0.14	0.098	0.102	0.175	0.118	0.145
Isoleucine	0.103	0.101	0.104	0.071	0.073	0.123	0.09	0.106
Histidine	0.181	0.497	0.2	0.151	0.142	0.342	0.228	0.232
Lysine	0.227	0.175	0.184	0.108	0.118	0.214	0.293	0.125
*Protein	2.21	2.57	2.23	1.68	1.78	3.01	1.98	2.44

Conclusions

There was no clear trend that would indicate that AMF inoculation has a significant effect on nutritional content of sweet potatoes. The largest effect of AMF inoculation was seen in the total sugars and total non-structural carbohydrates in the leaves for treatments WFSP 150 and WFSP 250 and a smaller increase of these substances in OFSP 150. AMF inoculation seemed to have a positive effect on the protein content of WFSP 150 AMF and all three OFSP AMF treatments.

CHAPTER 6 THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) AND WATER REGIMES ON GROWTH OF AFRICAN GINGER

Introduction

African ginger (*Siphonochilus aethiopicus*), also known as wild ginger or Natal ginger, is a highly valued medicinal plant native to southern and tropical Africa. Belonging to the Zingiberaceae family, this perennial herb is traditionally revered for its aromatic rhizomes, which have been used for centuries in African traditional medicine. African ginger is renowned for its diverse medicinal properties, including anti-inflammatory, anti-microbial, and pain-relieving effects, making it a key remedy for colds, flu, respiratory issues, and menstrual discomfort. Beyond its medicinal uses, the plant is prized for its culinary applications and its potential in the cosmetic and pharmaceutical industries. Despite its importance, African ginger faces challenges such as habitat loss and overharvesting, highlighting the need for sustainable cultivation practices to ensure its conservation and continued use. The effect of AMF inoculation on the growth and yield of African ginger was investigated in a greenhouse environment.

Materials and methods

The trial was conducted in a greenhouse at the ARC-VIMP (**Figure 6.1**). The trial consisted of 72 pots in total,4 pots per plot replicated three times. The treatments consisted of control (uninoculated) and AMF inoculated pots under three different irrigation regimes (see Chapter 4).

Figure 6.1 Ginger trial in green house.

Growth parameters and soil microbial activity were measured as described for the sweet potato trials (Chapter 4). Rhizomes/tubers were freeze dried and prepared for analyses as

described in chapter 5. Microbial diversity was determined using Biolog Ecoplates as described in chapter 4.

Results and Discussion

Results of growth parameters are given in **Figures 6.2** to **6.7**. The plant height results showed differences between the treatment and control in plant height during the cropping season in response to the different irrigation regimes (**Figure 6.2)**. African ginger under 30% ADL showed a greater plant height with AMF treatment compared to the control, whereas plant height between the AMF and control treatments under 70% ADL did not show any clear trend.

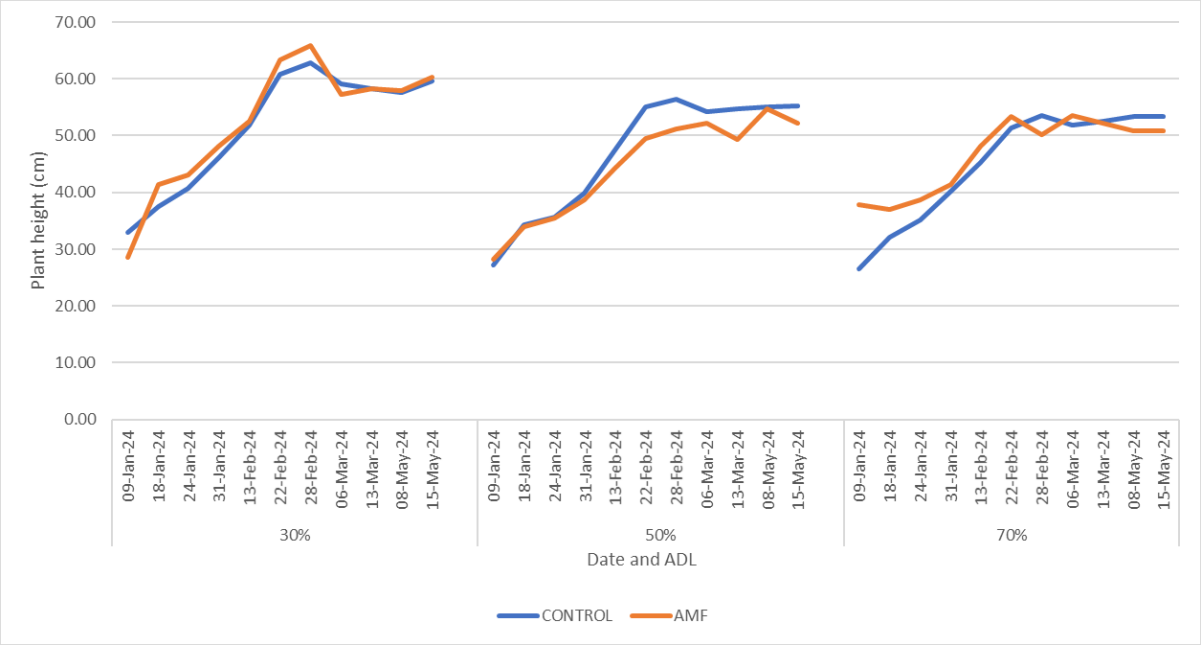


Figure 6.2 Plant height (cm) of African ginger in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) during 2023/24 season.

The AMF inoculated treatments have consistently higher root biomass (**Figure 6.3**) in all treatments as well as higher biomass in tubers of 50% and 70% irrigation treatments. The differences in rhizome/tuber mass between control and AMF is not as significant, however AMF had a positive effect under 70% ADL.

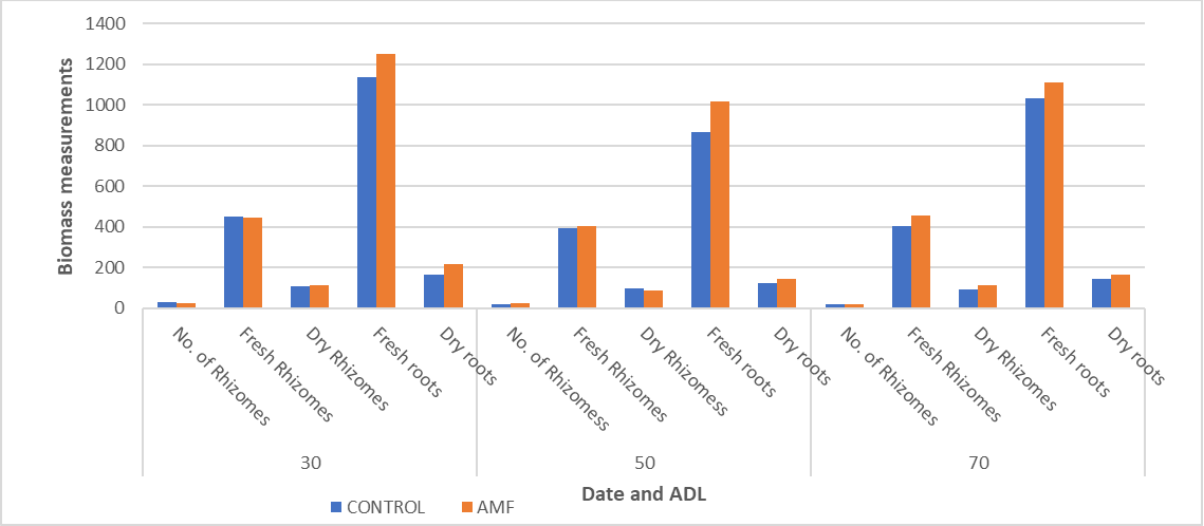


Figure 6.3 Biomass measurements of African ginger in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) for the 2023/24 season.

Figure 6.4 Ginger roots harvested from pots, showing differences between treatments.

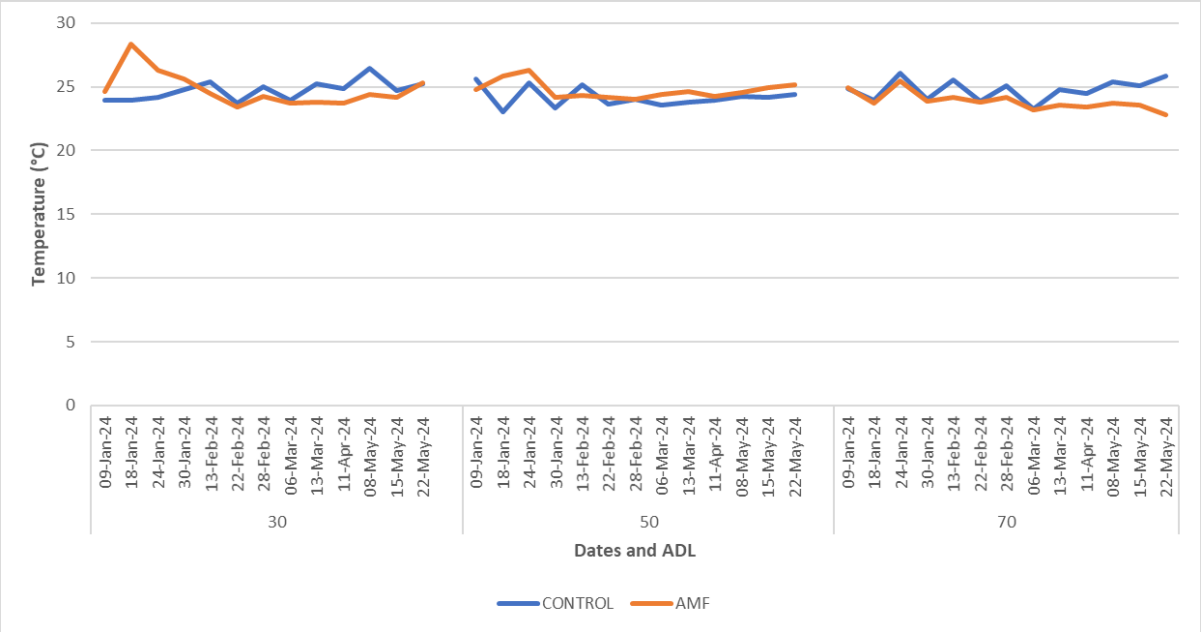


Figure 6.5 Canopy temperature of African ginger in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) for the 2023/24 season.

Canopy temperature was higher under 50% ADL, and lowest under water-stress (70% ADL). Under 30% ADL, the canopy temperature increased in the beginning of the growing period but decreased as the season continued.

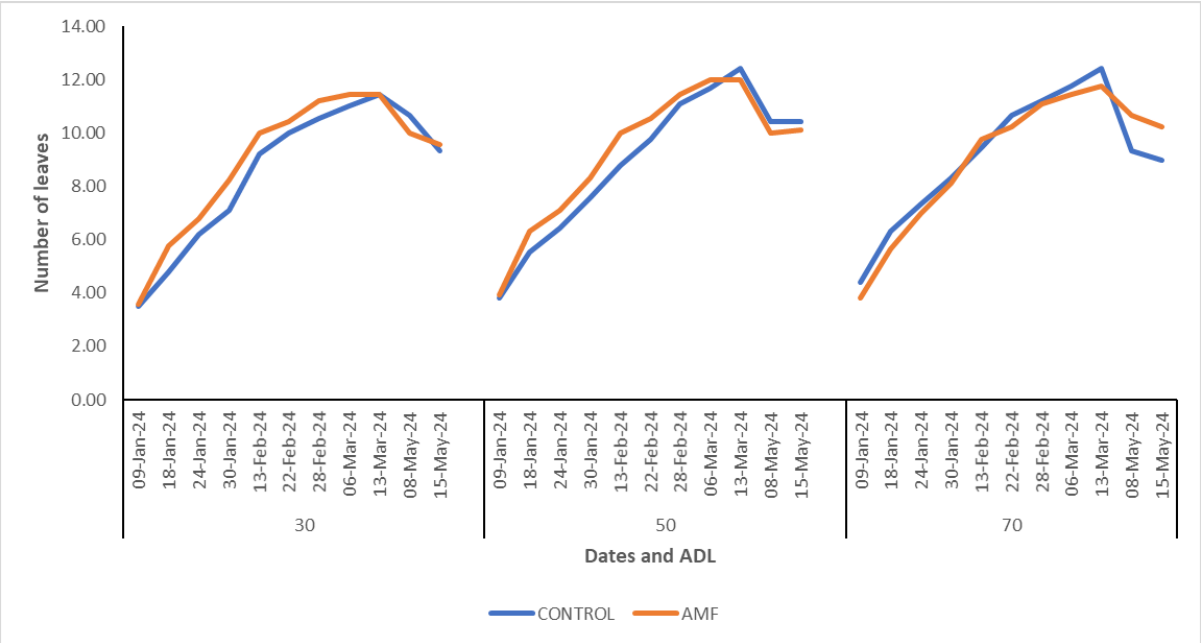


Figure 6.6 Number of leaves of African ginger in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) during 2023 season.

There were no clear trends in the number of leaves between the inoculated and uninoculated treatments or between the different irrigation regimes.

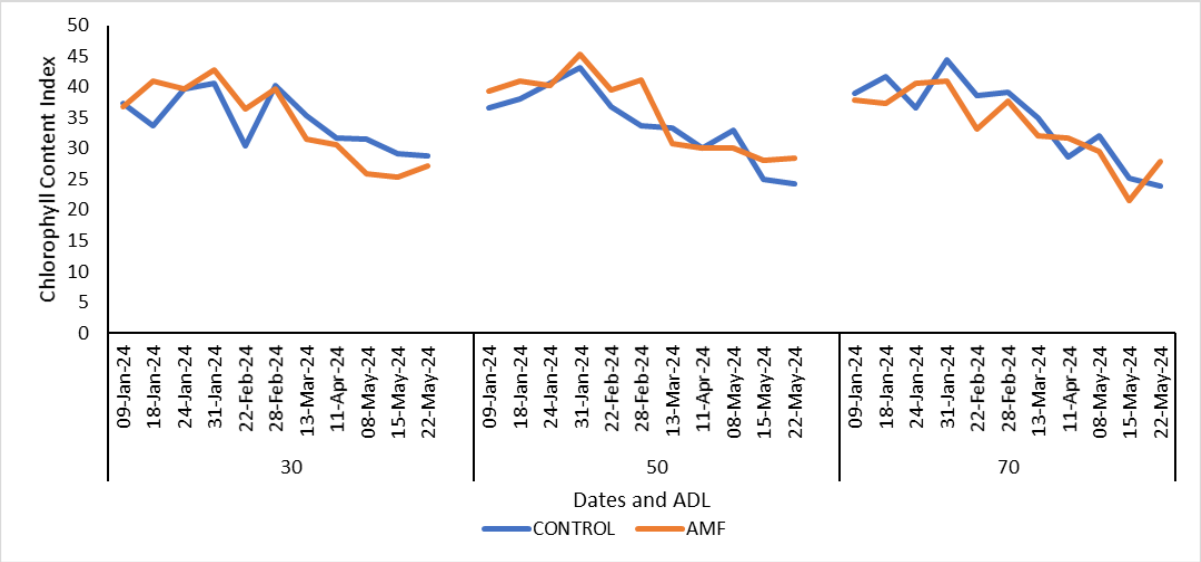


Figure 6.7 Chlorophyll content reading of African ginger in response to arbuscular mycorrhizal fungi (AMF) application and irrigation regime (ADL) for the 2023 season.

Chlorophyll content readings under both 30% and 50% ADL were higher in the beginning of the growing season compared to the end of the growing season, with lower readings under 70% ADL.

The phenolic and flavonoid content of tubers inoculated with AMF under 50% and 70% water stress decreased compared to the controls while the antioxidant activity of AMF inoculated treatment under 50% water stress increased significantly (**Table 6.1**).

Table 6.1 Total phenolic and flavonoid content as well as antioxidant activity of African ginger.

Treatment	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CE/g)	Antioxidant activity (µg/ml)
AMF 30	2.16	1.09	18.19
AMF 50	1.80	0.85	89.28
AMF 70	1.41	0.68	18.45
Control 30	2.14	1.98	10.91
Control 50	2.32	1.38	33.04
Control 70	2.12	0.86	56.93

Minerals elements calcium, copper, iron, potassium and zinc, increased under 70% water stress under both inoculated and control treatments (**Table 6.2**). There was no significant effect between inoculated and uninoculated treatments.

Table 6.2 Mineral composition of African ginger tubers in greenhouse trial.

Treatment	Mineral element (mg/100 g dry sample)							
	Ca	Cu	Fe	K	Mg	Na	P	Zn
AMF 30	9.05	0.97	775.64	6379.76	753.69	1609.68	628.75	7.07
AMF 50	7.48	0.8	95.2	10638.07	606.4	2027.34	539.62	7.17
AMF 70	10.04	1.08	1306.43	11118.67	764.46	2152.03	613.45	8.57
Control 30	8.53	1.08	904.32	10645.64	795.38	2047.3	698.12	7.07
Control 50	8.49	1	1013.64	10564.73	698.7	2171.15	612.37	6.17
Control 70	10.66	1.4	1875.01	13233.09	794.11	2065.39	661.11	11.89

Effect of AMF on soil microbial activity and richness

Average well colour development was highest under 50% ADL in the AMF treatment, similar to that of the control under 70% ADL (water-stress). However, under 70% ADL, the AMF treatment showed the lowest AWCD. Utilization of the five major groups of carbon sources was highest in the control under 50% ADL compared to the other watering regimes. In the AMF treatment, utilization of the carboxylic acids and amines was greatest under 50% and 70% ADL.

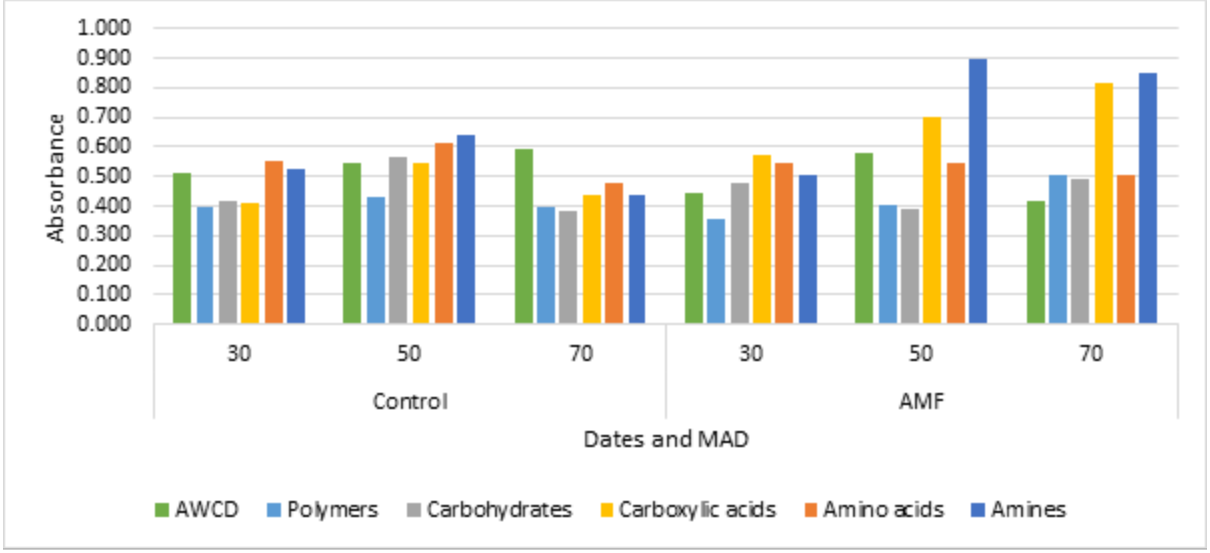


Figure 6.8 AWCD values that show the extent of utilization of five major groups of carbon sources by microbial communities of soil samples collected from the ginger trial 2023/24 season.

The highest diversity was observed in the AMF treatment under 30% and 70% ADL (**Figure 6.9**). Diversity was relatively similar between the AMF and control treatments under 30% and 50% ADL. Substrate richness increased in the control treatment from the 30% ADL to 70% ADL, whereas the opposite effect was seen in the AMF treatment, with a decrease in richness as the level of water-stress increased.

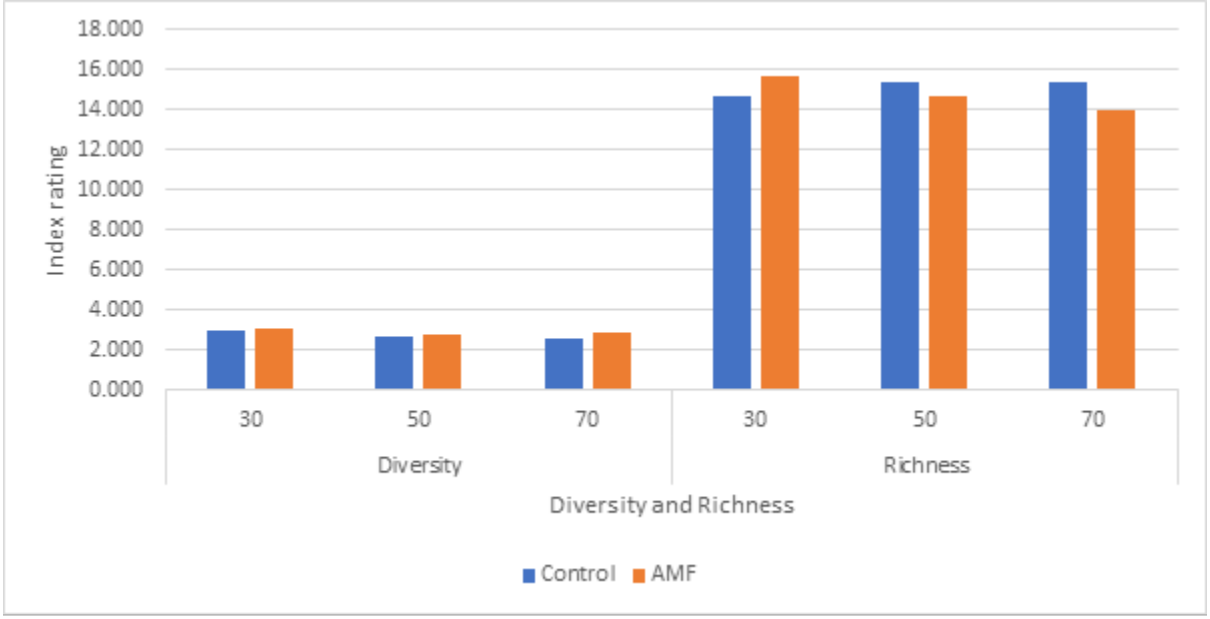


Figure 6.9 Shannon Weaver diversity index and Richness of microbial composition of soil samples collected from the ginger trial after the season.

Conclusions

On-farm produced AMF led to slight, but not significant, increases in African ginger rhizome/tuber and root yield. While no clear differences were observed in above ground growth during the season, the yield and especially antioxidant activity under 50% water stress showed promising increase in AMF inoculated treatments.

Similar to results from the sweet potato trial, AMF inoculation showed best results with moderate water stress (50%), but different from sweet potato it had a measurable effect on fresh root yield, even with severe water stress (70%).

CHAPTER 7 IMPLEMENTATION AND EVALUATION OF AMF PRODUCTION SYSTEM AT SMALLHOLDER FARM

Introduction

All agricultural systems depend heavily on soil microorganisms, which have various effects on food yield and quality. These effects range from harmful (like pathogenic fungi and bacteria) to beneficial (like plant growth promoters and pathogen antagonists), especially in low-input systems (Franco et al., 2011). Rhizosphere and endophytic microorganisms are receiving more and more attention since they are essential to plant growth and health. It has been stated that “the ultimate agricultural goal in studies of the biology of the soil-root interface must be the manipulation of microorganisms in this zone to increase plant health and growth” (Rovira, 1979).

When compared to imported inoculum, the on-farm production of AMF inoculum avoids some production and transportation costs, and farmers can easily transfer the technology (Douds et al., 2006). It may also help address the issue of high market inoculum prices, low quality, and inadequate delivery mechanisms associated with production and storage conditions. According to Enkhtuya et al. (2000), there are numerous situations in which producing inoculum on-farm from locally isolated adapted species may be more effective than introducing them. Furthermore, as opposed to commercial inoculum, which might only contain one species (Douds et al., 2005), a taxonomically functionally diverse inoculum can be produced (Smit et al., 2000; Hart and Reader, 2002). Since a single strain might not withstand particular environmental changes, a formulation containing a consortium of AMF strains would have several advantages over single-isolate AMF inoculum (Adholeya et al., 2005).

The goals of on-farm experimentation are multifaceted. It first enables farmers and researchers to collaborate on the advancement of technology. The likelihood that a practice will be adopted increases with farmers' involvement in the technology development process, both in terms of frequency and timing. On-farm trials are crucial for learning about farmers' opinions of a practice, getting their suggestions for changes, and seeing their innovations (Franzel and Coe, 2002).

Furthermore, testing conducted on-farm offers a more comprehensive evaluation of a practice's biophysical performance than can be obtained on-station. This is particularly significant because research stations' flora, fauna, and soil type frequently differ from those on nearby farms. Thirdly, on-farm trials crucial to get accurate input-output data for financial analysis. Financial analyses of on-station experiments are different from those of farm trials for three reasons: (1) yield response is frequently skewed upward; (2) labour estimates used by station labourers on small plots are not representative of the farming community; and (3) operations frequently vary, such as when tractors are used to prepare land rather than oxen or hoes (Franzel and Coe, 2002).

Lastly, testing conducted on farms offers crucial diagnostic data regarding issues faced by farmers. Interacting with farmers in on-farm trials can teach researchers much about their issues, preferences, and livelihood strategies-even if diagnostic surveys and appraisals have already been completed. Because trials are based on farmers' actions rather than just their

statements, they have significant advantages over surveys (Franzel and Coe, 2002). The aims of this study were to introduce the on-farm AMF inoculum production to a smallholder farmer, assist with inoculating a crop and evaluate the success of the system.

Materials and Methods

Starter soil was collected from an undisturbed area on the smallholder farm near Cullinan (25°36"S; 28°36"E; elevation 1 333 m). The vegetation was cleared away and digging down to a depth of about 25 cm, the soil and as many fine roots as possible were collected. Stony soil was sieved to get rid of large stones.

A ‘trap-pot’ system was used to multiply inoculum. Several large (5 litres) plastic pots were used for the pot system. The containers were filled with a mixture of red topsoil from the farm and compost at a ratio of 5:1 and the starter soil was added at approximately 5% v/v to each container and mixed with the top 15 cm of soil. Four to five Bahia grass (*Paspalum notatum*) seedlings were planted into each pot (**Figure 7.1**). Maintenance consisted of watering and weeding as needed. Plants were grown for at least three months for inoculum production.

Figure 7.1 Pots with Bahia grass seedlings for multiplication of mycorrhiza.

Watering was discontinued ten days before harvesting the inoculum. In doing so, the fungus was tricked into releasing proliferating spores while the plants were killed. Following a 10-day period, the inoculum was prepared by chopping off the tops of the grass and extracting the bait plants' roots. These were then roughly chopped into 1 cm pieces and re-incorporated into the soil from the trap-pot or trough (**Figure 7.2**). This soil-root mixture functioned as the inoculum.

Figure 7.2 Inoculum being prepared, showing roots from Bahia grass in pots.

Quantification and characterisation of AMF produced

Isolation and characterisation of produced AMF as well as the starter soil (veld soil) and the soil from the field where the trial was planted, were conducted as described in Chapter 3.

Study site and treatment details

The study was conducted at a smallholder farm north of Cullinan (Gauteng). The field was ploughed before planting, however no fertiliser was applied before or at planting. A single fertiliser application was done on 12 December 2023 of approximately 7 mL (one cold drink bottle cap) of KAN/LAN per plant.

Weather data (**Figure 7.3**) for the area during the period of the trial was obtained from ARC-NRE (Moeletsi et al., 2022).

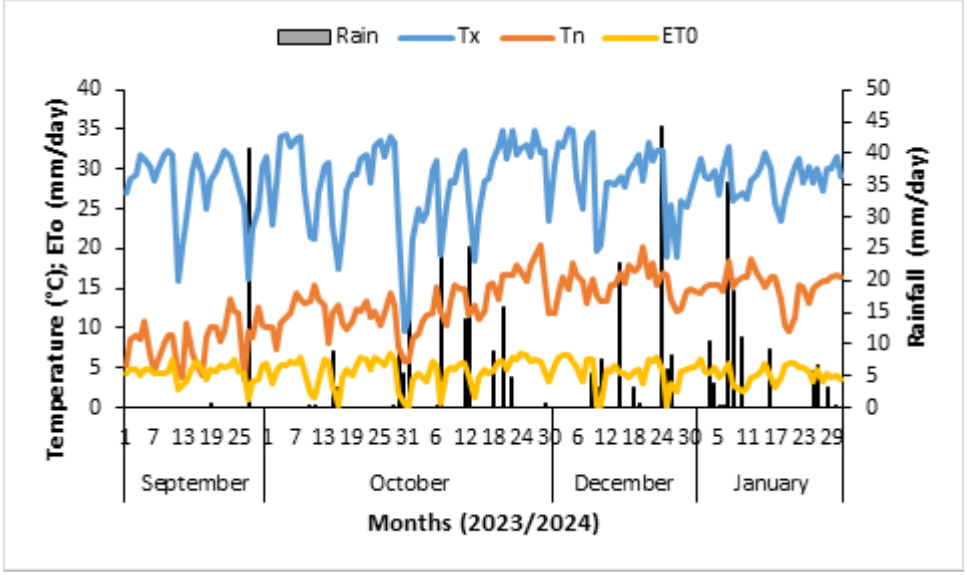


Figure 7.3 Total rainfall (mm), Evapotranspiration, average maximum (Tx) and minimum (Tn) temperatures collected from the nearest weather station at the experimental site.

Application of Arbuscular mycorrhizal fungi (AMF) in the field

AMF inoculum was prepared on-farm during the winter and was applied on the day of planting (26 October 2023). A total of 1 200 bell pepper seedlings (**Figure 7.4**) were planted. Inoculation was done by opening holes, planting a bell pepper seedling and adding 250 mL of AMF inoculum (**Figure 7.5**), after which the hole was closed using soil. Control plants were planted without AMF inoculum.

Figure 7.4 Tray of bell pepper seedlings before planting.

Figure 7.5 AMF inoculum applied with bell pepper seedling.

Plant growth parameters measured

Chlorophyll content in plant leaves was measured using a SPAD (Soil Plant Analysis Development), which provides an indication of the plant's health and photosynthetic activity. Chlorophyll is a pigment essential for photosynthesis, and its concentration can give insights into the plant's ability to produce energy through sunlight absorption. Fifteen plants were selected per treatment and readings were taken at each plant. Plant growth was measured by taking the measurement of each of 15 plants from soil level up to the top leaf with a measuring tape.

Quantification of AMF present in soil after trial

AMF was isolated and characterised as described in chapter 3.

Results and Discussion

The Bahia grass in the pots did not seem to grow very vigorously when looking at the leaves, however, good root systems were observed when the inoculum was prepared. Bahia grass is valued for outstanding drought and heat tolerance and as such makes it good candidate for AMF inoculum production as it needs very little care. The number of sporocarps observed from each soil sample are given in **Table 7.1**.

Table 7.1 The number of sporocarps recovered from 100g of soil.

Source	Number of sporocarps
Veld soil (starter soil)	1257
Trial site (farmer's field)	625
Trap pot inoculum	1242

The veld soil and trap pot had similar numbers of sporocarps, while the soil in the tunnel had half of the number of sporocarps. This indicates that the soil in the tunnel has degraded quality due to the intensive farming being conducted.

Sporocarps observed (**Figure 7.6**) could be broadly classified into 10 morpho-groups based on colour and size. Based on morphological identification, most of these isolates belonged to the Glomeraceae family.

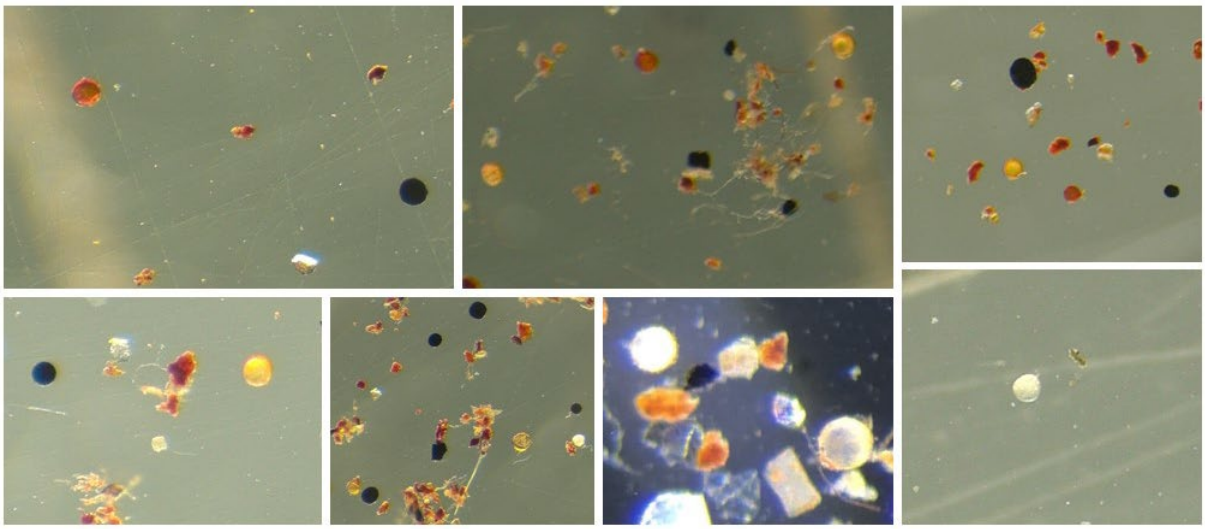


Figure 7.6 Diversity of AMF sporocarps observed.

The variety of AMF found in the natural veld soil was successfully trapped by the trap pot method used to multiply the AMF for inoculum. It is challenging to identify AMF species at the species level solely by morphology; nevertheless, the majority of morphological groupings fall under the Glomeraceae family. Numerous genera, including *Glomus*, *Rhizophagus*, *Funneliformis*, and *Septoglomus*, are abundant in the Glomeraceae family. These genera are the most frequently found in commercial inoculants and have been reported on all continents (Basiru et al., 2021). Different Glomeraceae members have demonstrated varying degrees of success in colonizing host roots and functioning in varied field environments. For example, species of *Glomus* and *Rhizophagus* have reportedly performed better under various management strategies than other genera, like *Gigaspora* and *Scutellospora* (Veresoglou et al., 2011). Therefore, the variety of AMF found in the generated inoculum ought to increase the likelihood of crop plants successfully colonizing various environments.

Plant growth parameters

The average chlorophyll content of the AMF inoculated and control plants are given in **Figure 7.7**. There are no statistical differences between the treatments, however the AMF inoculated plants had initially slightly lower chlorophyll content, compared to the control treatments. This was expected as it is known that energy investment for establishment of AMF symbiosis requires an input of around 20% of photoassimilated carbon (Jakobsen and Rosendahl 1989).

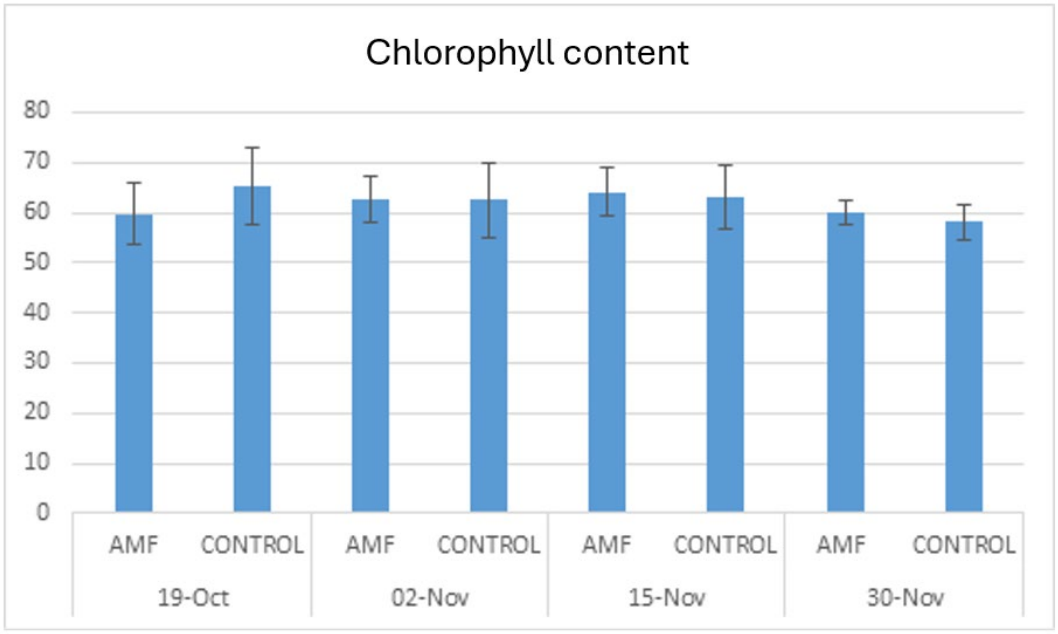


Figure 7.7 Average chlorophyll content of AMF inoculated and control plants.

The average plant length of AMF inoculated and control plants are given in **Figure 7.8**. The AMF inoculated plants were consistently taller than the uninoculated plants and had to be trellised shortly after planting to prevent it from toppling over, especially after fruit started forming.

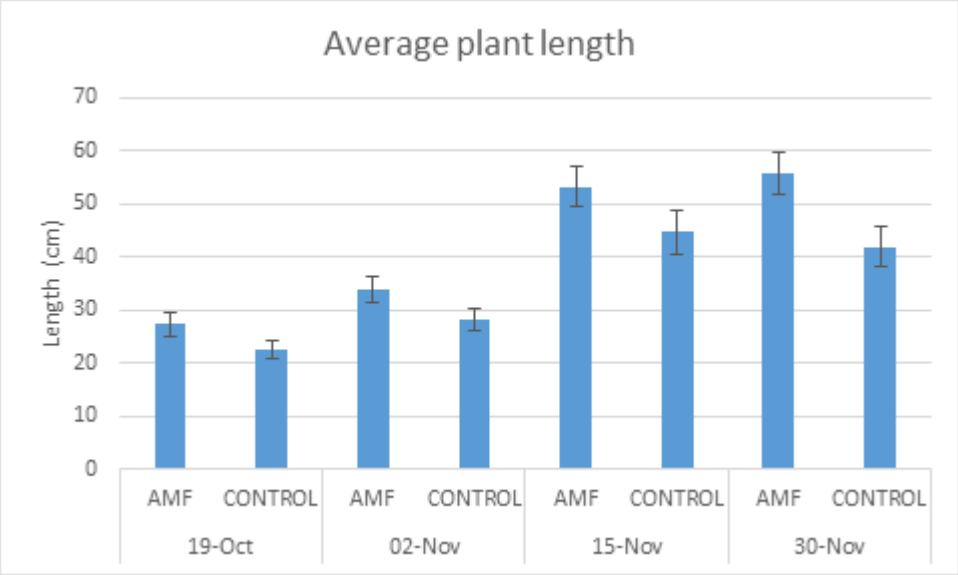


Figure 7.8 Average plant length of AMF inoculated and control plants.

Yield

Harvesting was conducted throughout December 2023 and January 2024. Although the plants were still bearing fruit in February, the farmer had decided to stop harvesting as the quality of the fruit had decreased due to sun scald. Unfortunately, the farmer did not keep separate records of fruit harvested of the inoculated and uninoculated plants, as was requested. The total amount of packets (4 bell peppers), boxes and crates harvested in December and January is given in **Table 7.2**.

Table 7.2 Total number of containers of bell peppers sold

	Price	Number of containers in December	Number of containers in in January	Total
Packet (4)	R10.00	120	200	R3 200.00
Box	R60.00	55	120	R10 500.00
Crate	R120.00	20	10	R3 600.00
				R17 300.00

Figure 7.9 Large, healthy bell pepper produced from an AMF inoculated plant.

AMF composition in soil after harvest

The average number of sporocarps recovered from soil in the inoculated treatments were 1 042 while that in the control was 726. The initial a number of sporocarps in the field before planting was 625 and the number in the inoculum was 1 242. In general, the number of sporocarps increased both in the inoculated and uninoculated treatments. The increase in sporocarps in the control shows that the native mycorrhizae present in the soil effectively colonised the plants. However, the differences in plant growth were still noticeable in the inoculated plants, indicating that the higher amount of AMF added from the inoculum were effective.

Farmer perception of AMF production and effect

In general, the farmer found the production and use of AMF easy and effective. The plants grew exceptionally well and were very healthy and vigorous with only a single fertiliser application through the growing season. No diseases were observed on the inoculated or uninoculated plants.

Plants with AMF produced more fruit that was generally larger. However, the AMF inoculated plants were more prone to falling over and had to be trellised (Figure 7.10a), while the uninoculated plants were more stocky and sturdy (Figure 7.10b).

Figure 7.10 Inoculated plants grew higher and had to be trellised while b) uninoculated plants were shorter and more stocky

Ms Carolisen indicated that she would repeat the process with other crops as it gave her good yields with minimal inputs.

Conclusions

The Bahia grass can multiply the AMF for inoculum. The history of the soil plays an important role when one uses it as a multiplication medium. Degraded or over-cultivated soils are not suitable candidates for the multiplication of AMF. Smallholder farmers are advised to use either undisturbed soils, such as the soil from the veld as for inoculum production or a combination of red topsoil (20-35% clay content) and compost mixed at a ratio 5:1 to which the starter soil (collected from undisturbed veld) is added. Plant height of AMF inoculated plants were significantly higher than the control plants and had to be trellised sooner.

The farmer found the process of inoculum production and application easy and understandable and felt that it added value to her production.

CHAPTER 8 GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The root-associated microbiome is the collection of various microorganisms that are linked with plant roots, such as bacteria, fungi, viruses, protists, and archaea. Among them, mycorrhizal fungi penetrate host roots to enhance their uptake of nutrients, primarily nitrogen and phosphorus. Plants provide photosynthetic carbon to the fungus that are colonising them in return. This nutrient exchange has a significant impact on the ecosystems of microorganisms and plants because it influences important soil processes, the carbon cycle, and plant health.

The framework for the regulation and exchange of nutrients between arbuscular mycorrhizal (AM) fungi and host plants has been studied by various researchers. Mycorrhizae are among the most significant biological interkingdom interactions. The only families that do not appear to form such interactions are Brassicaceae, Amaranthaceae, Caryophyllaceae and Chenopodiaceae. (Genre et al., 2020). To be used as biofertilisers and bioprotectors for sustainable agriculture and forestry management, we must have a greater understanding of mycorrhizal fungi and mycorrhizosphere microorganisms and their synergistic effects.

Plant roots and mycorrhizal fungal hyphae attract different microorganisms into their mycorrhizospheres during symbiosis. Plant interactions with the mycorrhizosphere microbiome, which supports plant development, nutrient absorption, and stress tolerance from biotic and abiotic sources, can be mediated by mycorrhizal fungi (Nouri et al., 2014; Zhang et al., 2022). Mycorrhizae's main contribution to plant growth is its massive nutrient-supply to the host plant in nutrient-deficient soils.

In sub-Saharan Africa, where soil fertility is low, using inorganic fertiliser is crucial for maximizing crop productivity. However, this practice has been associated with increased crop production costs, nitrate leaching contaminating surface and/or groundwater, and phosphate runoff that eutrophicates surface water. Additionally, cropping systems have become weaker due to secondary effects on the biotic community of the soil and soil impoverishment, which has increased their reliance on external chemical fertilisers. Effective management of plant nutrition should lead to increased and sustainable agricultural production and environmental protection. Enhancing the production and utilization of bio-inoculants, like AMF, is a developing technique in soil fertility management that can raise and reasonably enhance crop yields (Mukhongo et al., 2016).

Farmers in SSA have less access to mycorrhizal inoculants due to the lack of AMF inoculant production units in the area; these units are only known to exist in Kenya (Dudutech) and Mycoroot (Pty) Ltd in South Africa. (Mukhongo et al., 2016). This demonstrates that the majority of AMF inoculum used in SSA is imported, accounting for the high market prices brought on by production and transportation expenses (Mukhongo et al., 2016). Because imported inoculants are expensive, producing AMF inoculum on-site is viewed as an appealing alternative to importing inoculants (Douds et al., 2005).

The increased uptake of nutrients by AMF and the consequent better growth, health and yield of plants are especially important for smallholder farmers who cannot afford fertilisers. However, using AMF in sustainable agriculture also has a lot of drawbacks. High fertiliser applications prevent colonisation (Jensen and Jakobsen 1980; Kahiluoto et al., 2001),

whereas ploughing of the soil disturbs mycelial networks and reduces the range of fungi that may survive (Kabir et al., 1997; Helgason et al., 1998; Daniell et al., 2001). Basically, a lot of crop plants, particularly cereals, grudgingly establish arbuscular mycorrhizas, most likely due in part to breeding practices that have created an environment unfavourable to the symbiosis (e.g. ploughed soils or high nutrient levels). These problems are, however, mostly circumvented by the lack of high-tech equipment and fertilisers by smallholder farmers, which makes AMF an even more attractive solution for these farms.

Despite multiple reports on the importance of bio-inoculum in sustainable agriculture, the production and adoption of AMF in SSA smallholder systems is still limited due to a lack of awareness and understanding of bio-inoculum (Ijdo et al., 2011). As a result, the sector has not developed well (Schulte-Geldermann 2013). Gaining insight into how these obstacles impact bio-inoculant production and uptake could enhance the advantages of AMF inoculum for smallholder farmers in Sub-Saharan Africa. The primary obstacles to the production and adoption of AMF inoculums are research capacity and technological challenges (Mukhongo et al., 2016).

The trap pot system (either in greenhouse or outside) is effective in multiplying a diverse range of locally adapted mycorrhizae. The trap pots inside the greenhouse showed the best results with a 39-fold increase in sporocarps. The outside trenches also showed increase in sporocarps but had 30% less than the pots in the greenhouse. Both methods captured most of the diversity of AMF from the veld soil.

AMF inoculation under different watering regimes gave inconsistent results between the two season's trials. While clear increases in yield were seen for OFSP and WFSP under 30 and 50% ADL in season one, this was only the case for OFSP under 30% ADL in season two. While OFSP had increased yield under 50% and 70% ADL in season two, the increase was small (2.5 and 4 t ha^{-1}, respectively). This trial should be repeated.

No clear benefit was seen in the soil microbial diversity after inoculation with AMF and it changes in these communities may only occur after several years of managing soil health. Increasing soil health includes practises like crop rotation, including cover crops, increasing organic matter in the soil as well as inoculation with beneficial organisms like AMF.

AMF inoculation of African ginger in the greenhouse lead to an increase in fresh root weight under 30%, 50% and 70% ADL. When compared to the controls, the phenolic and flavonoid content of tubers inoculated with AMF under 50% and 70% water stress decreased but the antioxidant activity of the AMF-inoculated treatment under 50% water stress dramatically increased. There were no significant differences in the soil microbial composition of the AMF inoculated and uninoculated soil.

The implementation of the on-farm production at the smallholder farmer was successful. Although the pots were outside during the winter and the plants did not appear to grow well, the belowground root formation of the Bahia grass were sufficient to trap and increase the diversity of the AMF from the veld soil. Green pepper plants inoculated with the AMF grew taller than the control plants. Good yields were recorded although the farmer unfortunately did not record the differences between the inoculated and control plants.

Roll out of the on-farm AMF production to multiple localities in South Africa is recommended. Further studies on the effect of AMF inoculation on disease suppression of common soil-borne pathogens, especially under drought stress conditions, should be conducted. Trials on other crops than sweet potato and African ginger should be conducted. More cultivars of the crops that were already evaluated should be added, as it was clear that the WFSP and OFSP showed different responses to AMF under different watering regimes. The on-farm AMF production should be included in training sessions for farmers and extension officers.

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Appendices

Appendix A - Conference outputs

SASAT - South African Society for Agricultural Technologists held 22-25 October 2024

Abiotic and biotic factors influence on production of white flesh (Blesbok) and orange flesh (Bophelo) sweet potato cultivars

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Introduction

Sweet potatoes (*Ipomoea batatas*) is a crop recognised worldwide for its nutritional benefits and versatility even under suboptimal conditions. Arbuscular mycorrhizal fungi (AMF) have been shown to increase plant resilience and subsequent yield in adverse conditions. This research investigated the impact of abiotic (temperature and soil moisture) and biotic factors (AMF) on the yield of white flesh (Blesbok) and orange flesh (Bophelo) sweet potatoes.

Materials and methods

The experiment was conducted in a rain shelter and set up in a in a randomised complete block design (RCBD). The combined factors consist of three watering regimes (30%, 50% and 70% Allowable depletion level (ADL), two sweet potato cultivars and addition of AMF (250g) and without AMF; all replicated three times. Neutron probe access tubes were placed to monitor the soil moisture content at the desirable ADL.

Results and discussion

The study found that maintaining a 30% ADL combined with AMF enhanced the production of marketable tubers in orange-fleshed cultivars. A moderate water stress of 50% ADL optimized yield across both cultivars.

Conclusion

The study results indicated that inoculating crops with AMF under drought stress conditions can improve sweet potato resilience and productivity, making it a promising method for sustainable farming of sweet potato in water-limited environments, especially in regions with water shortages.

Keywords: Arbuscular Mycorrhiza Fungi (AMF), Allowable Depletion Level

Acknowledgements

The Water Research Commission for funding (C2022.2023-00918) and Agricultural Research Council for supporting the project implementation.

Abiotic and biotic factors influence on production of white flesh (Blesbok) and orange flesh (Bophelo) sweet potato cultivars

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Introduction

Sweet potato (*Ipomoea batatas*) is a major crop worldwide, known for its nutritional benefits and capacity to thrive in various environmental conditions. Nevertheless, the growth and productivity of sweet potatoes are affected by both environmental conditions and agronomic practices. Research has demonstrated that water stress impacts the growth of sweet potatoes (Agili et al., 2012). Sweet potato growers rely on seasonal rainfall to sustain the crop, and if there is insufficient rainfall, the crop's nutritional water productivity may suffer. Rainfall in South Africa is unstable and unreliable; incorporating beneficial microorganisms, such as arbuscular mycorrhizal fungi (AMF), into sweet potato production could increase crop yield even in adverse conditions, increasing drought tolerance and improving plant growth.

Objectives

- To determine the effects of AMF and different water regimes on the growth and yield of orange (Bophelo) and white flesh (Blesbok) sweet potato cultivars.
- Evaluation of AMF on nutritional content and phytochemicals of orange and white flesh sweet potato cultivars.

Materials and Methods

The experiment was conducted in a rain shelter and set up in a (3 x 2 x 2) randomized complete block design (RCBD). The combined factors consisted of: three water regimes (irrigation 70%, 50%, 30% Allowable depletion level (ADL); two sweet potato cultivars (Orange Flesh Sweet Potato "Bophelo", White flesh sweet potato "Blesbok"); with AMF (250g) and without AMF; all replicated three times. On planting day (Fig. 1A), holes were created, sweet potato cuttings were inserted, and 250 grams of AMF crude inoculum was added. Neutron probe access tubes were placed to monitor the soil moisture content.

Figure 1. Illustration of A) Planting of sweet potato, B) freeze-dried, ground samples for nutritional analysis, C) White flesh tubers, and D) Orange flesh tubers.

Results and discussion

Table 1. Total fresh biomass yield and water use of two sweet potato cultivars subjected to different irrigation regimes.

Sweet potato cultivars	Irrigation (%)	Fresh biomass Yield (t ha^{-1})	Seasonal crop water use (mm)	Crop water productivity (t ha^{-1} mm^{-1})
Orange flesh	30	35.36^{a}	389.93^{a}	0.09
	50	22.76^{b}	269.98^{b}	0.08
	70	8.84^{c}	195.56^{c}	0.05
White flesh	30	27.29^{b}	236.48^{b}	0.12
	50	25.38^{b}	188.97^{c}	0.13
	70	26.15^{b}	218.74^{c}	0.12

Results (continued)

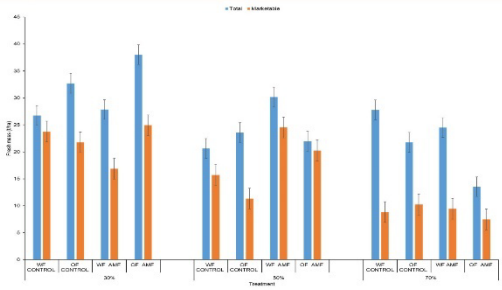

Figure 2. Total fresh mass and marketable yield of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhiza fungi (AMF) application and irrigation regimes (ADL).

Table 2. Total phenolic, total flavonoid, and condensed tannins content of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhiza fungi (AMF) application and irrigation regimes (ADL).

Cultivars	Irrigation (%)	Treatment	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CE/g)	Condensed tannins (mg cyanidin chloride/g sample)
White flesh	30	CONTROL	20.44	4.15	1.43
	50		17.05	5.23	3.96
	70		15.57	4.73	0.55
	30	AMF	16.94	3.33	0.20
	50		16.79	2.88	2.84
	70		13.89	3.19	0.17
Orange flesh	30	CONTROL	14.31	2.42	0.28
	50		14.45	3.37	2.67
	70		12.47	2.44	1.30
	30	AMF	13.33	2.92	0.40
	50		9.80	3.18	0.56
	70		13.56	3.87	0.06

The results revealed that compared to other treatments, well-watered treatment of 30% ADL for orange flesh sweet potato had significantly higher biomass yield and water use. In contrast, white flesh had higher water productivity than orange flesh, as shown in Table 1. Orange flesh sweet potato under 30% ADL combined with AMF had higher total fresh mass and marketable yield, followed by 50% ADL of white flesh sweet potato, as shown in Figure 2. In terms of phytochemical content, white flesh combined with 30% ADL control and with AMF resulted in higher flavonoid and phenolic content as compared to 70% ADL treatment, and orange flesh had lower phenolic as compared to white flesh in all treatments, as shown in Table 2. Arbuscular mycorrhizal fungi can enhance yields and sustainability by reducing agricultural water and fertilizer requirements and improving the farming process.

Conclusion

The study indicates that maintaining a 30% ADL combined with AMF enhanced the production of marketable tubers in orange-fleshed cultivars. However, the White flesh cultivar had higher nutritional content on AMF and 30% ADL compared to orange flesh.

Reference

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- Agricultural Research Council (ARC) for support
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- Tshwane University of Technology (TUT)

Combined congress - 19-24 January 2025

ABIOTIC AND BIOTIC FACTORS INFLUENCE ON PRODUCTION OF WHITE FLESH (BLESBOK) AND ORANGE FLESH (BOPHELO) SWEET POTATO CULTIVARS

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INTRODUCTION

Sweet potatoes (*Ipomoea batatas*) are a globally acknowledged crop known for their nutritional advantages and adaptability, even under substandard environments. Water stress has been demonstrated to impact the growth of sweet potatoes. Sweet potato cultivators depend on seasonal precipitation to support the crop, and inadequate rainfall may adversely affect the crop's nutritional water output. Rainfall in South Africa is erratic and inconsistent; integrating beneficial microbes into sweet potato cultivation may enhance crop yield despite unfavourable conditions (Dawwam et al., 2013). Arbuscular mycorrhizal fungi (AMF) enhance plant resistance and yield under unfavourable conditions. This study examined the influence of abiotic parameters (temperature and soil moisture) and biotic factors (Arbuscular mycorrhizal fungi) on the production of white-fleshed (Blesbok) and orange-fleshed (Bophelo) sweet potatoes.

MATERIALS AND METHODS

The experiment was conducted in a rain shelter and set up in a randomised complete block design (RCBD). The combined factors consist of three watering regimes (30%, 50%, and 70% Allowable depletion level (ADL)), two sweet potato cultivars, and the addition of AMF (250g) and without AMF; all replicated three times. Neutron probe access tubes were placed to monitor the soil moisture content at the desirable ADL.

RESULTS AND DISCUSSION

The study found that maintaining a 30% ADL combined with AMF enhanced the production of marketable tubers in orange-fleshed cultivars. A moderate water stress of 50% ADL combined with AMF optimized yield across both cultivars.

CONCLUSIONS AND RECOMMENDATIONS

The study results indicated that inoculating crops with AMF under drought stress conditions can improve sweet potato resilience and productivity, making it a promising method for sustainable sweet potato farming in water-limited environments, especially in regions with water shortages.

REFERENCE

Dawwam, G.E., Elbeltagy, A., Emara, H.M., Abbas, I.H. and Hassan, M.M., 2013. Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. *Annals of Agricultural Sciences*, *58*(2), 195-201.

Keywords: Allowable Depletion Level, Arbuscular Mycorrhiza Fungi

ACKNOWLEDGEMENTS

The Water Research Commission for funding (C2022.2023-00918) and the Agricultural Research Council for supporting the project implementation.

INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON PRODUCTION OF WHITE FLESH (BLESBOK) AND ORANGE FLESH (BOPHELO) SWEET POTATO CULTIVARS

Ndivhuwo Mulaudzi[1,2]*, Puffy Soundy[2], Kafua M Lodama[1], Elsie M Cruywagen[1], Hintsa T Araya[1], Mariette Truter[1]

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INTRODUCTION

Sweet potatoes (*Ipomoea batatas*) are widely recognised for their nutritional benefits and remarkable adaptability, even under challenging environments. However, water stress has been demonstrated to impact the growth of sweet potatoes. Sweet potato cultivators depend on seasonal precipitation to support the crop, and inadequate rainfall may adversely affect the crop's nutritional water output. Given South Africa's unpredictable and inconsistent rainfall patterns, incorporating beneficial microbes into sweet potato farming practices offers a promising strategy to improve yield under adverse conditions (Dawwam et al., 2013). Arbuscular mycorrhizal fungi (AMF) enhance plant resistance and yield under unfavourable conditions. This study examined the influence of abiotic parameters (temperature and soil moisture) and biotic factors (AMF) on the production of white-fleshed (Blesbok) and orange-fleshed (Bophelo) sweet potato.

OBJECTIVES

- To determine the effects of AMF and different water regimes on the growth and yield of orange (Bophelo) and white flesh (Blesbok) sweet potato cultivars.
- Evaluation of AMF on nutritional content and phytochemicals of orange and white flesh sweet potato cultivars.

MATERIALS AND METHODS

The experiment was conducted in a rain shelter and set up in a (3 x 2 x 2) randomized complete block design (RCBD). The combined factors consisted of: three water regimes (irrigation 70%, 50%, 30% allowable depletion level (ADL); two sweet potato cultivars (orange flesh "Bophelo", white flesh "Blesbok"); with AMF (250g) and without AMF; all replicated three times. On planting day (Fig. 1A), holes were created, sweet potato cuttings were inserted, and 250 grams of AMF crude inoculum was added. Neutron probe access tubes were installed to monitor the soil moisture content.

Figure 1. Illustration of A) Planting of sweet potato, B) White flesh tubers, C) Orange flesh tubers.

RESULTS AND DISCUSSION

The results revealed that compared to other treatments, well-watered treatment of 30% ADL for orange flesh sweet potato had significantly higher biomass yield and water use. In contrast, white flesh had higher water productivity than orange flesh, as shown in Table 1. Of treatments with AMF, orange flesh sweet potato under 30% ADL had the highest total fresh mass and marketable yield, followed by 50% ADL for white flesh sweet potato (Figure 2). In terms of nutritional content, white flesh combined with 30% ADL without AMF and with AMF resulted in higher flavonoid and phenolic content as compared to 70% ADL treatment, and orange flesh had lower phenolic as compared to white flesh in all treatments, as shown in Table 2. Arbuscular mycorrhizal fungi can enhance yields and sustainability by reducing agricultural water and fertilizer requirements and improving the farming process.

Table 1. Total fresh biomass yield of tubers and water use of two sweet potato cultivars subjected to different irrigation regimes during the 2022/23 growing seasons.

Sweet potato cultivars	ADL (%)	Fresh biomass Yield (t ha^{-1})	Seasonal crop water use (mm)	Crop water productivity (t ha^{-1} mm^{-1})
Orange flesh (Bophelo)	30	35.36^{a}	389.93^{a}	0.09
	50	22.76^{b}	269.98^{b}	0.08
	70	8.84^{c}	195.56^{c}	0.05
White flesh (Blesbok)	30	27.29^{b}	236.48^{b}	0.12
	50	25.38^{b}	188.97^{c}	0.13
	70	26.15^{b}	218.74^{c}	0.12

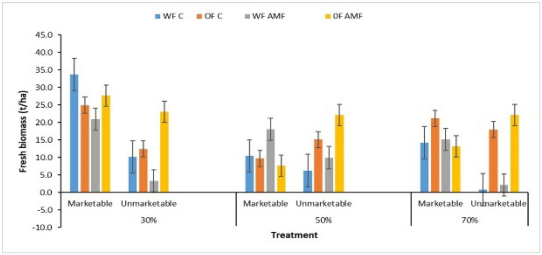

Figure 2. Marketable and unmarketable fresh tuber biomass yield of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhiza fungi (AMF) application and irrigation regimes (ADL).

Table 2. Total phenolic, total flavonoid, and condensed tannins content of orange flesh (OF) and white flesh (WF) sweet potato cultivars in response to arbuscular mycorrhiza fungi (AMF) application and irrigation regimes (allowable depletion level, ADL).

Cultivars	ADL levels (%)	Treatment	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CE/g)	Condensed tannins (mg cyanidin chloride/g sample)
White flesh (Blesbok)	30	CONTROL	20.44	4.15	1.43
	50		17.05	5.23	3.96
	70		15.57	4.73	0.55
	30	AMF	16.94	3.33	0.20
	50		16.79	2.88	2.84
	70		13.89	3.19	0.17
Orange flesh (Bophelo)	30	CONTROL	14.31	2.42	0.28
	50		14.45	3.37	2.67
	70		12.47	2.44	1.30
	30	AMF	13.33	2.92	0.40
	50		9.80	3.18	0.56
	70		13.56	3.87	0.06

CONCLUSIONS

The study results indicated that inoculating crops with AMF under drought stress conditions can improve sweet potato resilience and productivity, making it a promising method for sustainable sweet potato farming in water-limited environments, especially in regions with water shortages.

REFERENCE

Dawwam, G.E., Elbeltagy, A., Emara, H.M., Abbas, I.H. and Hassan, M.M., 2013. Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. Annals of Agricultural Sciences, 58(2), 195-201.

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- Agricultural Research Council (ARC) for support
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- Tshwane University of Technology (TUT)

Appendix B - Student project abstract

Sweet potato (*Ipomoea batatas*) is an essential global crop, ranking seventh in production. It is highly nutritious, providing carbohydrates, fiber, vitamins B6, D, and beta carotene, making it vital for food security, particularly in Southern Africa. Various cultivars are grown, with orange-fleshed varieties like Impilo and Bophelo praised for their high beta-carotene content, which helps prevent vitamin A deficiency.

Sweet potatoes thrive in warm climates (21-29 °C) and can tolerate drought, making them suitable for regions facing water scarcity. However, abiotic stresses like drought, heat, and salinity can reduce yields, with some cultivars, such as Bophelo and Blesbok, showing better drought tolerance. Biotic stresses, including pests and diseases, also impact productivity.

Arbuscular mycorrhiza fungi (AMF) have shown potential in enhancing sweet potato growth by improving water and nutrient uptake, especially under drought conditions. Research suggests AMF inoculation can increase biomass production, water use efficiency, and nutrient absorption, but further studies are needed to confirm its impact on yield. Encouraging drought-resistant, nutrient-rich crops like sweet potatoes could help combat food insecurity and malnutrition in developing regions.

Research on bio-symbiont microorganisms that enhance soil fertility and crop nutrition is gaining attention. Arbuscular mycorrhiza fungi (AMF), soil microorganisms from the phylum Glomeromycota, form symbiotic relationships with most plants, improving water uptake and nutrient exchange. They help roots adjust osmotically, allowing better water absorption, but their precise role in enhancing plant water relations remains unclear. While AMF is known to aid plant survival in water-limited environments, little is known about its impact on sweet potato yield and water productivity. However, there is no existing data on the water use efficiency, yield, and nutritional water productivity of sweet potatoes inoculated with such microorganisms. This study aimed to evaluate these factors in two AMF-inoculated sweet potato cultivars, Bophelo and Blesbok.

The experiment was conducted during the 2022/23 cropping seasons and repeated in the following season. The trial was laid out in (3x2x2) factorial design (RCBD), replicated 3 times. Where there were 3 irrigation regimes (control - soil refilled to field capacity when 30% of available soil water (ASW) was depleted, irrigation treatment 2 - refilled to field capacity when 50% of ASW was depleted and irrigation treatment 3 - refilled to field capacity when 70% of ASW was depleted) two cultivars of sweet potato (Bophelo - orange flesh (OFSP) and Blesbok - white flesh (WFSP)) and 2 treatments namely with AMF and without AMF.

Plant growth parameters collected included chlorophyll content, canopy temperature, photosynthetic efficiency as indicated by chlorophyll fluorescence (CF) was measured using a Handy Plant Efficiency Analyzer (PEA), leaf area index measured with Decagon Lp-80 Ceptometer. A root scanner was installed to investigate root and symbiosis development. During 2022/23 a hydroprobe (model 503DR Campbell Pacific Nuclear Inc., California, USA), was used to measure the soil moisture content. Yield was determined as total and marketable yield.

White flesh sweet potato under 30% and 50% allowable water depletion level (ADL) showed longer vines than the other treatments. Plant height at low to moderate water stress resulted in similar heights for both cultivars, while the severely stressed plants were stunted most. The chlorophyll content leaf readings on the treatment for both cultivars revealed lower values for 50% and 70% ADL.

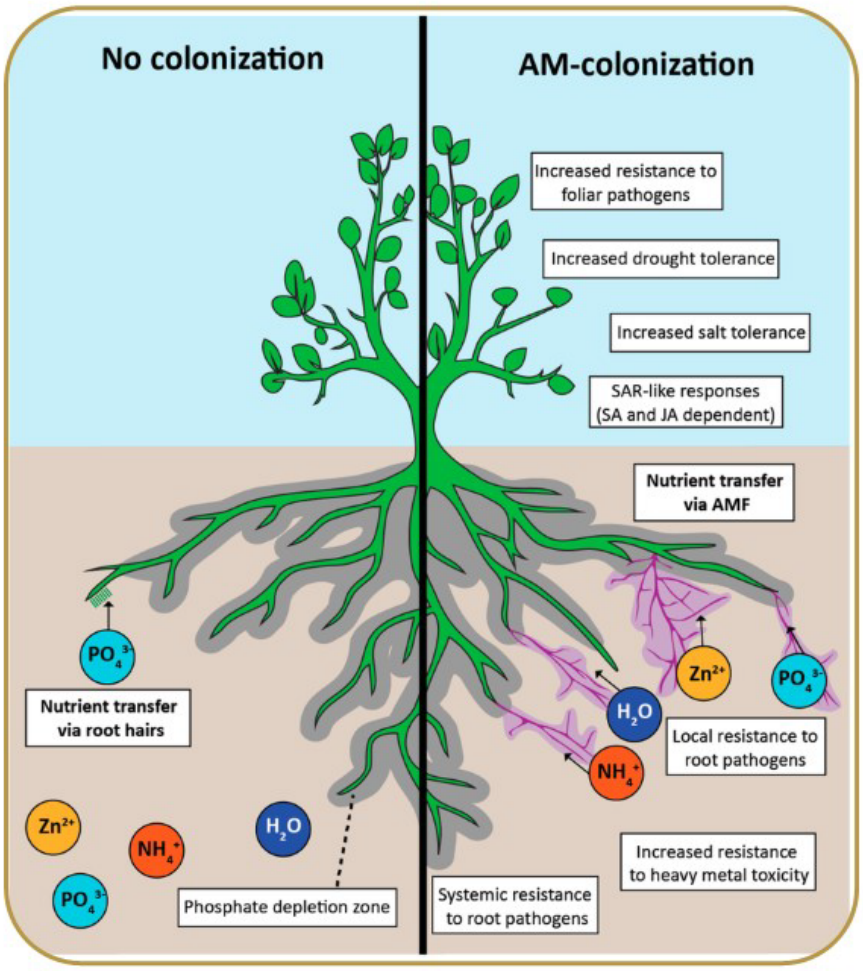
The AMF-inoculated WFSP produced 10, 18, and 8.8 t ha^{-1} more in season one under 30, 50, and 70% ADL, respectively. In season two, however, all of these treatments produced lower yields than the uninoculated controls. Season one yields for the OFSP were 8.7 and 10 t ha^{-1} under 30 and 50% ADL, respectively, and season two yields were 12.7 and 2.5 t ha^{-1}.

When compared to other treatments, the well-watered treatment (30% ADL) for OFSP demonstrated noticeably higher fresh biomass yield and water use. For both cultivars, water use dramatically dropped as water stress levels rose from 30% to 50% ADL. Crop water use was not significantly impacted by an additional increase in stress to 70% ADL, though.

According to the study's findings, inoculating crops with AMF during drought stress can increase the resilience and productivity of sweet potatoes, making it a viable technique for sustainable sweet potato farming in water-limited environments, particularly in areas with water scarcity.

Appendix C - Infographic

ON-FARM MYCORRHIZA PRODUCTION



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- Mycorrhiza - broad range of mutualistic associations formed between plant roots and fungi
- About 80% of plants form associations with AMF
- Mycorrhizal fungi - increase plant tolerance to abiotic stresses
  - Significantly increase water and nutrient uptake compared to plants without these associations
- Most plants can grow without these fungi, plants colonised with mycorrhizae generally grow much better than those without mycorrhizal symbionts

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Appendix D - Video link; On-farm production of mycorrhizae

https://youtu.be/m_9wcPrdQDY?si=jkCv4Fpk8QrUa2I_