Response of Tissue Cultured Giant Cavendish Banana to Inoculation with Kenyan Isolates of Arbuscular Mycorrhizal Fungi

Robinson Jalang'o Juma

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature ---------- Date ----------

Robinson Jalang'o Juma

This thesis has been submitted for examination with our approval as university supervisors.

Signature ---------- Date ----------

Dr. Losenge Turoop

JKUAT, Kenya

Signature ---------- Date ----------

Professor Leonard Wamocho

Masinde Muliro University, Kenya

Signature ---------- Date ----------

Dr. Joyce Jefwa

Tropical Soil Biology & Fertility, Kenya

DEDICATION

To my supportive classmates, friends and relatives whose encouragement and advice has contributed immensely towards achieving this noble task. Also, I wish to dedicate this work to all the professionals who introduced me to the world of Arbuscular Mycorrhizal Fungi and tissue culture bananas. You guys have made me understand the roles this tiny organisms can do and I appreciate the benefit this knowledge will bring to my society and I in the near future.

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LIST OF ACRONYMS AND ABBREVIATIONS

AMF:	Arbuscular Mycorrhizal Fungi
DW:	Dry Weight
FAO:	Food and Agricultural Organization
FAOSTAT:	Food and Agricultural Organization's Statistics
GE:	*Glomus etunicatum*
IITA:	International Institute for Tropical Agriculture
INIBAP:	International Network for the Improvement of Banana and Plantain
KARI:	Kenya Agricultural Research Institute
Masl:	Meters above sea level
MOA:	Ministry of Agriculture
RMD:	Relative Mycorrhizal Dependency
TC:	Tissue culture
WK:	Week

ABSTRACT

Mycorrhization of tissue cultured bananas is known to improve their growth and establishment under field conditions. Studies were carried out to evaluate the effects of inoculating tissue cultured Giant Cavendish banana with Kenyan isolates and *Glomus etunicatum*. Performance of inoculated plants in conventional nursery medium and sterile sand was also evaluated. A crude inoculum comprising of about 400 spores in 20 g soil was put into each tray cell during the weaning phase of tissue cultured banana plantlets. The content of each cell was emptied into a polythene sleeve containing 800 g of the respective medium eight weeks after inoculation. The experiment was laid out in Split-plot Design in the screenhouse. Plant growth parameters including height, number of leaves, leaf length and width were measured on a weekly basis for four months. Four destructive samples were taken at monthly intervals beginning at eight weeks after inoculation. Shoot and root fresh and dry weights, leaf surface area, number of spores per 100 g of medium and plant tissue nutrient analysis was assessed at each sampling stage. Results showed that indigenous isolates enhanced growth and nutrient uptake more uniformly in both media. *Glomus etunicatum* enhanced growth and nutrient uptake better than the indigenous isolates for plants established in conventional nursery medium. However, the isolate was less effective in sterile sand medium than indigenous isolates. Root colonisation had a direct impact on uptake of the three primary elements (N, P, and K) analysed. It is therefore evident that *Glomus etunicatum* is more preferable for inoculating tissue cultured bananas in conventional medium whereas the indigenous isolates can perform satisfactorily in both media.

CHAPTER 1

1.0 GENERAL INTRODUCTION

Production of Bananas (*Musa spp.)* in the tropics is commonly done on both large and small scales (Elsen, 2002). Bananas are mainly consumed as a fresh fruit making it one of the world's major food crops ranking fourth after rice, wheat and maize in terms of gross value of production (VanDyk, 2005). Banana is regarded as a staple commodity, which forms an essential component of many cooking dishes in Uganda, the Caribbean and Latin America (Karugaba and Kimaru, 1999). In 2004, India was the world's largest banana producer, with a total of 16.82 million tonnes (FAOSTAT, 2004). Eighty five percent of this production is consumed locally as a staple food while 15% of dessert bananas are exported. Uganda is the second largest producer of bananas in the world, with an annual output estimated at 10.5 million metric tonnes, accounting for 10% of world output (FAO, 2001).

In Kenya, the area under banana cultivation over the last 20 years has stagnated at around 80,000 hectares, covering about 1.7% of the country's total arable land area (FAOSTATs, 2005). It is grown both for subsistence and commercial purposes. Approximately 25% of bananas are produced for subsistence and the remaining 75% sold (Qaim, 1999).

Conventional method of producing bananas has been through corms and suckers (Qaim, 1999). The corms or suckers are large and frequently harbour pathogens and are often in short supply especially when many new plantings are required. Germplasm maintenance in the field is expensive and space consuming. Micropropagation helps to alleviate these

problems by ensuring mass multiplication of clean plantlets for large-scale production purposes (Frison and Putter, 1989). Tissue cultured plantlets are weak and have to go through acclimatization process during which they increase absorption of water and minerals and their photosynthetic rate (Yano-Melo et al., 1999) for ease of field establishment. Bananas growing in the field have long been known to be colonized by Arbuscular Mycorrhizal Fungi (AMF) (Umesh et al., 1988). The endophytes involved may enhance growth and uptake of soil bound water and mineral nutrition of the host plants (Schroeder and Janos, 2005). Arbuscular mycorrhizal fungi are known to have a positive effect on the growth and development of micropropagated banana through improvement of mineral nutrition, induction of root system modifications and increased resistance or tolerance to pathogen (Jaizme-Vega and Azcon, 1991; Declerck et al., 1995).

The technique of utilizing indigenous AMF species common to banana farms in Kenya has not been commercialized. There was therefore, a need to explore the extent of benefits tissue cultured bananas can acquire as a result of inoculation with the indigenous AMF isolates with a view to commercialization. The growth response of tissue culture banana plantlets due to inoculation with indigenous and exotic commercial isolates was studied and details of the relationship between growth and colonisation was evaluated as contained in chapter 2 of this thesis. The effect of media types on AMF colonisation of tissue cultured bananas was also evaluated in terms of AMF sporulation and primary nutrient uptake by inoculated plantlets is discussed in chapter 3 of this thesis.

1.1 Statement of the problem

Pests and diseases are a major hindrance to banana production in the tropics. Black sigatoka, Fusarium wilts and banana weevils pose the greatest threat in the tropics and sub-tropics (Boshart, 2004). Nematodes also cause a considerable yield loss in banana production. In nematode-infected banana, the root system is damaged hence the plant topples and the bunch is lost before maturity (Rillig, 2004). Increase in land pressure due to overpopulation has resulted to a more intensified land use, which has reduced soil fertility (Smith and Read, 1997; Muok and Ishii, 2005) . Low soil fertility and poor farm management practices have led to high disease incidence and pest infestation due to establishment of vulnerable plants (Nowak, 1998).

Unreliable rainfall patterns in the sub-tropical regions hinder optimal banana production, as the plant requires a lot of water for growth and maturity (Yano-Melo et al., 1999).

1.2 Justification

Arbuscular Mycorrhizal Fungi (AMF) colonization at nursery stage has been shown to improve acclimatization and establishment of tissue cultured bananas after transplanting (Jaizme-Vega et al., 2002b). Micropropagated plantlets have become a material of choice in Kenya (Wambugu and Kiome, 2001). Mycorrhizal inoculation has been shown to improve growth and post-transplant performance of several micropropagated plant species (Jaizme-Vega and Azcon, 1995; Yano-Melo et al., 1999). Introducing AMF before plants are taken to the field will give them an advantage in colonizing the plant which will subsequently offer potential benefits such as; enhancing phosphorus

(P) uptake in soils with low available P, increase tolerance to environmental stress, pest infestation and disease infection (Wamocho et al., 2000).

Tissue cultured banana plantlets provides for the establishment of clean (disease free) banana plantations (Strosse et al., 2004). Tissue cultured bananas has proved to benefit from exotic commercial Arbuscular mycorrhizal Fungi (mainly *Glomus* genus) colonization (Mwashasha, 2005). However, the response of tissue cultured bananas to indigenous AMF isolates has not been perfected. The research aimed at evaluating the performance of indigenous cultures of AMF common to banana farms in Kenya against that of a known exotic commercial isolate, *Glomus etunicatum*. A positive performance of indigenous isolates would result to a more convenient, cost effective and adaptable use of these isolates by commercial nursery producers. Since the practice of inoculation using indigenous isolates of AMF has not been perfected, investigating the media of choice for nursery AMF inoculation of tissue culture bananas was a priority need for this research. A comparison between sterile sand and conventional nursery media to determine the preferable media for nursery inoculation was envisaged to be important for successful AMF colonization.

1.3 Objectives of the Study

The overall objective of the study was to evaluate the response of tissue cultured banana plantlets to inoculation with indigenous AMF cultures common to banana farms in Kenya. The specific objectives were: 1) To determine the effects of indigenous AMF isolates and *Glomus etunicatum* on growth of tissue cultured giant Cavendish bananas.

2) To evaluate the effects of sterile sand and the conventional nursery media on AMF colonization of tissue cultured giant Cavendish bananas.

1.4 Research Hypotheses

Commercial AMF inoculants improve growth and nutrient uptake better than indigenous AMF inoculants in all media types.

Conventional nursery medium (Forest soil) nourishes tissue culture bananas better than sterile sand under similar environmental conditions.

1.5 LITERATURE REVIEW

1.5.1 Economic importance of Bananas

Consumption of Bananas is mainly as a fresh fruit: - making it one of the world's major food crops ranking fourth after rice, wheat and maize in terms of gross value of production (INIBAP, 1991). Export Bananas, mostly of the Cavendish variety, are also called "dessert" bananas. Banana is regarded as a staple commodity, which forms an essential component of many cooking dishes in Uganda, the Caribbean and Latin America (Karugaba and Kimaru, 1999). Through the processing of bananas, it is possible to obtain dried bananas, also called 'chips', banana puree as the basis for baby food or for dairy products, banana flour and powder, banana juice or even banana alcohol (Vanlauwe et al., 2005). Bananas are considered to be good for the treatment of gastric ulcer and diarrhea (IITA, 1995). They contain vitamin A, which acts as an aid to digestion. They are high in vitamin B6, which helps to reduce stress and anxiety (IITA, 1998). In many countries, banana leaves are used for wrapping food when cooking. In some cases, banana crop is used to give shade to other crops that need it, such as coffee and cocoa (Vanlauwe et al., 2005). The crop is harvested throughout the year thus ensuring food and income security at the household level (Lusty and Smale, 2002). Over 70% of Uganda's farmers rely on bananas as a major food crop and source of income (IITA, 1995), and they cover over 30% of utilized agricultural land in Uganda, which makes it the most important food security crop. Banana is an important food and cash crop in Tanzania and in terms of major crops consumed; it ranks third after maize and cassava (Karugaba and Kimaru, 1999). About 2% of Kenya's arable land is under

banana production, which plays an important role in the horticultural industry as a source of income (MoA, 1997).

1.5.2 Banana growth requirements

Banana is a tropical herbaceous evergreen plant that has no natural dormant phase. It has a high leaf area index and a very shallow root system (Robinson, 2000). Depending on the prevailing climatic conditions, estimates of the annual evapotranspiration (ET) of banana plants range from 1200 to 2690 mm (Robinson, 2000). The crop has a high water demand, the minimum being 25 mm per week whereas an average annual rainfall of 2000 to 2500 mm evenly distributed throughout the year is satisfactory. Relative humidity should be greater than 80% (Robinson, 1996). Banana is well adapted to well-drained, loamy soil that is rich in organic matter. Temperatures between 27 to 30ºC are most favourable to the crop. However, the crop can do well at a temperature range of 25–32ºC (Robinson, 1996). Banana grows at sea level up to an altitude of 1,800 meters. It is susceptible to root rot when exposed to too much water. Banana can survive on varied soil types but deep, well-drained loams with high fertility and organic matter content are ideal. The roots are fragile, with low penetrating power and have high demand for oxygen (KARI, 2004). A soil pH range of 5.8-6.5 with potassium range of 200-350mg K/kg is suitable for bananas. Phosphate levels of 4mg P/g is sufficient since plants can readily absorb P (INIBAP, 2001). Nitrogen fertilization levels of 100-150mg N/kg is recommended for tropical soils. Excessive salinity increases sodium content of the soil hence reducing K uptake and subsequent yields (Robinson, 1996).

1.5.3 Tissue culture technique

Banana is one of the crops where the tissue culture technique is widely used in the commercial production of planting materials (Krik-Orian, 1990). Tissue culture technology provides an avenue for mass production of clean planting materials (Frison and Putter, 1989). It enables uniform plant establishment and the reduction of soil-borne pests and diseases (Swennen and Vulysteke, 2004). Mass production of plantlets ensures availability of clean planting materials throughout the year. The axenic nature of the plant's production eliminates beneficial microorganisms such as the AMF, which may confer some resistance to biotic and abiotic stresses in the field (Pereira et al., 1999). Tissue cultured banana plantlets are widely accepted and used as propagation material of choice in Kenya (Wambugu and Kiome, 2001).

1.5.4 Arbuscular Mycorrhizal Fungi Symbiosis with bananas

Arbuscular Mycorrhizal fungi form symbiotic relationship freely with about 80% of terrestrial plants (Fitter, 2000). The main physiological basis for mutualism is bi-directional nutrient transfer (Smith and Smith, 1997), where plants supply the AMF with sugars, and the fungi enhance the plants' ability to scavenge for scarce and immobile nutrients, particularly P. In AMF inoculated plant, P_i (as orthophosphate) can be absorbed both directly at the soil-root interface through root epidermis and root hairs, as well as indirectly via the external mycorrhizal hyphae in the soil. These hyphae absorb P; translocate it rapidly to AMF structures in the roots (intercellular hyphae, intracellular coils, and highly branched intracellular arbuscules) where it is released to

the interfacial apoplast adjacent to root cortical cells (Smith and Smith, 1997). Arbuscular mycorrhizal fungi association is characterized by formation of intracellular structures known as arbuscules (Smith and Read, 1997) on plant's root hairs. Root hair extensions (arbuscules) are the primary sites for nutrients and carbon exchange between the symbionts (Rillig, 2004).

Root colonization by AMF can alleviate both deficiency and toxicity of micronutrients such as copper and zinc (Quilambo, 2003). AMF has been shown to increase tolerance to Aluminium toxicity (Jaizme-Vega et al., 2002a) and biotic stress caused by nematodes (Elsen et al., 2001). AMF increases resistance to *Fusarium oxysporum fsp*. Cubense (Jaizme-Vega et al., 1998).

Mycorrhizal inoculation has been shown to improve growth and post-transplant performance of several plant species (Wamocho et al., 2000). Bananas show a positive response to AMF inoculation (Reyes et al., 1995). Arbuscular mycorrhizal fungi is known to increase biomass and tolerance to abiotic and biotic stresses of bananas (Smith and Read, 1997; Muok and Ishii, 2005). A number of investigations have demonstrated AMFs positive impact on nutrient uptake and growth of tissue cultured bananas (Jaizme-Vega and Azcon, 1995; Yano-Melo et al., 1999).

Tissue cultured bananas generally lack proper root hair development both in the nursery and after field establishment (Jaizme-Vega et al., 1997). Inoculation with AMF has been shown to increase the survival, growth and establishment of micropropagated plant species (Wamocho et al., 2000; Elsen, 2002; Muok and Ishii, 2005).

The fungus absorbs nutrients such as P, Ca, Cu, N, and transfers them to the root hairs for assimilation into plant cells. Tropical soils have greater tendency for P fixation thus rendering it unavailable. Presence of AMF in the banana roots is shown to improve P

availability in such soils (Yano-Melo et al., 1999; Wamocho et al., 2000).

Many diseases and pests have been reported to cause high yield reduction in any banana production season (Karugaba and Kimaru, 1999) and plant parasitic nematodes such as the burrowing type (*Radopholus similis*), root knot (*Meloidogyne spp*.), spiral (*Helicotylencus multicinctus)* and lesion type *(Pratylenchus goodeyi*) cause high yield losses in E. Africa (Elsen, 2002). Panama disease which is caused by *Fusarium oxysporum fsp* Cubense is threatening productivity of bananas in the tropics. Arbuscular mycorrhizal fungi can decrease the severity of diseases caused by fungi, e.g. *Phytophthora spp*. in citrus, *Cylindrocarpon spp*. in strawberry, *Cylindrocladium spp.* in banana and Fusarium spp. in tomato and banana (Elsen, 2002). Presence of AMF suppressed the nematode population build-up and the damage in the roots was significantly less when AMF were growing in the roots (Elsen et al., 2001). Early mycorrhization appeared to increase banana tolerance to *Pratylenchus goodeyi* (Cobb) Sher (Vigo et al., 2000).

Arbuscular mycorrhizal fungi reduce the number of pathogenic infection loci in mycorrhized plant roots, excluding the fungal pathogen from the roots (Vigo et al., 2000). Arbuscular mycorrhizal fungi's strategy for biocontrol of nematodes is via exclusion since AMF hyphae act as root hair extensions; thus shielding nematodes from establishing any direct contact with root hairs (Elsen et al., 2001).

Glomus etunicatum is known to enhance growth and establishment of tissue culture banana plantlets (Lesueur et al., 2001; Mwashasha, 2005) thus investigating its impact on Giant Cavendish AAA cultivar was important.

1.5.5 Effect of media on AMF colonization

The development and survival of introduced AMF propagules depends on edaphic conditions and soil types where the symbiosis is established (Plenchette, 2000). Media aeration is the single most important factor for successful AMF colonization (Six et al., 2004). Arbuscular mycorrhizal fungi require aerobic conditions (sufficient oxygen) for effective colonization and growth of hypha and other mycorrhizal structures (Diaz-Zorita et al., 2002). Soil drainage influences aeration which is a key determinant for the survival of aerobic micro flora and fauna. Arbuscular mycorrhizal fungi's infective structures require well drained media conditions for maximum sporulation and growth of root hair extensions (Six et al., 2002). Soil structure influences many biotic, physical and chemical aspects of soil. It is the basic setting in which processes take place in soil (Six et al., 2004). Arbuscular mycorrhizal fungi are important in influencing soil structure at the macroaggregate level, where direct hyphal involvement is thought to be most pronounced mechanism (Miller and Jastrow, 2000). The dense interlocking network of hypha (mycelia) binds soil macroaggregates giving them a completely new structural look. Mycorrhiza root hair extensions penetrate macroaggregates creating pores which improve soil aeration (Rillig et al., 2002).

CHAPTER 2

EFFECTS OF INDIGENOUS AMF ISOLATED FROM BANANA FARMS IN KENYA AND COMMERCIAL ISOLATE (*Glomus etunicatum)* ON GROWTH OF TISSUE CULTURED GIANT CAVENDISH BANANAS

Abstract

Performance of the indigenous isolate and *Glomus etunicatum* on growth of tissue cultured giant Cavendish bananas was evaluated in both sterile sand and conventional nursery media. It was expected that *G. etunicatum* will enhance growth better than indigenous isolate in both media. 20 g of soil inoculum containing 400 single spores of the two AMF types was sandwiched into each tray cell with a volume of $8cm^3$ to form the middle layer. Six week old tissue cultured banana plantlets (cv. Giant Cavendish) were planted into the tray cells with respective inocula and their growth monitored for a period of twenty weeks. Plant growth was assessed on a weekly basis. Four destructive samples were taken on a monthly basis, beginning at 8 weeks after inoculation. Indigenous glomus species significantly ($p<0.05$) enhanced growth of inoculated plants in both media achieving a mean increase in growth of 41.7% and 13.3% in sterile sand and conventional media, respectively. *Glomus etunicatum* significantly enhanced growth of plants established in conventional medium with a 14.8% increase in growth while its performance in sterile sand medium was not significantly ($p>0.05$) different from control. The indigenous isolate proved to be the most successful growth enhancing inoculum in both media.

2.1 Introduction

Tissue culture technique (TC) has gained global acceptance as the ideal method of producing banana planting material for the establishment of new or replacement of existing plantations (Wambugu and Kiome, 2001). The reasons for using such material are the rapid production of high quality disease-free and uniform plantlets (Jaizme-Vega et al., 2003). Micropropagated species lack beneficial microbial inhabitants of roots such as Arbuscular Mycorrhizal Fungi (AMF), which are known to improve plant growth and performance under environmental stress conditions (Smith and Read, 1997). Arbuscular mycorrhizal fungi facilitate plant adaptation to nursery through enhancing tolerance to both biotic and abiotic factors (Jaizme-Vega and Azcon, 1995).

Mycorrhiza inoculation during the weaning phase of TC plantlets is beneficial to several tropical species such as papaya, avocado, pineapple (Jaizme-Vega and Azcón, 1991) and banana (Declerck et al., 1995; Jaizme-Vega and Azcón, 1995; Yano-Melo et al., 1999; Jaizme-Vega et al., 2002a). Various sources of inoculums such as spores, colonized root fragments, rhizospheric soils and in-vitro root organ culture technique have been successfully used for nursery AMF inoculation (Jaizme-Vega et al., 2003).

Successful inoculation of AMF at the beginning of the acclimatization period (Granger et al., 1983; Brazanti et al., 1992; Guillemin et al., 1995) or during in-vitro cultivation (Mathur and Vyas, 1995) has been demonstrated. The beneficial effect of the symbiosis formed with the root system of plants from tissue culture manifests itself in the development of vigorous plants with high photosynthetic and transpiration rates, improved absorption of nutrients and water; and increased stress tolerance (Azcón-Aguilar et al., 1997; Jaizme-Vega et al., 1997).

Inoculation of micropropagated plants with AMF during initial growth *in vivo* may contribute to high colonization rates through positive mycorrhizal symbiotic effects on the activity of the root meristem. Berta et al. (1995) demonstrated that AMF association altered the branching pattern of roots of *Prunus cerasifera*. The inoculum type used in the acclimatization is important. Fortuna et al. (1992) recommended the use of infective and efficient species of AMF which promote rapid increase in plant growth. While comparing the efficiency of two AMF species in promoting growth of micropropagated *P. cerasifera*, the author demonstrated that the infectivity of the fungi influenced their effectiveness. Greater fresh and dry matter, and height increases were found with plants inoculated with *Glomus mosseae* than with *G. coronatum*, but at the end of the experiment both groups of plants showed similar growth.

Indigenous AMF isolates have the potentials to establish effective colonization in most cases than pure commercial isolates. This is because indigenous isolates easily adapt to the environment of their use and persistence of their infective structures can be guaranteed. Indigenous isolates may be easily accessed by commercial nursery producers at a shorter notice since they are locally available. The above assumptions may not be necessarily correct.

Yano-Melo et al. (1999) reported a 57% increase in leaf area, 32% increase in plant height and a 64% increase in dry matter of tissue culture banana plantlets inoculated with *Glomus etunicatum* than their non-inoculated counterparts. *G. etunicatum* was reported to be very efficient in enhancing Phosphorus (P) uptake by *Senna macranthera*, a woody species (Flores-Aylas et al., 2003). Several studies have reported that *G. etunicatum* enhances nutrient uptake and performance of micropropagated bananas (Yano-Melo et al., 1999; Millner et al., 2001; Flores-Aylas et al., 2003;

Cornejo et al., 2006). The response of TC bananas to inoculation by indigenous AMF isolates in Kenya has not been documented. This study aimed at evaluating the growth response of tissue culture bananas to inoculation with indigenous AMF cultures isolated from banana farms in Kenya.

2.2 MATERIALS AND METHODS

2.2.1 Study site

The study was carried out in a screenhouse at Jomo Kenyatta University of Agriculture and Technology (JKUAT) (latitude: 1°10′48′S; longitude: 37°07′12′E; 1525 masl) located in Juja, Kiambu County of Kenya between the months of March to August, 2007 and 2009. The screen house was covered with screen net which permits 60% penetration of light intensity into the chamber. The daily average light intensity recorded in the screen house during the experiment was 720µmol$m^{-2}s^{-1}$.

2.2.2 Source of inoculums

Indigenous inoculum comprised of a mixture of four *Glomus* species isolated from 10 banana farms in Maragwa District (00°47′S, 037°07′E; 2175 masl), Muranga County of Kenya. The four indigenous glomus species were purified then mixed in the ratio of 2:1:1:1 parts of AMF inoculants with the best sorghum growth enhancing isolate having two parts. The species used for the experiment were found to be common in all the ten farms sampled. Indigenous inoculum was cultured and bulked up at NMK mycology laboratory from which it was obtained for use during the experiment. *Glomus*

etunicatum was obtained from Tropical Soil Biology and Fertility (TSBF-CIAT) molecular biology laboratory who imported it from United Kingdom.

2.2.3 Source of banana plantlets

Tissue culture Giant Cavendish banana plantlets were obtained from the Institute of Biotechnology Research (IBR) laboratory of Jomo Kenyatta University of Agriculture and Technology (JKUAT). Plantlets had three fully developed leaves and well-developed root system formed *in vitro* using MS (Murashige and Skoog, 1962) liquid culture medium after six weeks of acclimatization in the laboratory. Root induction was done in the biotechnology laboratory. Regenerated shoots were cultured in rooting media containing half strength MS medium supplemented with 1.0 mg/l of Indole Butaric Acid (IBA). It took a period of 30 days for an average of 3 roots to be regenerated.

2.2.4 Media preparation and treatment

Two media types (conventional nursery medium and sterile sand) were used during the experiment. Conventional nursery medium comprised of a native forest soil (obtained from Kieni forest, Nyeri County, Kenya) and sand mixed in a ratio of 3:1w/w respectively. The soil was collected from 5 to 30 cm depth in the middle of the forest. The media was sterilized using carbofuran at the rate of 80g/100kg of soil (to ensure that any existing mycorrhiza and pathogen present in the media was eliminated) then covered with black polythene sheet on a bench for four weeks to achieve maximum

sterilization before use. Selected properties of the soil mixture used in the experiment were established (Table 1).

Table 1: Selected chemical properties of the conventional nursery medium used in the experiment

Chemical property considered	Value
Available P (mg/kg soil)	4.22
pH (H_2O)	5.82
Soil organic matter (g/kg)	280
K (cmol(+)/kg)	8.68
N (mg/kg)	18.71
Ca (cmol(+)/kg)	9.63
Mg (cmol(+)/kg)	1.33
CEC (cmol(+)/kg)	24.33

Sterile sand medium was obtained after thorough washing of river sand using distilled water and 5% (v/v) formaldehyde repeatedly. The sand was then steam sterilized at a temperature of 120^{o}C and pressure of 1.5 bars for 90 minutes and left to cool for two weeks before potting and transplanting. The sand was collected from River Mwala in Machakos County, Kenya.

2.2.5 Preparation of plantlets for inoculation

Using long tweezers, the plantlets were carefully removed from the culture bottle and placed into buckets containing double distilled water. Agar was washed off carefully

from the roots to prevent bacterial and fungal contaminants. Plantlets were then separated into single plants ensuring that, as many roots as possible remained intact.

2.2.6 Inoculation of banana plantlets

Mycorrhization was done at hardening stage when the banana plantlets were six weeks old. The inoculum comprising of a homogenous mixture of the rhizosphere sand with spores, mycelium and colonized sorghum root fragments of *Glomus etunicatum* or indigenous mixed isolate was used. Twenty grams consisting of about 400 spores was obtained from a homogenized inoculum. Inoculum was sandwiched in the media to form a middle layer of 1 cm depth out of the full tray cell depth of 2 cm. The AMF inoculum-soil mixture was moistened and holes made on the moist media inside hardening trays before lowering the plantlets carefully into the cells to a depth of 0.5 cm into the inoculum layer. Banana plantlets were inoculated with the two AMF inocula while the control was treated with an equivalent quantity of dead inoculum in order to take care of any nutritional variation which may result from the inoculum. A total of 126 plantlets were deflasked into six polystyrene hardening trays (measuring 50 × 30 cm with a total of 153 cells, each cell having a volume of 8 cm^3) for eight weeks. Misting using distilled water was done regularly to maintain the humidity. During the hardening phase (first four weeks), modified Hewitt nutrient solution with low P (0.027 ml in 1L of water) was applied twice per week in the sterile sand trays. Plants were maintained under this condition for a period of four weeks after which they were gradually hardened for two more weeks in preparation for potting.

2.2.7 Preparation of Modified Hewitt nutrient solution

Modified Hewitt nutrient solution (Stock solution) was prepared using procedure (Appendix 1) developed for experiments with *Musa,* Mycorrhiza and Nematodes (Hewitt, 1966). For the first four weeks of hardening process, nutrient solution with low P (0.027 ml in 1L of H_2O) was applied twice per week at the rate of 100 ml/pot. After four weeks, nutrient solution with higher P (0.272 ml/L) was applied twice every week at the above rate throughout the experimental period. The seedlings shoots were misted with distilled water whenever their leaves showed signs of desiccation. Four weeks after inoculation, the nutrient solution application frequency was reduced to once a week and media kept moist appropriately to achieve full acclimatization before potting in week eight.

2.2.8 Potting

Potting was done eight weeks after inoculation. The plantlets were transplanted into 5 x 9 x 10 cm polythene sleeves. The sleeves were filled with the respective media (sterile sand and conventional media) in each case then watered. Holes were made using a sterile spatula in all the pots in readiness for transplanting. Using a sterile spatula, the whole content of the tray cell was scooped out and placed into the hole made inside the media filled sleeves. The eight week old plantlets were gently hand firmed into the sleeves and plantlets established on sterile sand were irrigated with a high P (0.272 ml/L) modified Hewitt nutrient solution.

2.2.9 Experimental design

Three AMF treatment levels (*G. etunicatum*, indigenous and non-inoculated plants) and two media levels (conventional and sterile sand) were laid out in a Split-plot design with two media types in the main plots and AMF treatments in the sub-plots. Each treatment combination contained 21 plants replicated four times.

2.3.0 Data collection

Plant growth parameters which include shoot height, number of leaves, leaf width and leaf length measurements were recorded from individual plants on a weekly basis beginning from eight weeks after inoculation. Leaf width was measured across the midpoint of the youngest fully expanded leaf whereas leaf length was measured along the midrib from the end of stalk to the tip of the same leaf.

Four destructive harvests were conducted on a monthly basis beginning from eight weeks after inoculation during which the following data were taken: fresh and dry weights of shoots and root, surface area of the broadest leaf per plant was measured using AAC-400 leaf area meter, (Hayashi Denkoh Co., Ltd, Tokyo, Japan) and sub-sampling roots for AMF colonization assessment was done according to procedure of Gemma and Koske (1989). AMF colonization was assessed under two categories: 1. Intensity of colonisation which was expressed as the proportion of a 1cm root containing mycorrhiza infective propagules; 2. Frequency of colonisation was assessed as percentage number of root samples colonised out of the total number of samples assessed. Estimation of the percentage root AMF colonisation frequency and intensity

was done using the subjective visual technique by Kormanik and McGraw (1982) commonly referred to as the slide method.

2.3.1 Sampling for colonisation assessment

Four plants were sampled from each treatment combination every month for four months beginning from 8 weeks after inoculation. The roots were separated from the shoots using a sterile scapel. Fresh weight of shoots were taken using a weighing balance before being oven dried at 70^{o}C for 72 hours. The root of each plant sample was divided into two portions; one part was weighed then placed in the oven to dry while the second part was cleaned and preserved in 70% ethanol awaiting AMF colonisation assessment.

2.3.2 Root clearing and staining

Banana root samples (100 pieces) were washed under running tap water to remove soil debris and then processed according to procedure by Gemma and Koske (1989) to assess mycorrhiza colonisation. The roots were cleared with 2.5% KOH (25 g KOH in 1000 ml water) by heating in an oven at 70ºC for one hour and then rinsed with tap water. To remove phenolic substances, alkaline hydrogen peroxide (60 ml of 28-30% NH_4OH, 90 ml of 30% H_2O_2 and 840 ml distilled water) was added and roots placed in the oven at 70ºC for twenty minutes for all the samples. The roots were rinsed with tap water and acidified with 1% HCl and left for 30 minutes. The HCl was decanted and without rinsing the roots, 0.05% trypan blue in acid glycerol (500 ml glycerol, 450 ml

water, 50 ml of 1% HCl and 0.5 g trypan blue) staining reagent was added and roots placed in the oven for 1 hour at 70ºC. The stain was decanted and de-staining solution comprising of acid glycerol (500 ml glycerol, 450 ml water, and 50 ml of 1% HCl) was added.

2.3.3 Assessing mycorrhiza colonization

Fine root segments were cut into 1 cm-long pieces and 10 pieces randomly picked, mounted on slides and observed under a dissecting microscope (400X) to assess the frequency and intensity of AMF colonization. Presence of arbuscules, vesicles, internal and external hyphae and spores was examined. The frequency of AMF was recorded as the number of root fragments infected with AMF and expressed as a percentage of total number of root pieces observed (20 pieces). The intensity of AMF colonization was recorded as percentage cover of AMF infective propagules in each 1cm root fragment.

2.3.4 Data analysis

Plant growth data and biomass was subjected to ANOVA using the procedure GLM (SAS institute, 2003). Percentage data for AMF colonisation frequency and intensity was arcsine transformed before ANOVA was performed. Means were compared using Tukey's HSD test. All tests were performed at $\alpha = 0.05$.

2.4 RESULTS

2.4.1 Plant growth parameters

Plant height

The height of plants inoculated with the two inocula was not significantly different ($p<0.05$) for plants established in conventional nursery medium twenty weeks after inoculation. *Glomus etunicatum* (FE) achieved better plant height than indigenous isolates (FA) in conventional medium however, the above result did not differ significantly ($p>0.05$) (Fig.1). In both cases, inoculated plants were significantly taller than non-inoculated (FO) ($p<0.05$). Plants inoculated with *G. etunicatum* (SE) were significantly shorter than those inoculated with indigenous inoculum (SA) in sterile sand medium. Although SE were slightly taller than control (SO) for the first 18 weeks after inoculation, that difference was not significant throughout the experiment ($p>0.05$) (Fig.1). Inoculation of tissue cultured banana plants with any of the two inoculum resulted in significant increase in plant height when established in conventional nursery medium but not in sterile sand medium. Indigenous inoculum caused significant increase in plant height ($p<0.05$). However, the effects of AMF did not depend on the media used as indicated by a non-significant ($p>0.05$) two-way interaction.

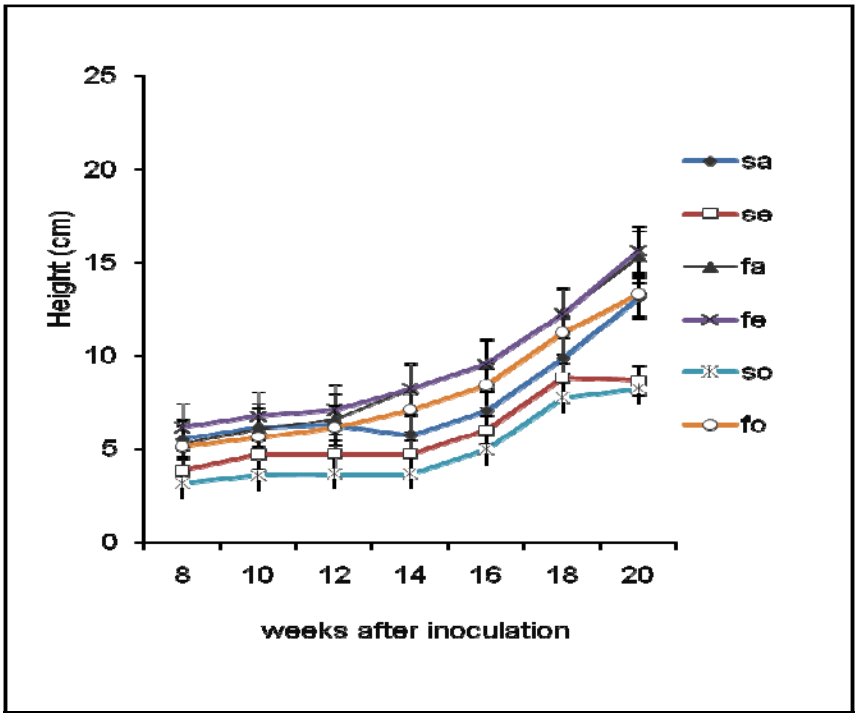


Fig. 1: Effect of inoculation on plant height. Points represent means and standard errors of heights of 21 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

Number of leaves

Twenty weeks after inoculation, the number of leaves was significantly influenced by AMF compared to the control ($p<0.05$). However, the difference between treatments FE (*Glomus etunicatum* inoculated plants established in conventional medium) and FA (indigenous AMF inoculated plants grown in conventional medium) were not significantly ($p>0.05$) different. Indigenous isolates attained a higher ($p<0.05$) number of leaves in sterile sand medium than GE. Number of leaves for treatment SE (*Glomus etunicatum* inoculated plants established in sterile sand) was not significantly different ($p>0.05$) from SO (non-inoculated plants established in sterile sand medium) (Fig. 2). Results indicate that indigenous inoculum generated more leaves than commercial inoculum (*Glomus etunicatum*) in both media during the experimental period of twenty

weeks. Generation of leaves for SE plants was not different ($p>0.05$) from that of control (SO).

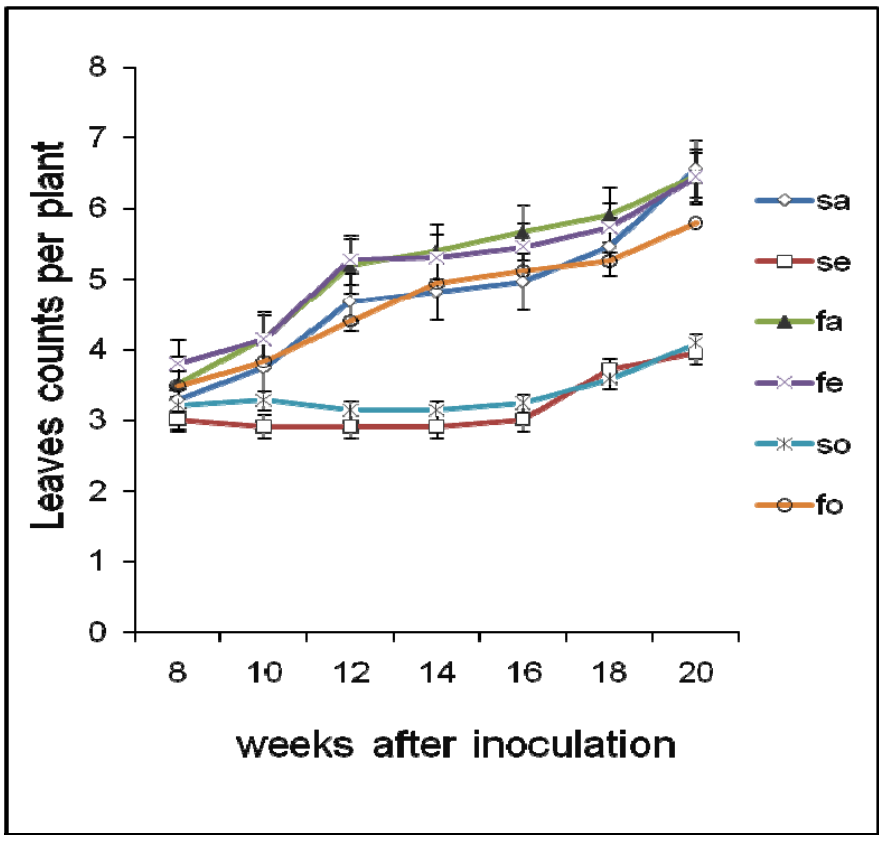


Fig. 2: Effect of inoculation on generation of leaves. Points represent means and standard errors for number of leaves of 21 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

Leaf expansion

Leaf expansion was measured in terms of width and length of the broadest new leaf which, in most cases was the youngest fully expanded leaf. The leaf width did not show any significant differences ($p>0.05$) between treatments FE and FA twenty weeks after inoculation. Both inocula enhanced leaf expansion similarly in conventional medium. FE and FA achieved better mean leaf width ($p<0.05$) than the control (FO). The indigenous inoculum enhanced leaf width more than ($p<0.05$) the commercial isolate

(SE) and the control (SO) in sterile sand medium. The commercial inoculum's performance was not different from that of control in sterile sand medium (Fig.3). In all treatments (except SA), leaf expansion increased steadily with time up to week 18 before decreasing in weeks 19 and 20 of the experiment (Fig.3). The scenario was attributed to root balling which negatively affected the growth of the new leaves.

The two inoculums did not show any differences ($p>0.05$) in promoting leaf expansion in plants established in conventional nursery medium. However, FE and FA were significantly different ($p<0.05$) from the control. Indigenous inoculum significantly ($p<0.05$) enhanced leaf elongation in sterile sand than commercial inoculum whose mean leaf length was not different from the control twenty weeks after inoculation (Fig.4).

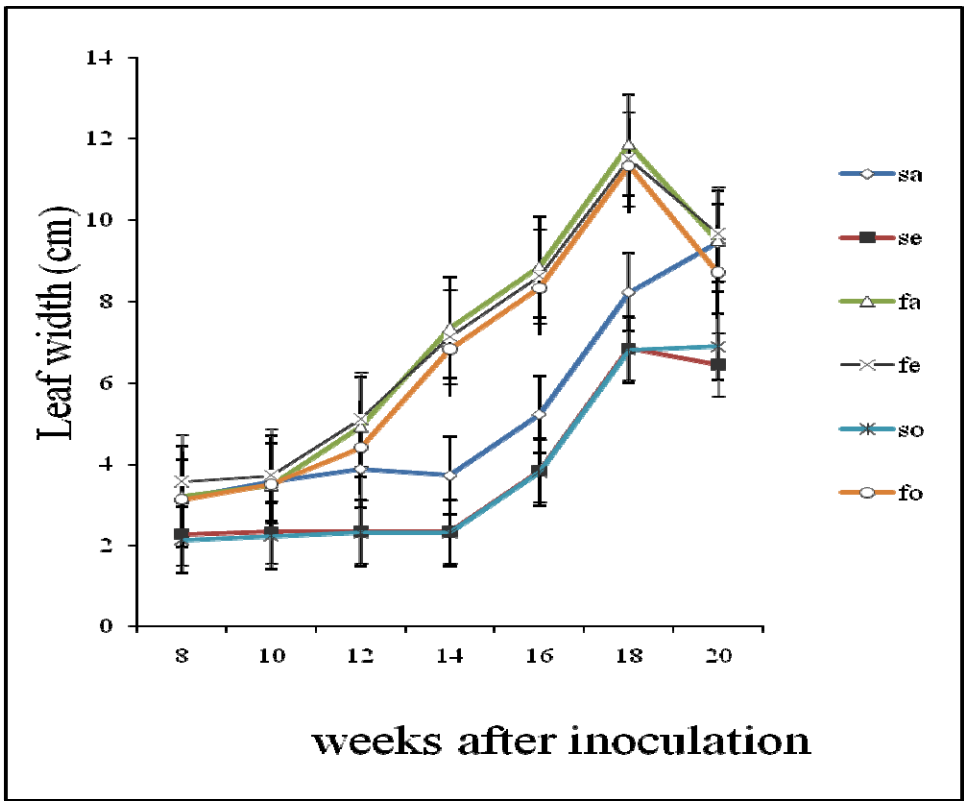


Fig. 3: Effect of inoculation on broadest leaf width. Points represent means and standard errors of broadest leaf width of 21 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

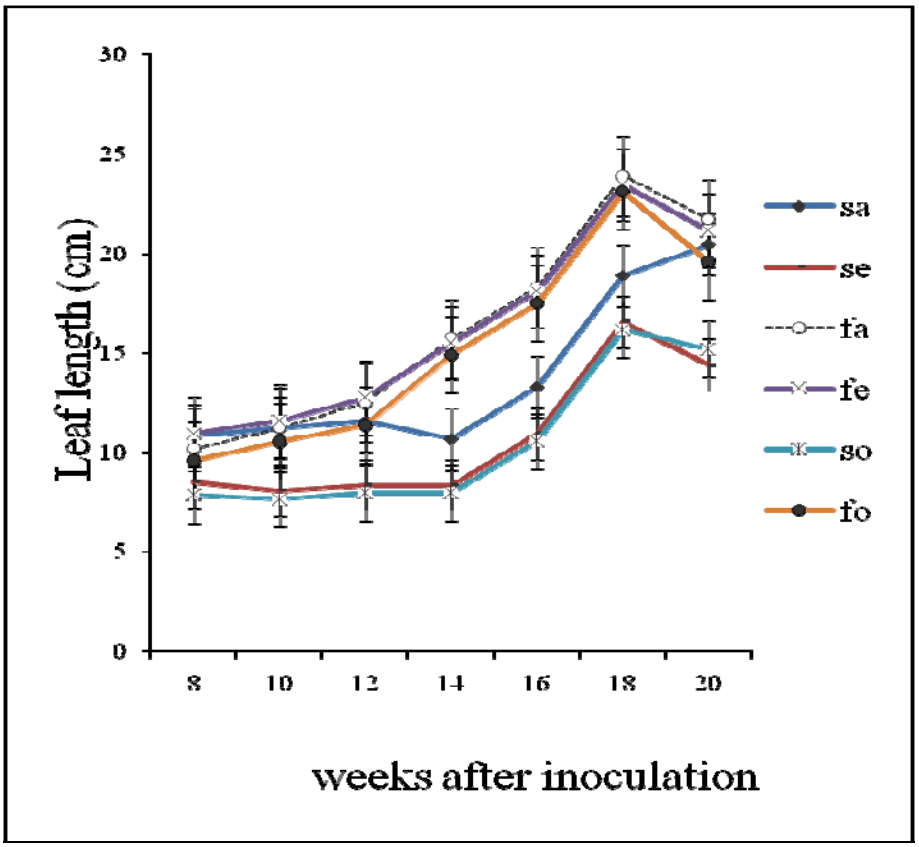


Fig. 4: Effect of inoculation on broadest leaf elongation. Points represent means and standard errors for broadest leaf length of 21 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

2.4.2 Plant biomass accumulation

Shoot fresh weight

Glomus etunicatum enhanced shoot fresh weight significantly ($p<0.05$) more than indigenous inoculum in conventional medium (Fig.5) twenty weeks after inoculation. Treatment FE had a significantly heavier ($p<0.05$) shoot fresh weight than control. Shoots of plants inoculated with commercial inoculum had a lower fresh weight than

the control in sterile sand medium, although the difference was not significant ($p>0.05$). The indigenous inoculum enhanced shoot fresh weight more ($p<0.05$) than the commercial inoculum in sterile sand medium. Commercial inoculum performed better than indigenous inoculum in conventional nursery medium.

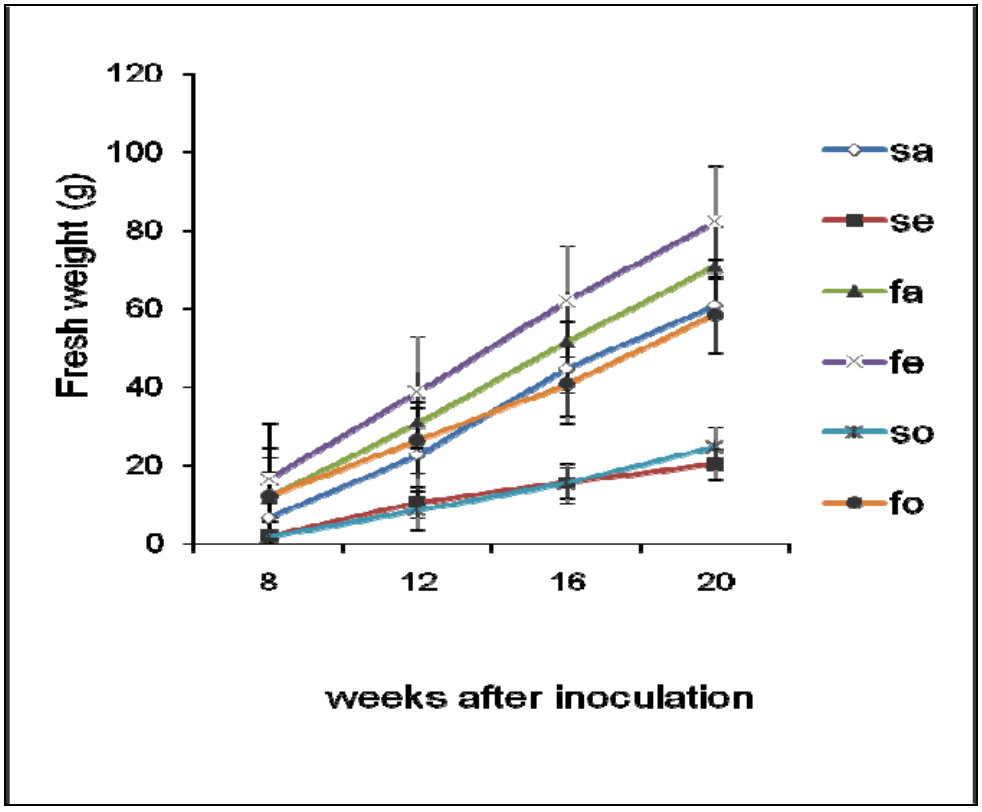


Fig. 5: Effect of inoculation on shoot fresh weights. Points represent means and standard errors for shoot fresh weight of 21 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

Shoot dry weight

Shoots of plants inoculated with commercial isolate had a higher dry weight than those of plants inoculated with indigenous inoculum in conventional medium eighteen weeks after inoculation. The two treatments (FE and FA) were not significantly different from

each other during the experiment ($p>0.05$). At time of termination of the experiment (i.e. week 20), FA had a higher dry weight than FE. This result contrasts that of shoot fresh weight (Fig.5) which showed that FE was significantly heavier than FA throughout the experimental period. Indigenous inoculum (SA) enhanced shoot dry weight more significantly ($p<0.05$) than commercial inoculum in sterile sand medium. Dry weight of shoots of plants inoculated with commercial inoculum (SE) were lighter than those of non-inoculated plants in sterile sand medium however, the difference was not significant. It is not clear why dry weight of SO was higher than that of SE.

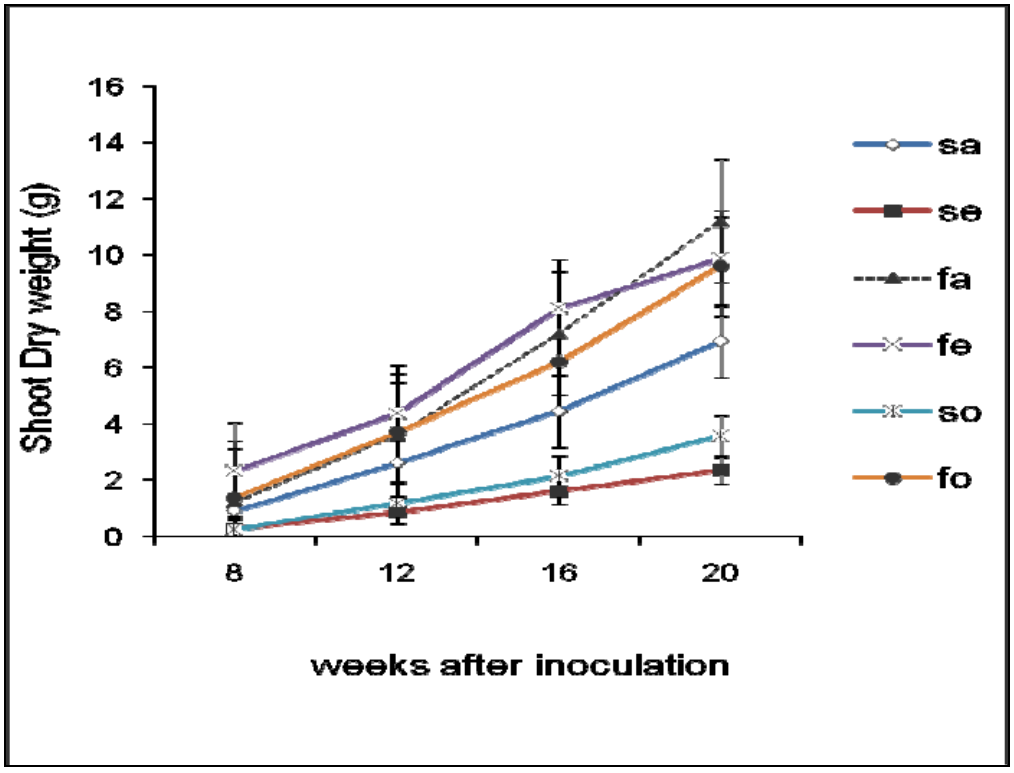


Fig. 6: Effect of inoculation on shoot dry weight. Points represent means and standard errors for shoot dry weights of 21 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

Leaf Area

Glomus etunicatum significantly ($p<0.05$) enhanced leaf area more than indigenous isolates twenty weeks after inoculation in conventional medium. Commercial inoculum however, did not show any significant ($p>0.05$) differences from the control for plants established in sterile sand medium throughout the experiment. Indigenous glomus species significantly ($p<0.05$) enhanced leaf area more than commercial inoculum in sterile sand medium (Fig.7). Leaf area showed clear treatment differences between inoculated plants and non-inoculated ones in all treatments except SE (Fig.7). The interaction means (media*AMF) was not significant ($p>0.05$).

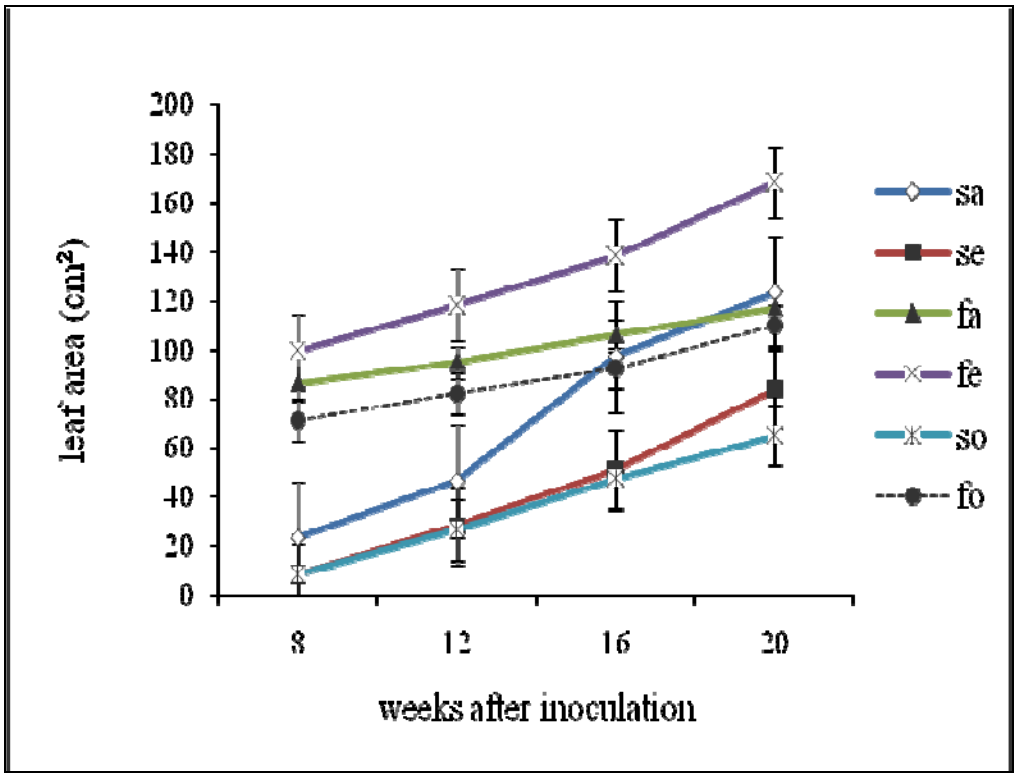


Fig. 7: Effect of AMF inoculation on leaf area. Points represent means and standard errors of leaf areas of 21 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

Root fresh weight

Glomus etunicatum enhanced root fresh weight more than indigenous glomus species in conventional nursery medium throughout the experiment (Fig.8). The two treatment means were significantly different from each other and the control in the entire experimental period ($p<0.05$). The effectiveness of the commercial isolate depended on the type of media used for establishment of inoculated plantlets ($p<0.05$).

Glomus etunicatum yielded a significantly ($p<0.05$) higher fresh weight than indigenous inoculum in plants established in conventional medium whereas its performance in sterile sand was far much less than that of the indigenous inoculum. Plants inoculated with indigenous isolates had significantly ($p<0.05$) higher root fresh weight than *Glomus etunicatum* irrespective of the media for establishment.

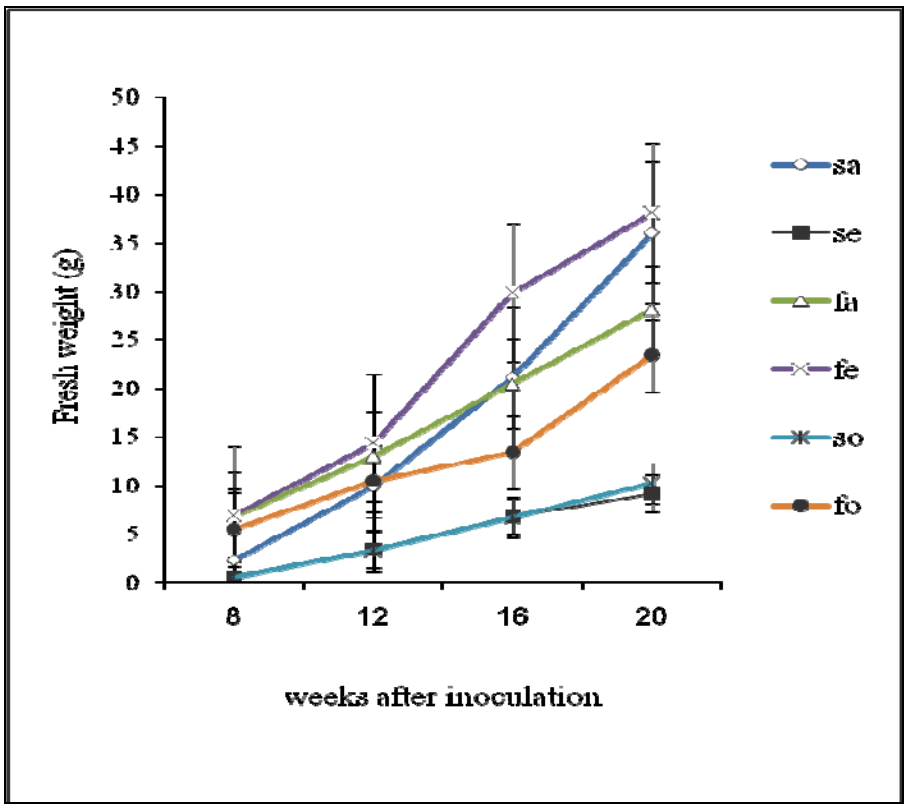


Fig. 8: Effect of inoculation on root fresh weight. Points represent means and standard errors for root fresh weights of 21 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

Root dry weight

Root dry weight was significantly higher for plants inoculated with *Glomus etunicatum* and established in conventional medium ($p<0.05$). The same inoculum however, produced results which were not significantly different from the control in plants established in sterile sand medium. Performance of indigenous glomus isolates was similar ($p>0.05$) in both media. Neither did performance of indigenous inoculum differ significantly from those of commercial inoculum nor control in conventional medium (Fig.9). Indigenous isolates enhanced dry matter in inoculated plants equally

irrespective of media for establishment at stated probability level ($p<0.05$). The indigenous isolates were more adapted to prevailing conditions in both media.

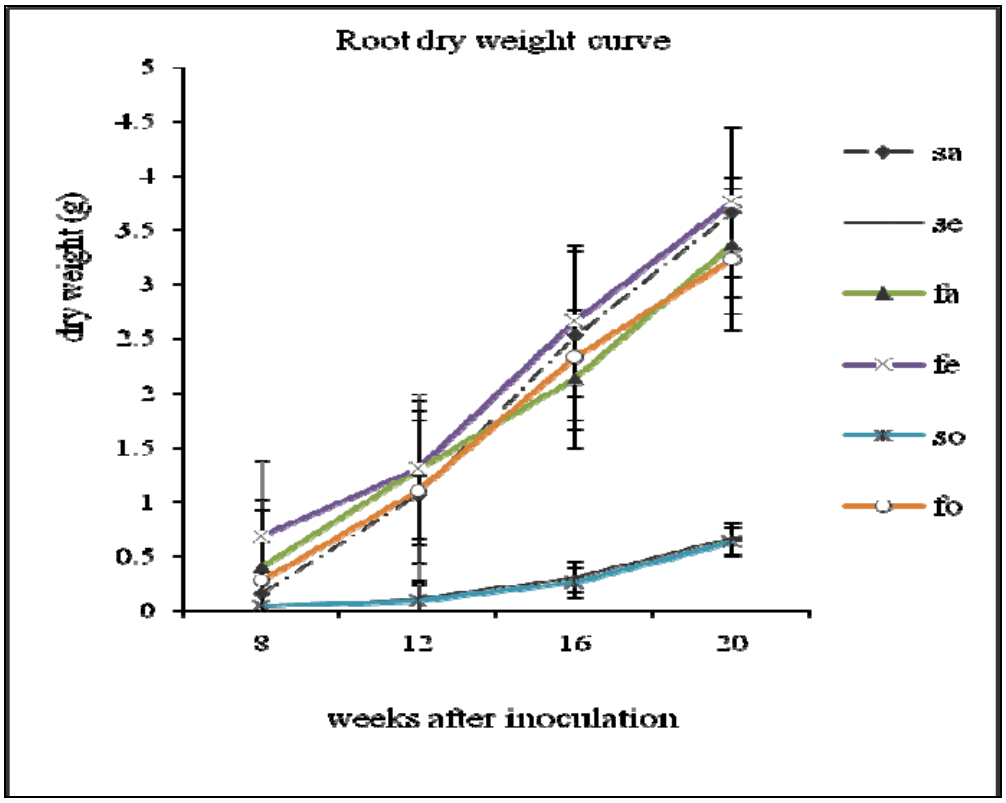


Fig. 9: Effect of inoculation on root dry weight. Points represent means and standard errors of root dry weights of 21 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

2.4.3 Assessment of AMF colonization

2.4.3.1 Intensity of root colonization

The commercial inoculum's colonisation of roots in conventional medium was observably different from that of indigenous inoculum, though the difference was not

statistically significant ($p>0.05$). *Glomus etunicatum* established a more intense colonization in conventional medium than indigenous glomus species. However, Indigenous inoculum established a more intense colonisation in sterile sand medium than the commercial isolate. Both controls (FO and SO) were not colonised throughout the experiment (Fig.10). Intensity of colonisation for the indigenous inoculum was not significantly different in both media. The intensity of colonisation by indigenous glomus species did not depend on type of medium for establishment of inoculated plants.

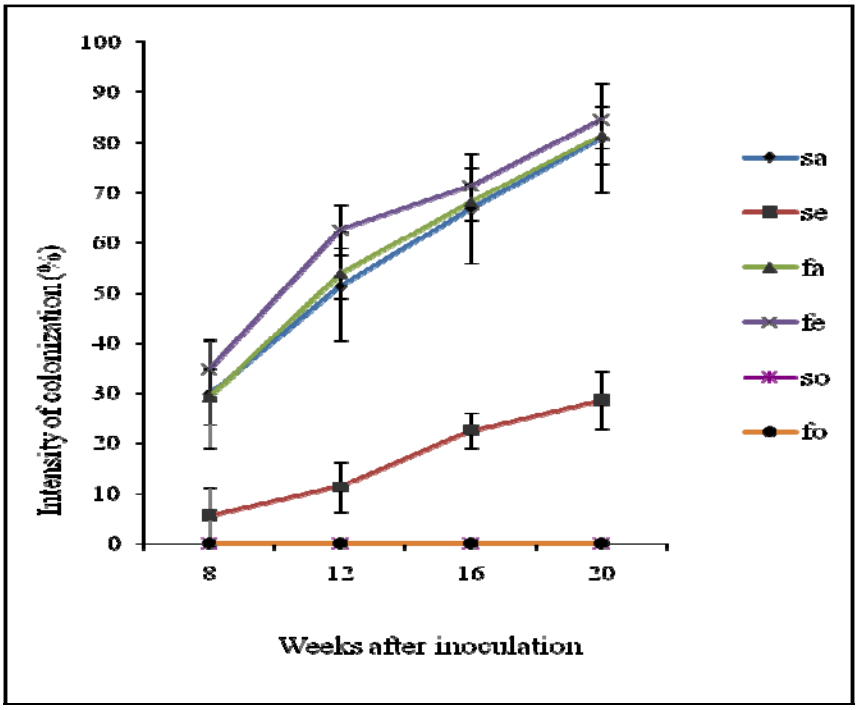


Fig. 10: Effect of AMF inoculation on intensity of root colonisation. Points represent means and standard errors for percent intensity of root colonisation of 12 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

2.4.3.2 Frequency of root colonisation

Root colonisation was more frequent in plants inoculated with commercial inoculum than it was for plants inoculated with indigenous inoculum twenty weeks after establishment in conventional medium ($p<0.05$). Frequency of root colonisation was not different ($p>0.05$) in both media for plants inoculated with indigenous inoculum throughout the experiment. Indigenous inoculum showed significantly higher frequency of colonisation ($p<0.05$) from commercial inoculum in plants established in sterile sand medium throughout the experimental period of twenty weeks (Fig.11). Both control treatments (FO and SO) showed zero percent frequency of colonisation during the entire experiment (Fig.11).

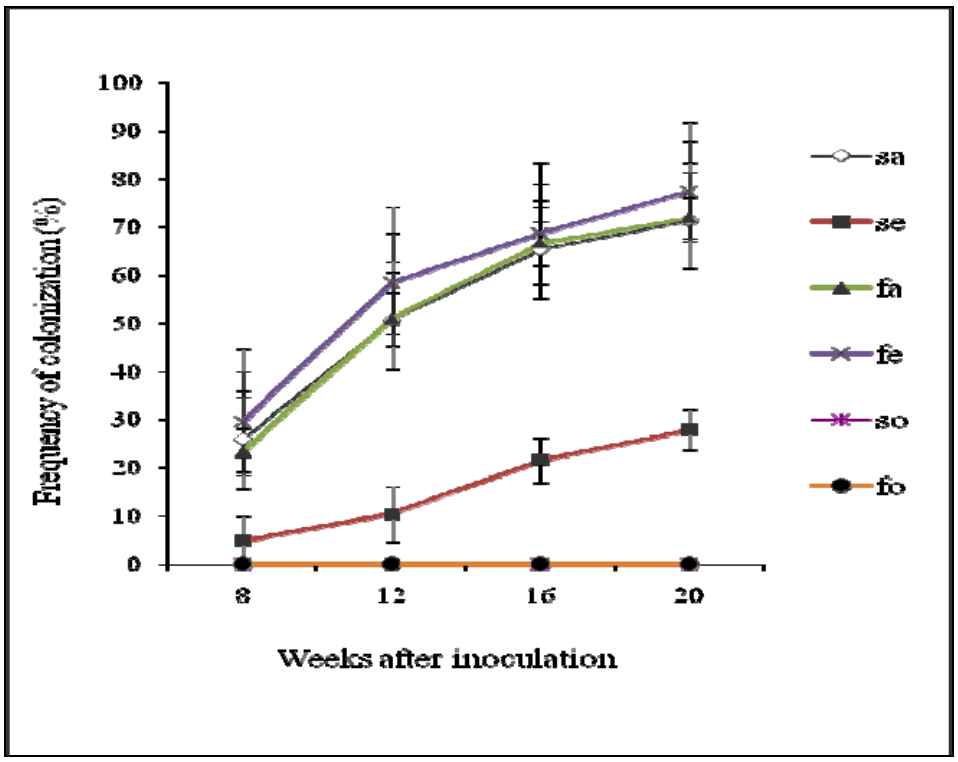
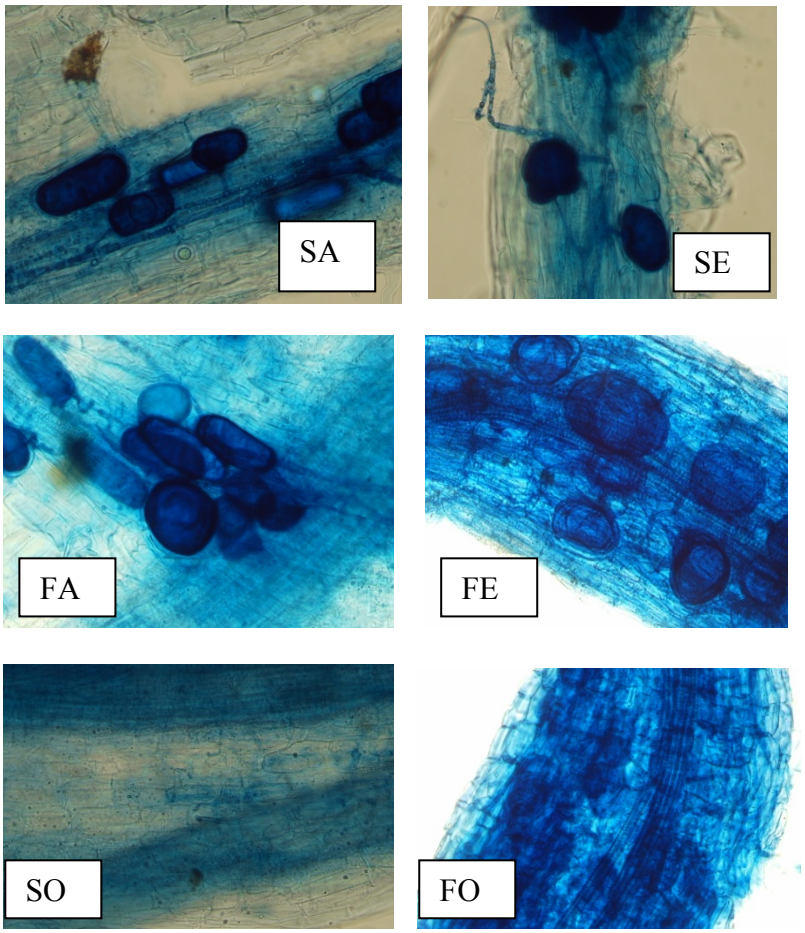


Fig. 11: Effect of AMF inoculation on frequency of root colonisation. Points represent means and standard errors of percent frequency of root colonisation of 12 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

2.4.3.3 Visual observation of AMF colonisation assessment

Representative plates for all treatment combinations showing the extent of AMF infection of banana roots are displayed in plate 1 below. The information reported on frequency and intensity of AMF colonisation (section 2.4.3 of this thesis) was extracted from twenty plates per treatment combination.



Key: A- arbuscules; S- spores; V- vesicles and H- hyphae. SA, SE, FA, FE, SO and FO represents the six treatments. SO and FO are controls (non-inoculated).

Plate 1: Visual comparison of AMF root colonisation showing mycorrhiza infective structures as observed under electron microscope (Nikon 59533, Japan) at magnification of 400X after staining with 0.05% trypan blue stain. The letters S-represent sand, F-conventional medium, A-indigenous isolates, E-*Glomus etunicatum* and O-non-inoculated controls.

2.5 Relationship between growth and colonization

The intensity of AMF colonisation and growth parameters shoot fresh and root dry weights were strongly correlated (r=0.9891 and 0.9823 respectively). All growth parameters measured were significantly ($p<0.05$) affected by frequency and intensity of AMF colonisation. There was a strong positive linear relationship (R=0.8) between intensity of AMF colonisation and growth (plant height) (Fig. 12). The more frequent

AMF structures appeared, the more intense the colonisation was in all AMF inoculated treatments.

Table 2: Correlation between growth and AMF colonisation parameters.

	Shoot fw	Shoot dw	Root dw	L. area
Intensity	0.98916	0.97553	0.9823	0.97605
Frequency	0.95732	0.92976	0.94561	0.94432

NB: fw is fresh weight; dw is dry weight; L. area refers to leaf surface area. The figures represent Pearson's correlation coefficients, r at 5% level of significance.

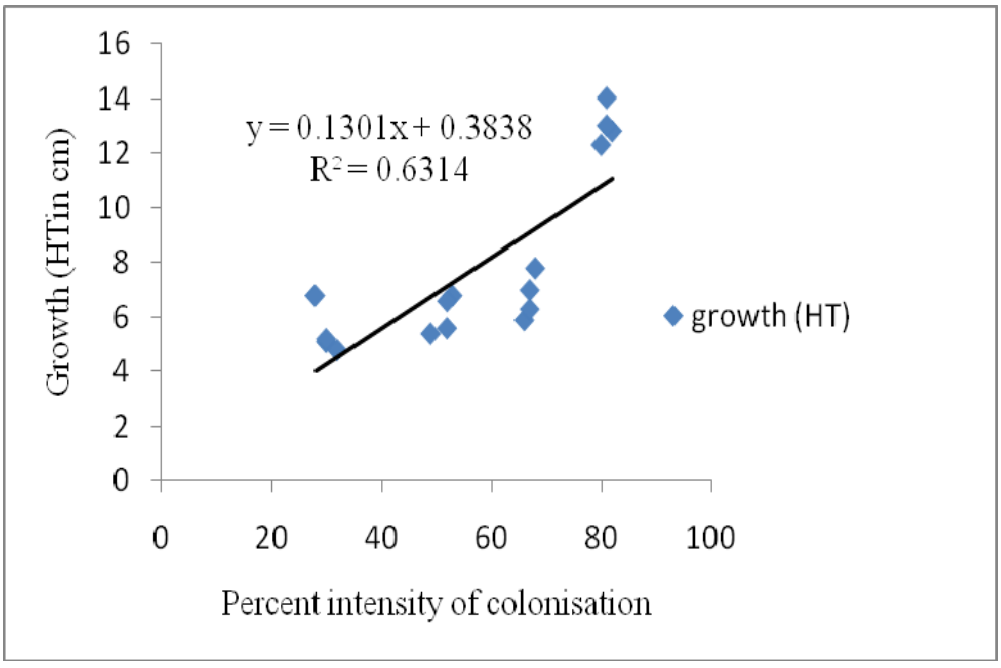


Fig. 12: Regression analysis showing the relationship between pooled data for the intensity of AMF colonisation and plant growth response after 20 weeks of inoculation with indigenous AMF species and *Glomus etunicatum*.

2.6 DISCUSSION

This study demonstrated that inoculation of tissue cultured banana plantlets with AMF significantly ($p<0.05$) increased growth of all plants established in conventional nursery medium. All AMF treatments established in conventional medium showed vigorous growth in most of the parameters measured, and was significantly ($p<0.05$) different from control (non-inoculated plants). The extent of increase in growth varied with different mycorrhiza inoculum (commercial and indigenous) used for the experiment. *Glomus etunicatum* inoculated plants registered the highest (14.8%) increase in growth in all the parameters (height, number of leaves, leaf width and leaf length) measured, whereas indigenous inoculum (glomus species isolated in Kenya) stood at 13.3% increase in growth against control. *Glomus etunicatum* is reported to have been an excellent growth enhancer under tropical and sub-tropical conditions (Azcon et al., 1992; Cuenca and Azcon, 1994; Tobar et al., 1994; Azcon et al., 1996; Cornejo et al., 2006). *G. etunicatum* enhances plant root system which increases plant's chances of accessing more extensive soil water and nutrient reserves (Sanchez-Diaz and Honrubia, 1994). This may have resulted in vigorous growth habit observed in plants inoculated with *G. etunicatum*. Plant sizes were fairly larger than those of indigenous inoculum in all parameters measured. Results of the present experiment were in agreement with those of Yano-Melo et al. (1999) who reported a 57% increase in leaf area, 32% increase in plant height and a 64% increase in dry matter of tissue culture banana plantlets inoculated with *Glomus etunicatum*. However, percentage increases in growth obtained during this experiment were lower than those of Yano-Melo et al. (1999). The cause of such variation is not clear but it can partly be attributed to the difference in size

of experimental containers or pots. Yano-Melo et al. (1999) used three litre containers which carried a larger volume of medium than the one litre pots used during this experiment (Fig. 3). The roots of plants grown in 1L pots began to intertwine after sixteen weeks of inoculation indicating that the root mass had exceeded the pot volume. Increase in growth parameters measured reduced with time between weeks 16 and 20 after inoculation. This might have been responsible for the variation in data reported by Yano-Melo et al. (1999) and those of the current study.

Indigenous inoculum can be used with sterile sand as a medium of choice by commercial nursery banana producers. Indigenous inoculum achieved a 41.7% increase in growth as opposed to *Glomus etunicatum* (SE) which posted an average of 3.6% increase in growth in sterile sand medium (Fig. 3).

Indigenous inoculum was able to withstand the loose structure and coarse texture of sand medium to accelerate the growth of tissue culture banana plantlets more significantly ($p<0.05$) than the commercial isolates (Fig. 9). The success of indigenous inoculum over commercial isolate in sterile sand may have been due to the fact that indigenous inoculum was well adapted to the prevailing media and environmental conditions. The inoculum (indigenous) was therefore able to tolerate harsh conditions in sterile sand medium better than *Glomus etunicatum*. Similar results have been reported by Bouamri et al. (2006) from his study on AMF association with date palm (*Phoenix dactylifer*) in the sandy Ziz valley of Morocco. Bouamri et al. (2006) noted that all the indigenous glomus isolates used in the experiment enhanced growth of date palm more than *Glomus mosseae* and all other four commercial isolates used during the experiment. Similar observations have also been made by Rurangwa et al. (2010) who reported that indigenous isolates (isolated in Kibungo and Rubona areas in Rwanda)

enhanced growth and survival of micropropagated bananas more than the commercial isolate, *Glomus mosseae*. Although the commercial isolate (*Glomus etunicatum*) was expected to exhibit some negative effects due to harsh conditions in sterile sand medium, 3.6% increase in growth fell far much below expectation (Figs. 10 and 11). Performance of *G. etunicatum* in sterile sand medium contradicts results by Mwashasha (2005) who reported a 53.1% growth promotion of giant Cavendish banana cultivar with maximum frequency and intensity of colonisation of 70% and 80% respectively, twenty two weeks after inoculation. The cause for drastic decline during the current experiment was not clear since the same inoculum was used to inoculate plants in conventional medium which gave a 14.8% increase in growth (Figs. 10 and 11). The disparity in the growth response due to inoculation by *G. etunicatum* between the two experiments can be attributed to poor colonisation the isolate established in sterile sand medium in the present study. However, it remains unclear why the commercial isolate established poor colonization in sterile sand medium.

The ability of the two inocula in enhancing growth of tissue culture bananas in the respective media for establishment was demonstrated by data from biomass accumulation. Information obtained from shoot and root dry weights (Figs. 6 and 8) were consistent with those of plant height, number of leaves, leaf width and leaf length (Figs. 1 - 4). *Glomus etunicatum* recorded an average of 34% (65-31) increase in shoot dry weight more than control plants (31%) in conventional medium. The performance of the commercial inoculum was not different ($p>0.05$) from that of indigenous inoculum which resulted in a 29% (60-31) increase in shoot dry weight in conventional medium. Variation in root dry weights for plants treated with the two inoculums in conventional medium was 2% with commercial inoculum posting the highest increment

in dry weight of 32% (58-26). Root mass for plants inoculated with the two inoculums in conventional medium were different from their control (FO). From analysis of plant biomass, it was observed that the two inocula enhanced plant biomass accumulation nearly 2.2 times better than they increased plant growth in conventional medium. Such results have also been observed by Yano-Melo et al. (1999) who reported an increase in growth (plant height) and biomass of 32% and 64% respectively when they inoculated tissue culture banana cultivar Pacovan using *Glomus etunicatum*. According to Douds et al. (1998), the physiological response of a plant is a result of interactions between environment, plant, and fungus genotype. Glomus spp. usually enhances plant shoot dry matter between 1.5 - 2.5 times better than observable growth parameters under favourable environmental conditions as was reported by Guillemin et al. (1992). Guillemin's observation provides the explanation for the 2.2 times disparity between growth and biomass increment, since both inocula used were of glomus genus. Plant biomass data from inoculated plants established in sterile sand medium reflected those of plant growth parameters whereby indigenous inoculum registered a 44% increase in shoot dry weight whereas commercial inoculum recorded 5% increase in growth. The possible reasons for the success of indigenous inoculum may be as was discussed earlier on in this section.

Correlation analysis revealed a strong positive relationship (R=0.8) between plant growth and intensity of AMF colonisation (Table 2). The Pearson's correlation coefficient (r) was significant ($p<0.05$) for all the five parameters (shoot fresh & dry weight, and leaf surface area) tested. The results suggest that the magnitude of increase in growth depended on the frequency and intensity of AMF colonisation. The more frequent and intense the colonisation was, the greater the increase in growth. This

argument was true in all treatments where plants were inoculated (treatments SA, SE, FA and FE). Many studies reported similar results (Jaizme-Vega et al., 1997; Monteiro et al., 1991; Declerck et al., 1995; Yano-Melo et al., 1999; Cornejo et al., 2006) suggesting that plant shoot growth is directly proportional to intensity of AMF colonisation.

Performance of the indigenous isolate in promoting growth during this experiment offers potential for commercial utilization of indigenous mycorrhiza species in commercial tissue culture banana nurseries in Kenya. The hope for commercialization is further emphasized by the isolate's ability to transfer nutrients to host plant tissues as discussed in the next chapter.

CHAPTER 3

EFFECTS OF STERILE SAND AND CONVENTIONAL NURSERY MEDIA ON AMF COLONISATION AND NUTRIENT UPTAKE OF TISSUE CULTURE GIANT CAVENDISH BANANAS

Abstract

The influence of sterile sand and conventional media on AMF colonisation and nutrient uptake in giant Cavendish bananas was evaluated. It was expected that colonisation would be higher in sterile sand whereas nutrient up could be higher in conventional medium. Four destructive samplings were made at an interval of one month beginning at 8 weeks after inoculation. Dry plant samples were conducted for analysis of nitrogen, phosphorous and potassium content. A 100 g media sample was taken around the root region of each plant sampled for destructive analysis. The wet sieving method was used to extract spores from respective media for counting. Arbuscular Mycorrhizal Fungi colonization and nutrient accumulation in plant tissues were higher in inoculated plants established in conventional medium than those established in sterile sand medium for both AMF isolates. *Glomus etunicatum* had a mean value of 4,162 spores compared to 3,841spores/100 g of soil for the indigenous inoculum after twenty weeks of inoculation and subsequent establishment in conventional medium. Indigenous inoculum had the highest number of spores in sterile sand medium with 3,706 spores compared with 1584 spores/100g of sand formed by GE in the same medium. Conventional medium recorded the highest amounts for N, P and K of 3,358, 160.9 and 6,114 mg/Kg,

respectively in tissues of plants inoculated with GE. In sterile sand, mean nutrient amounts of 2,874, 140.3 and 5,687 mg/Kg for N, P and K, respectively were recorded from plants inoculated with the indigenous isolates. Conventional medium is therefore, preferable for inoculation using both *Glomus etunicatum* and indigenous isolates. Further studies should be conducted to ascertain why *Glomus etunicatum* failed to substantially colonise plants established in sterile sand medium.

3.1 Introduction

Successful colonization and survival of introduced AMF infective structures depends on media conditions and soil types where the symbiosis is established (Plenchette, 2000). Media aeration is the single most important factor for successful AMF colonization (Six et al., 2004). AMF require sufficient supply of oxygen for effective colonization and growth of hypha, vesicles, arbuscules, appressoria, spores and other mycorrhizal structures (Diaz-Zorita et al., 2002). Soil drainage influences aeration which is a key determinant for survival of aerobic microflora and fauna. AMF infective structures require well drained media conditions for maximum sporulation and growth of root hair extensions (Six et al., 2002). Media structure influences many biotic, physical and chemical aspects of the media. It is the basic setting in which processes take place in soil (Six et al., 2004). AMF are important in influencing soil structure at the macroaggregate level, where direct hyphal involvement is thought to be most pronounced mechanism (Miller and Jastrow, 2000). The dense interlocking network of hyphae (mycelia) binds soil macroaggregates giving them a completely new structural look (Duponnois et al., 2001). Mycorrhiza root hair extensions penetrate macroaggregates creating pores which improve soil aeration (Rillig et al., 2002). Sand medium offers a conducive environment for AMF sporulation due to its loose structure. The looseness of sand allows sufficient oxygen circulation which encourages growth and maturation of fungal spores (Jones et al., 2004). The rate of AMF sporulation in sand is estimated to be 1.5 times that of soil due to less compaction of the sand medium (Dexter, 1987). Sand has poor moisture and nutrient retention capacity which calls for frequent irrigation. Depending on the amount of water used and frequency of watering,

a large proportion of extra radical spores can be lost during irrigation (Horn et al., 1994).

Soil structure is influenced by soil water content and its variation with time. A decrease in soil water content typically increases contact points between primary particles and organic matter, resulting to soil compaction (Horn and Dexter, 1989). Soil compaction affects AMF sporulation due to poor aeration and drainage giving rise to anaerobic conditions (Dexter, 1987).

Nutrient uptake from the soil depends on several edaphic factors including media moisture and nutrient retention capacity, texture and pH. There is likelihood that the difference in properties of the two media (conventional and sterile sand) will influence the extent of AMF colonization both inside and outside plant roots as well as amount of nutrients taken up by individual plants. Uptake of nutrients has a strong bearing on growth of plants. “It is assumed that the higher the nutrients absorbed by plant roots, the higher the growth”; a hypothesis which is dependent on several factors including properties of the growing medium. The objective of this research was to determine whether sterile sand and conventional nursery media influence (i) AMF colonisation and (ii) nutrient uptake of tissue culture bananas.

3.2 MATERIALS AND METHODS

3.2.1 Assessment of AMF root colonization

The details of study site, source of inoculum, source of plantlets, media preparation and treatment, inoculation, preparation of modified Hewitt nutrient solution and potting are same as those contained in sections 2.2.1 to 2.2.9 of chapter 2 of this thesis. Four destructive harvests were conducted on a monthly basis beginning at eight weeks after inoculation. Assessments of root colonisation frequency and intensity were done as outlined in the procedure in sections 2.3.0 to 2.3.3 of the previous chapter (chapter 2).

3.2.2 Assessment of AMF colonization of media around the root region

Three replicates each containing a 100g media were taken from the root rhizosphere for assessment of AMF sporulation in the media around the root region. Each plant sampled was accompanied with its respective media sample which was labelled according to treatment and replicate number. Media samples were put into 30 microns polythene sleeves and stored in the refrigerator at 4^{o}C before extraction of spores was done. Media sampling was done on a monthly basis for four months beginning at 8 weeks after inoculation.

3.2.3 Extraction of spores

Spore extraction was done according to Ingleby and Mason (1973). Two cores (20g of sand/soil) from each of the treatments were assessed by mixing the substrate with water, stirring to a uniform suspension passed through 710 and 45 µm sieves several times until suspension was clear. The sediment was back-washed from the 45µm sieve into six 50 ml centrifuge tubes and centrifuged for 5 minutes at 1750 revolutions per minute (rpm). The solution was allowed to settle for about 10 minutes before careful removal of floating debris without disturbing the pellet. The pellet was mixed with 48% sucrose (227g dissolved in 500 ml water) then tubes balanced by weight and shaken thoroughly a few seconds before centrifuging for 15 seconds at 1750 rpm. The suspension was poured quickly through a 45µm sieve before it settled. Suspension was back-washed to remove sucrose. Spore counting was done under a compound microscope (Nikon 59533, Japan) at 400X magnification.

3.2.4 Preparation of diagnostic slides of AMF spores

Characterization of spores was done to ascertain the persistence and survival of original inocula. Characterization was done according to the procedure developed by Ingleby (2007).

Spores were quashed on microscope slides to reveal details of spore wall layers and hyphal attachments. All preparatory stages were carried out under a dissecting microscope. Similar spores were cleaned and separated in Petri dish using a mounted needle (at least 30 spores were mounted). Spores were transferred into cavity dishes

using pipette. A microscope slide with very small drops of polyvinyl lactoglycerin (PVLG) and Melzer's reagent was prepared. Spores of one type were transferred onto a filter paper using a pipette. A mounted needle was dipped into appropriate mountant then used to pick half of the spores from the filter paper to a mountant on slide. The process was repeated for the other mountant using the remaining spores. Care was taken for the spores not to dry out on filter paper. Ensuring that the spores are at the centre of the mountant, a 13mm cover slip was lowered onto the mountant plus spores. A gentle pressure using a clean mounted needle was then applied on the cover slip to crush the spores. Spores were examined under a compound microscope for wall layers, hyphal features, properties, ornamentation, development features-saccule/scar, germination shield and reaction with Melzer's reagent. Each slide was labelled and the mountant was allowed to dry and shrink. After 10 days, a further mountant was added and the cover slip edges sealed with nail varnish and kept as a permanent slide.

3.2.5 Plant tissue nutrient analysis

Plant shoot samples were oven dried at 70^{o}C for a period of 72 hours at JKUAT's Horticulture laboratory. The shoot samples (pseudostems and leaves combined) were ground in a ball mill and the samples analyzed for primary macronutrients N, P and K. Plant samples were analyzed for nitrogen (Kjeldahl, 1994) on a semiautomatic nitrogen analyzer (M/S Gerhardt, Germany). Kjeldahl procedure is based upon the fact that on digestion with sulphuric acid and various catalysts, the organic material is destroyed and the nitrogen is converted to ammonium acid sulphate. On making the reaction mixture

alkaline, ammonia is liberated which is removed by steam distillation, collected, and titrated.

The phosphorus and potassium content were estimated in acid-digested samples according to procedure by Jackson (1958). The tissue is extracted with 2% trichloroacetic acid and the elements are determined on the filtrate by atomic absorption spectrophotometry.

3.2.6 Data analysis

The percentage root colonization and spore count data was arcsine and log transformed respectively, before being subjected to ANOVA using General Linear Model (GLM) procedure in SAS (SAS Institute, 2003). Separation of treatment means was done using Tukey's HSD test. Characterization of spores was done by visual observation under compound microscope (400X) while referring to spore characterization chart made by Ingleby (2007). All tests were performed at $\alpha = 0.05$.

3.3 RESULTS

3.3.1 Assessment of AMF root colonization

Conventional nursery medium was the most ideal medium for AMF colonization by both inoculums. The overall mean for percent colonization frequency and intensity in conventional medium was significantly higher ($p<0.05$) than that of sterile sand (Figs. 13 and 14). Examination of individual treatments indicated no difference between percent colonization frequency and intensity of treatments FE (*Glomus etunicatum* inoculated plants established in conventional medium), FA (indigenous glomus inoculated plants established in conventional medium) and SA (indigenous glomus inoculated plants established in sterile sand medium) (Fig. 13). Colonization of *Glomus etunicatum* was notably lower (17%) in sterile sand medium (treatment SE) than it was in conventional medium (63%). SE was significantly different (lower) from the rest three treatments FE, FA and SA ($p<0.05$). The two controls FO and SO were not colonized.

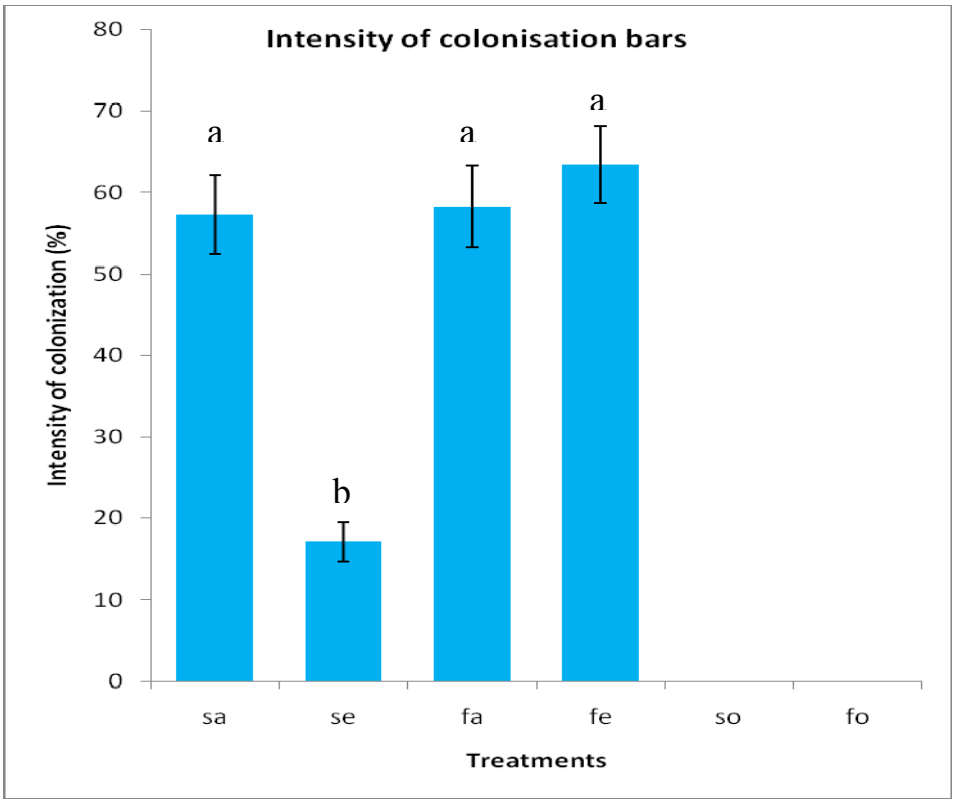


Fig. 13: Effect of media on intensity of AMF colonisation of banana roots. Bars represent means and standard errors of percent intensity of root colonisation of 12 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented with letter o.

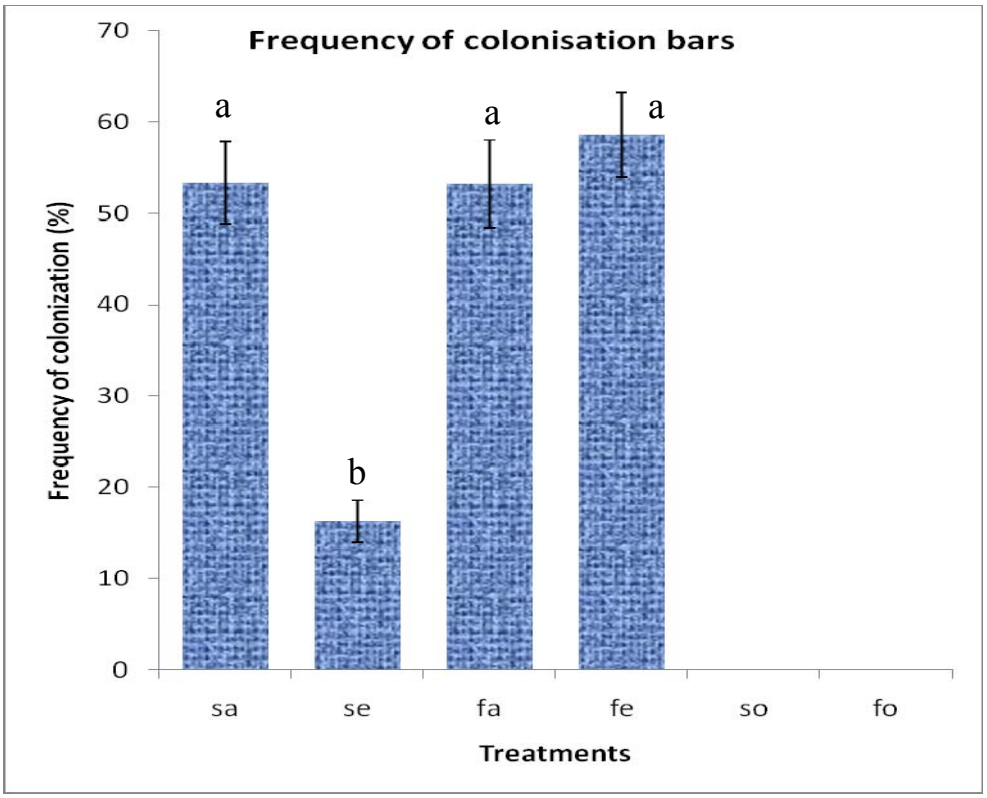


Fig. 14: Effect of media on frequency of AMF colonisation of banana roots. Bars represent means and standard errors of percent frequency of root colonisation of 12 banana plantlets taken from four replicates per treatment and measured over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented with letter o.

3.3.2 Assessment of AMF colonization of media around the root region

Sporulation was higher in conventional medium than in sterile sand ($p<0.05$) with *Glomus etunicatum* (FE) attaining the highest number of spores of 2,420.4/100g of soil, twenty weeks after inoculation in conventional medium (Fig.16). AMF did not depend on the media used as indicated by insignificant interaction (media*AMF, $P>0.05$). Sporulation in conventional medium was not significantly different ($p>0.05$) in both treatments FE and FA. Production of spores in sterile sand medium was significantly

higher ($p<0.05$) around plants inoculated with indigenous inoculum (treatment SA) than it was around plants inoculated with *Glomus etunicatum* (SE) throughout the experimental period (Fig.15). Media sampled from control treatments (FO and SO) contained no spores throughout the entire experiment.

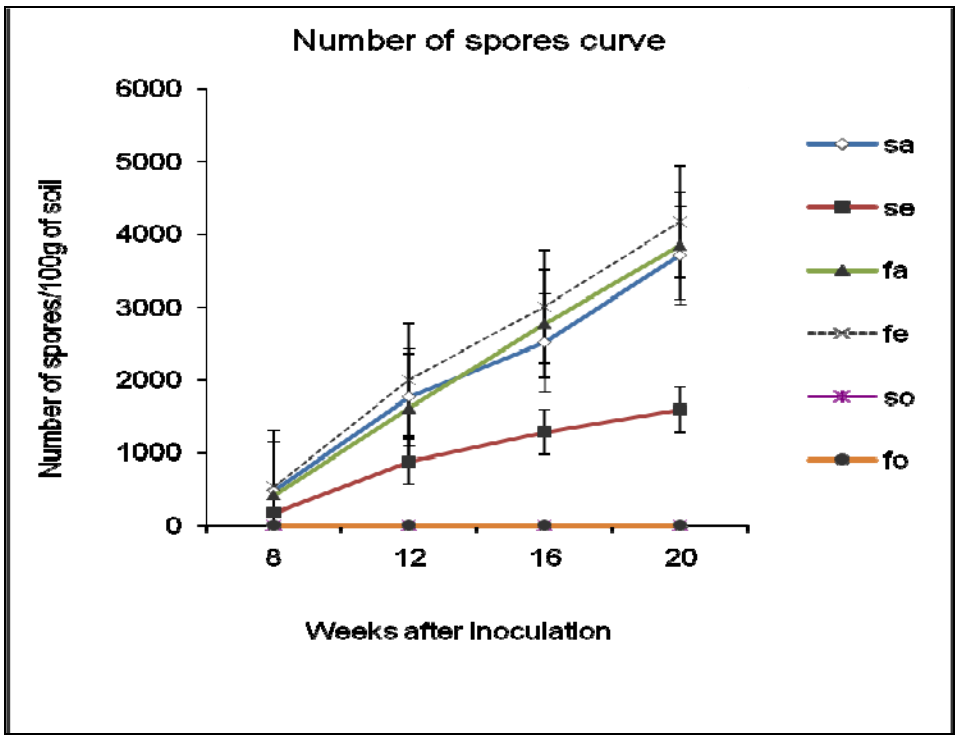


Fig. 15: Distribution of spore count per treatment across the four sampling stages. Points represent means and standard errors of number of spores per 100 g soil of 12 samples taken from four replicates per treatment monitored over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

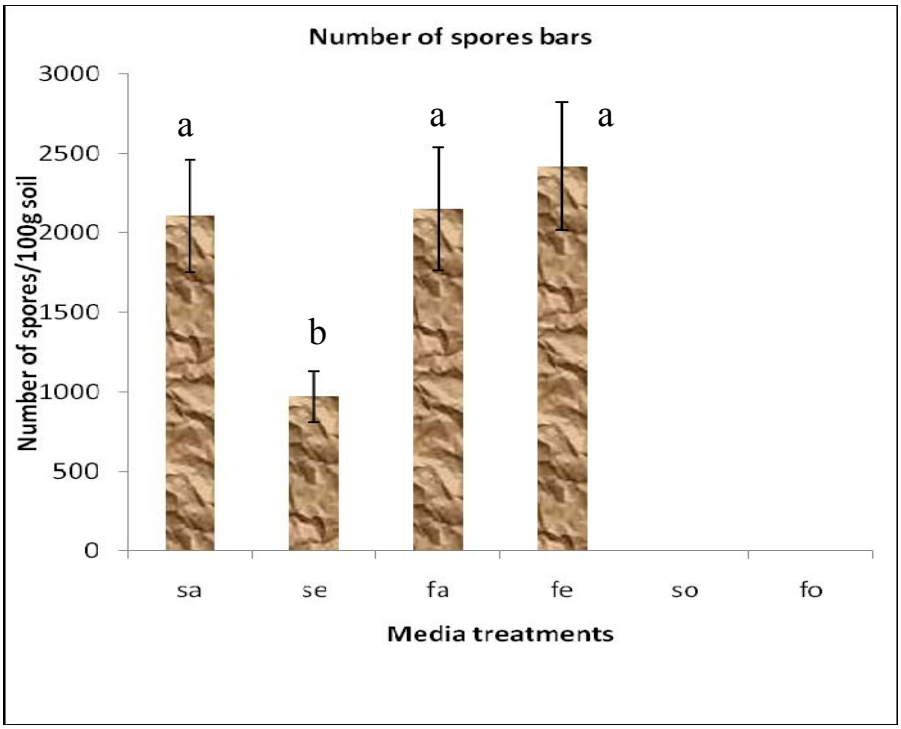


Fig. 16: Effect of media on rhizosphere sporulation of AMF inoculums. Bars represent means and standard errors of number of spores per 100 g soil of 12 samples taken from four replicates per treatment monitored over a period of twenty weeks after inoculation with *Glomus etunicatum* (e) and a mixed indigenous isolate (a) in sterile sand (s) and conventional nursery media (f). Control is represented by letter o.

3.3.3 Plant tissues nutrient analysis

Nitrogen (N)

Shoots of plants established in conventional nursery medium had higher levels ($p<0.05$) of nitrogen (N) than those of plants established in sterile sand medium (Table 3). Accumulation of nitrogen in shoots of plants established in conventional medium was significantly higher ($p<0.05$) than those of inoculated plants established in sterile sand medium at all sampling stages. *Glomus etunicatum* inoculated plants accumulated significantly higher amounts of nitrogen than those inoculated with indigenous glomus

species within twelve weeks after inoculation (Table 3). All treatments in conventional medium were significantly different ($p<0.05$) from their control (FO) counterparts. Plants inoculated with indigenous glomus species (SA) had higher levels of N than those inoculated with *Glomus etunicatum* (SE) in sterile sand medium ($p<0.05$). Both SA and SE were significantly ($p<0.05$) different from control, SO.

Table 3: Effect of AMF colonization and medium type on accumulation of Nitrogen (mg/plant dry weight)

Time WAI	8	12	16	20
AMF				
FE	251.9±0.99a	954.6±1.76a	1726.0±2.64a	3358.0±1.53a
FA	244.2±1.60a	862.3±3.18b	1661.6±3.28b	3106.0±4.73b
SA	220.6±0.74b	755.1±2.75c	1535.7±2.54c	2874.0±3.21c
FO	177.6±3.18c	701.0±7.00d	1498.0±8.33d	2675.6±39.98d
SE	138.0±1.15d	372.0±8.89e	884.3±10.41e	1424.6±11.33e
SO	106.3±3.71e	319.6±5.81f	801.0±6.92f	1271.6±43.32f

NB: MSD represents Tukey's minimum significant difference compared at 5% level significance; CV represent coefficient of variation. Figures represent treatment means ± standard errors. WAI represents weeks after inoculation.

Phosphorus (P)

Shoots of plants established in conventional nursery medium had significantly higher levels ($p<0.05$) of phosphorus (P) than those of plants established in sterile sand medium (Table 4). *Glomus etunicatum* inoculated plant shoots accumulated significantly higher amounts of P in conventional medium than plants inoculated with indigenous isolates at all sampling stages (Table 4). All treatments in conventional medium had significantly higher ($p<0.05$) amounts of P than their control (FO)

counterparts. Plants inoculated with indigenous glomus species (SA) had higher levels of P than those inoculated with *Glomus etunicatum* (SE) in sterile sand medium ($p<0.05$). Both SA and SE were significantly ($p<0.05$) different from control, SO.

Table 4: Effect of AMF colonization and medium type on accumulation of Phosphorus (mg/plant dry weight)

Time WAI	8	12	16	20
AMF				
FE	10.60±0.38a	48.90±0.64a	78.33±0.34a	160.93±0.73a
FA	9.40±0.11b	43.53±0.43b	74.50±0.32ba	152.66±1.20b
SA	8.86±0.12b	39.36±0.55c	70.16±0.52bc	140.26±0.54c
FO	7.70±0.25c	33.33±1.45d	68.66±1.76c	128.00±1.53d
SE	3.46±0.08d	22.66±0.88e	38.96±1.02d	60.33±1.45e
SO	2.86±0.20d	19.00±0.58e	31.00±1.15e	51.33±1.76f

NB: MSD represents Tukey's minimum significant difference compared at 5% level significance; CV represent coefficient of variation. Figures represent treatment means ± standard errors.

Potassium (K)

Shoots of plants established in conventional nursery medium had significantly higher levels ($p<0.05$) of potassium (K) than those of plants established in sterile sand medium (Table 5). Accumulation of K in shoots of plants established in conventional medium was significantly higher ($p<0.05$) than those of inoculated plants established in sterile sand medium at all sampling stages. *Glomus etunicatum* inoculated plants accumulated significantly higher amounts of K than plants inoculated with indigenous glomus species throughout the period of experiment (Table 5). Plants inoculated with indigenous glomus species (SA) had higher levels of K than those inoculated with

Glomus etunicatum (SE) in sterile sand medium ($p<0.05$). Both SA and SE had significantly higher ($p<0.05$) amounts of P than their control, SO counterparts.

Table 5: Effect of AMF colonization and medium type on accumulation of Potassium (mg/plant dry weight)

Time WAI	8	12	16	20
AMF				
FE	502.80±0.83a	1488.67±1.76a	2454.60±3.49a	6114.33±2.60a
FA	482.33±2.03a	1375.00±3.00b	2255.00±28.67b	5889.33±7.51b
SA	451.97±0.64b	1256.77±1.86c	2038.17±1.07c	5687.67±1.20c
FO	381.33±8.69c	1161.00±5.20d	1905.33±16.56d	5465.67±40.68d
SE	257.33±2.33d	601.67±5.24e	1248.00±9.02e	2689.00±21.78e
SO	220.33±5.78e	560.00±23.76e	1055.33±30.12f	2439.67±76.36f

NB: MSD represents Tukey's minimum significant difference compared at 5% level significance; CV represent coefficient of variation. Figures represent treatment means ± standard errors.

3.3.4 Relationship between uptake of nutrients and AMF colonization

There was a positive linear relationship ($R^2=0.67$) between intensity of AMF colonization and accumulation of phosphorus in inoculated tissue cultured giant Cavendish banana plant lets (Fig. 17). The more intense colonization was, the greater the accumulation of phosphorus in plant tissues. A strong positive relationship ($r=0.95$) was obtained when intensity of root colonization was correlated with uptake of N and P (Table 6). The relationship between root colonization and uptake of K ($r=0.88$) was however, not as strong as that of N and P. Uptake of P influenced the absorption of N

and K by mycorrhizal roots, both of which had a significant ($P<0.05$) positive linear relationship ($r=0.99$ and 0.98 respectively) with P (Table 6).

Table 6: Pearson's correlation coefficients showing the relationship between spore count, root colonization and nutrient (mg/plant dry weight) uptake tested at 5% level of significance

	Intensity of AMF colonization	Spores	N	P	K
Intensity	1	0.99	0.95*	0.95*	0.88*
Spores	0.99	1	0.97*	0.98*	0.92*

NB: Initials N, P and K represent Nitrogen, Phosphorus and Potassium respectively. Figures represent the Pearson's correlation coefficients tested at 5% level of significance.

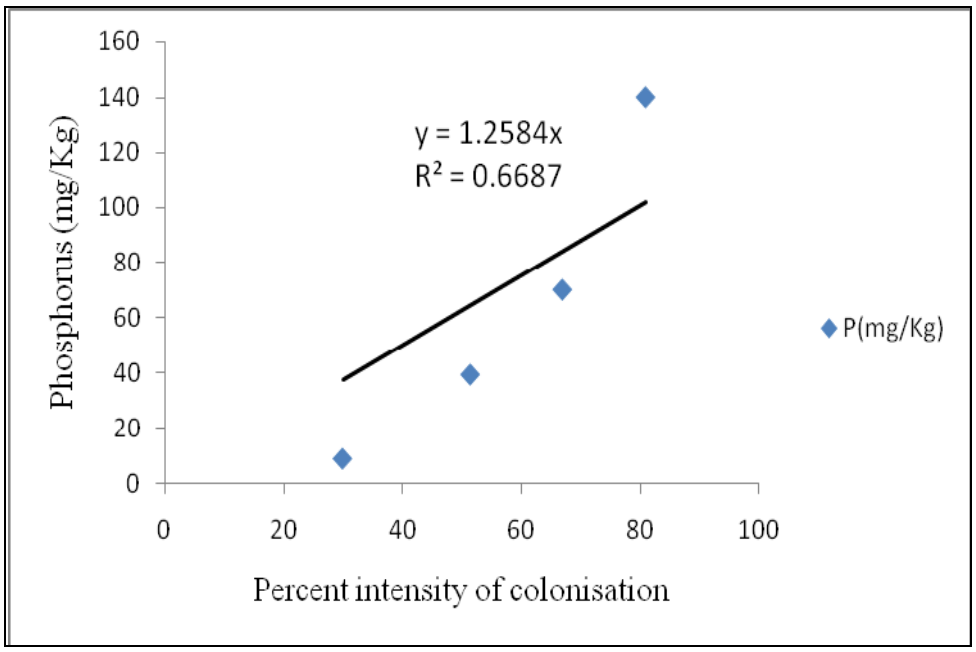


Fig. 17: Regression analysis of the relationship between percentage intensity of root colonisation and accumulation of phosphorus in inoculated banana tissues after 20 weeks of inoculation using both indigenous and commercial AMF types.

3.4 Diagnostic slides of the indigenous AMF spores

A sample slide for each of the four indigenous AMF isolates used during the experiment was captured under a compound microscope (400X) and the representative specimens were as shown in plate 2 below. The spore appearance was the single most important characteristic used for the identification of the isolates up to genera level. The isolates were all Glomus genus and were found to be common in all the 10 banana farms sampled in Maragwa District, Muranga County, Kenya.

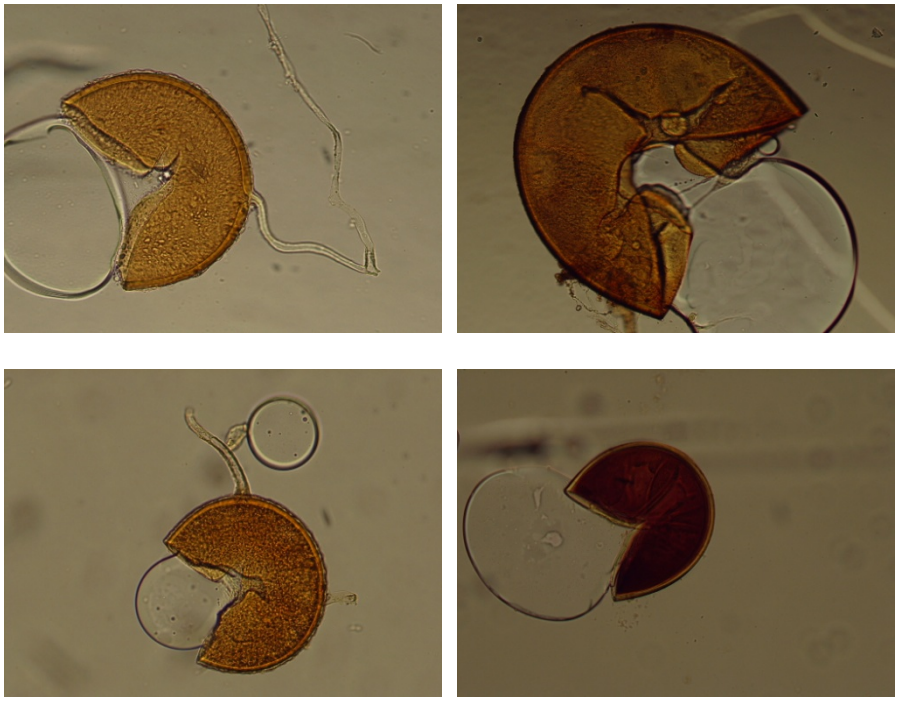


Plate 2: Pictorial representation of the four indigenous isolates spores as observed under compound microscope (Nikon 59533, Japan at 400X magnification) after reacting with melzer's reagent.

3.5 DISCUSSION

Results from this study indicate that media has a pronounced influence on AMF colonization of roots. A specific AMF species will only colonize significantly in a given media type. Both kinds of AMF studied during this experiment established effective colonization in conventional nursery medium as opposed to sterile sand. Both frequency and intensity of colonization were highest (58% and 63%, respectively) in conventional medium than they were (53% and 57%, respectively) in sterile sand medium for the two isolates tested. The highest frequency and intensity percentages in conventional medium were obtained from roots of plants inoculated with *Glomus etunicatum* whereas the highest values recorded in sterile sand medium were those of plants inoculated with indigenous glomus species. This shows that *Glomus etunicatum* has a specific preference ($P<0.05$) for conventional nursery medium where it managed to colonize 58 and 63% of the root samples whereas in sand, it could only colonize 16 and 17% frequency and intensity, respectively (Figs. 13&14). It is not clear why *G. etunicatum* established poor colonization of plant roots grown in sterile sand medium. Similar observations were made by Bouamri et al. (2006) who reported the inability of commercial isolates (specifically *G. mosseae*) to substantially colonize date palm grown in the sandy Ziz valley of Morocco. Specific inhibitory factor to *G. etunicatum*'s colonization of sand still remains unclear. On the contrary, *G. etunicatum* established 70% intensity of colonization of tissue culture banana roots in sterile sand medium (Mwashasha, 2005). The difference between colonization of plants established in conventional and sterile sand media by the indigenous glomus isolates in the current experiment was however, not significant ($P>0.05$). Frequency and intensity of colonization by indigenous inoculum stood at 53 and 58% in conventional medium

while in sterile sand medium, the same parameters stood at 53 and 57% respectively. These results indicate that colonization of plants inoculated with indigenous isolates does not depend on the type of medium for establishment under conditions in which the experiment was conducted. Indigenous inoculum seemed to be well adapted to the stressful conditions in sterile sand medium more than the commercial isolate (Jones et al., 2004). The success of indigenous glomus species in sterile sand medium may have been due to the fact that the species were isolated from a wide range of soil types including sandy-loam and alluvium soils. The four species making up the indigenous inoculum were common in all the ten banana farms sampled and were therefore, more adapted to survival under local edaphic conditions (Tsuchida and Nonaka, 2002; Bouamri et al., 2006). Colonization of medium around the root zone of inoculated plants followed a similar trend of internal root colonization. The highest number of spores was observed in conventional medium around roots of plants colonized by *Glomus etunicatum*. This observation is contrary to the expectation that sporulation would be much higher (1.5 times) in sand than in conventional nursery medium (Dexter, 1987). The loose structure of sterile sand medium ensures sufficient aeration, quicker water and nutrient percolation to the root region which facilitates faster root growth. However, the loose structure allows water and minerals to evaporate faster from the medium resulting in stress conditions which induces and accelerates sporulation by AMF. This hypothesis was confirmed by the trend of SA and FA curves after 12 weeks of inoculation whereby the mean number of spores in sterile sand (SA) was higher than that of conventional medium FA (Fig. 15) for the indigenous inoculum. The said mean (SA) however, declined after week 12 and remained permanently below that of FA throughout the experiment suggesting either a reduction in sporulation rate or loss of

spores. The root cause of the decline in number of spores was not quite clear but could be attributed to loss of spores via the drainage holes at the bottom of polythene sleeves during irrigation. Care was however taken to minimize run off during irrigation of sterile sand with both water and modified Hewitt nutrient solution. Possible loss of extra radical spores through irrigation water was also reported as a major cause for reduced spore counts in loose media (peat and sand) by Horn et al. (1994). Spore loss was so pronounced in Horn et al. (1994)'s experiment that at certain data points, the new count was less than the previous observation. The authors attributed the loss mainly to irrigation water and a lesser extent to entomological factors. High sporulation by AMF in sand medium was reported by Dexter (1987) and Jones et al. (2004) who related it to the looseness of the sand medium which, encouraged fresh air circulation and induced mild stress in the root rhizosphere; a condition which facilitates high spore production. Results of the current experiment, however, contradict those of Dexter and Jones perhaps due to the explained spore loss contributory factors; irrigation, pot's drainage holes and spore predators. Spore production in conventional medium was enhanced by inclusion of one-part sand during preparation of the 3:1 mixture of forest soil and sand. This may have negated the anticipated adverse effects of conventional medium (resulting from soil compaction) on AMF sporulation as was observed by Horn and Dexter (1989).

There were clear cut differences amongst all treatments as far as nutrient uptake was concerned. As was expected, inoculated plants established in conventional nursery medium accumulated higher ($P<0.05$) amounts of N, P and K than their counterparts in sterile sand medium. Conventional nursery medium has a higher nutrient retention capacity than sterile sand; therefore, it was expected that nutrient solution would be

retained longer around roots of plants grown in conventional medium which, may have ensured maximum uptake and accumulation of N, P and K in plant tissues. In conventional medium, plants inoculated with *Glomus etunicatum* (FE) had the highest mean amounts of N, P and K (mg/plant) which were all significantly different ($P<0.05$) from their corresponding means in sterile sand medium (Tables 3-5). Results show that *Glomus etunicatum* had a superior ability to explore nutrients in the conventional medium than indigenous glomus species. Indigenous glomus species was more superior to *Glomus etunicatum* in sterile sand medium with respect to uptake of N, P and K. The strong ability of indigenous isolates to access significant ($P<0.05$) amounts of nutrients in sterile sand medium than the commercial isolate may have been due to its high tolerance to the adverse conditions in sterile sand medium as was discussed earlier. Cornejo et al. (2006) reported increased uptake and accumulation of 14,950 mg/plant N and 3,237 mg/plant dry weight P in tissues of banana plants after 150 days (5 months) of inoculation with *Glomus etunicatum*. Cruz et al. (2003) reported enhanced accumulation of N, P and K in tissues of forest trees species established in nutrient deficient soils. Increased storage of N, P, K, Mg, Cu, and Zn in maize tissues was reported by Liu et al. (2000). Declerck et al. (1994) observed high shoot P and K content in banana plants inoculated with *G. mosseae* (a commercial isolate) compared to the non-inoculated plants. Declerck et al. (1994) however, reported significantly lower levels of N in inoculated plants; a result which is in contrast to those of the study in focus. Results of this study on shoot nutrient content further confirms those of Jaizme-Vega and Azcon (1995) who observed higher content of N, P, K in banana plantlets inoculated with other commercial isolates, specifically, *G. mosseae* and *G. fascicculatum*.

Analysis of the relationship between rhizosphere medium colonisation (spore count), root colonisation and nutrient uptake had a linear relationship (r=0.98), suggesting that the three parameters are interdependent. Spores build up in the rhizosphere had a direct impact on the extent of AMF colonisation of roots. An increase in number of spores in the medium around the root resulted to increase in AMF root colonisation intensity by a similar margin. The intensity of root colonisation has a direct influence on the uptake of nutrients from the media. The higher the extent of AMF colonisation (both inside roots and the surrounding medium), the higher the uptake of nutrients by the mycorrhizal roots will be. Similar observations were made by Sastry et al. (2000) who noted a strong relationship between uptake of nutrients (N and P) and the extent of colonisation of Eucalyptus hybrid roots by *Glomus intraradices*. Phosphorus and nitrogen are reported as the major macronutrients whose acquisition and translocation by mycorrhizal roots depend directly on the extent of colonisation (Smith and Read, 1997; Subramanian and Charest, 1999). External medium colonisation controls uptake of nutrients indirectly through modification of rhizosphere environment thus lowering pressure around the root hairs which, allows mineral solutions to access root hairs by mass flow (Harrison et al., 2002; Paszkowski et al., 2002).

The beneficial effect of AMF colonization on plant growth has been attributed to improved uptake of nutrients especially P (Smith et al., 1992). However in this study, N and K also seem to contribute to the improved growth status of the inoculated plants. The formation of an extensive network of mycorrhizal mycelium in soil increases the volume of soil accessible to plants and absorption by this mycelium effectively explore soil P (Kothari et al., 1991) and increases specific uptake rate of nutrients by colonized roots. The higher P content could be related to various mechanisms including enhanced

media exploration which reduces the distance of ion diffusion to the plant roots, faster movement of P into mycorrhizal roots and modification of root rhizosphere (Bolan, 1991).

This study demonstrated that conventional nursery medium is the ideal medium for inoculation by both AMF isolates. Whereas *G. etunicatum* showed a better performance than indigenous isolates in conventional medium, it showed a very poor colonisation in sterile sand medium (Figs. 13 and 14) which necessitates further investigations to ascertain the actual cause of its poor colonization. Performance of indigenous glomus species in both media carries the hope of commercializing the use of indigenous AMF isolates in tissue culture banana nurseries in Kenya. Indigenous isolates demonstrated the potential of using sterile sand as a medium of choice for commercial nursery AMF inoculation due to its enormous ability to enhance nutrient uptake in inoculated plants (Tables 3-5). It gives the hope of reaping the benefits of sterile sand medium which include disease free, no compaction, free root development and clean culture among many others.

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSION

4.1 Discussion

The growth promotion by the two inocula witnessed during this study corroborates several other studies (Rizzardi, 1990; Declerck et al., 1994; Reyes et al., 1995; Fogain and Njifenjou, 2002; Jaizme-Vega et al., 2002a; Elsen et al., 2003) which showed that bananas are excellent beneficiaries of AMF colonization by glomus genus. Commercial isolates are well developed and are expected to enhance growth more than indigenous cultures of AMF. Performance of *Glomus etunicatum* in conventional medium was significantly ($P<0.05$) higher than that of the indigenous glomus species for all the growth parameters considered. In sterile sand however, indigenous species enhanced plant growth far much better ($P<0.05$) than the commercial isolate. Increase in growth was found to have a strong positive correlation with frequency and intensity of AMF colonization of banana roots (Table 2). An increase in root colonization by one percentage unit resulted to subsequent increase in growth by 0.95 percent, reflecting a strong relationship between growth and AMF colonization. Performance of *Glomus etunicatum* in conventional medium was in agreement with many other studies (Flores-Aylas et al., 2003; Jaizme-Vega et al., 2003; Fuchs and Haselwandter, 2004; Cornejo et al., 2006; Janos, 2007) which illustrates the superiority of the isolate in enhancing growth and nutrient uptake of many terrestrial plant species. Flores-Aylas et al. (2003) reported a 40% increase in growth of tree species (*Senna macranthera*, *Guazuma ulmifolia*, *Senna multijuga*, *Solanum granuloso-leprosum*, *Schinus terebenthifolius* and

Trema micrantha) after 4 months of inoculation with *Glomus etunicatum* (GE). Several other reports (Yano-Melo et al., 1999; Millner et al., 2001; Jaizme-Vega et al., 2003; Fuchs and Haselwandter, 2004; Cornejo et al., 2006; Janos, 2007) support the ability of GE to enhance growth beyond 50% in crop plants.

Poor growth promotion by GE in sterile sand medium observed in the present research was not in agreement with results reported by Mwashasha (2005) on the same isolate, in which 70% growth promotion was reported. Indigenous isolates have been reported to be best performers in extreme stress conditions like those found in sand medium, dry grasslands and semi-aquatic environments. Tsuchida and Nonaka (2002) reported that glomus species isolated from native grassland promoted growth of orchard grass (*Dactylis glomerata*: grass O) established in four dry districts more than their commercial counterparts, namely, *Glomus clarum* and *Gigaspora margarita*. In their report, Tsuchida and Nonaka (2002) acknowledged that the commercial isolates performed better in a trial established during wet season. Miller (2000) reported that native mycorrhizal species tolerated effect of flooding more than commercial isolates and were able to substantially increase growth of two semi-aquatic grasses, *Panicum hemitomon* and *Leersia hexandra*, that grew along a wide hydrologic gradient in South Carolina, USA. A similar observation was made by Bouamri et al. (2006) who reported the inability of commercial isolates (specifically *G. mosseae*) to substantially colonize date palm grown in the sandy Ziz valley of Morocco. The three reports highlight the capacity of indigenous isolates to withstand harsh environmental conditions more effectively than their commercial partners. The edaphic conditions that prevailed in the sterile sand medium were just as stressful to both isolates as those reported by Bouamri et al. (2006).

Assessment of the two media types used during this experiment revealed conventional medium to be the most ideal medium for inoculation with the two AMF cultures. Information gathered from analysis of spores count represented the suitability of each medium for survival and development of AMF fungal infective structures. Colonization of the media around the root zone is presumed to have a strong relationship with internal root infection which confers enhanced nutrient uptake among other benefits (Rutto et al., 2002). In the present study, rhizosphere medium colonization, was positively related (r=0.98) to root infection and nutrient uptake. The external medium colonization is an indicator of the strength of AMF root infection which directly controls the uptake and accumulation of nutrients in plant tissues. Enhanced nutrient uptake is only realized when Phosphorus is limiting (less than 25ppm) in the medium (Bolan, 1991; Smith et al., 1992; Sastry, 2000). Intensive exploration of medium in search for P by AMF triggers maximum uptake of other minerals such as zinc, copper, magnesium and calcium (Berta et al., 1993). Mycorrhizal plants have been found to contain more nutrients per unit biomass than non-mycorrhizal ones (Stribley et al., 1980; Bolan, 1991), which can be interpreted as luxury accumulation (Smith and Gianinazzi-Pearson, 1988). The internal storage of nutrients (accumulation) is further increased by the volume of fungal hyphae (Bolan, 1991). These mechanisms could buffer the flow of nutrients from fluctuations in external nutrient concentration, which could be expected to act for the benefit of mycorrhizal symbiosis under variable concentration of external nutrients (Cui and Caldwell, 1996a, b).

4.2 Conclusions

The indigenous glomus isolates are more successful for inoculating tissue cultured banana plantlets in both media. While *Glomus etunicatum* achieved better results than indigenous isolates in conventional medium, it failed to colonize plants established in sterile sand medium any better than non-inoculated plants. Therefore, growth promotion as a result of inoculation using the commercial isolate in sterile sand medium was far much less than that of the indigenous inoculum. In all the growth and colonization parameters assessed in plants established in sterile sand, GE inoculated plants exhibited better growth than the non-inoculated plants (SO), although the difference was not statistically significant in many cases. The difference between inoculated plants and control (non-inoculated) in sterile sand medium was not significant in all parameters measured except for leaf area, which was significant. In most cases, plants inoculated with indigenous isolate were significantly larger ($P<0.05$) than their control counterparts in both media.

Conventional nursery medium was found to be a preferable medium for nursery AMF inoculation of tissue culture banana plantlets. Both AMF isolates achieved their best performance in conventional medium, with GE achieving better colonization, nutrient uptake and growth. Sterile sand proved to be a conducive medium for sporulation, root colonization and growth promotion for the indigenous isolate but not for the commercial isolate. There is some hope of using sterile sand as a medium for commercial nursery establishment of tissue cultured banana plantlets through inoculating the plants with indigenous isolates used in this study. This implies that developing and refining the use of indigenous AMF species should be the greatest priority of local research scientists in

the field of Agricultural biotechnology since this research offers credible information on the potential commercial use of the isolates. The information from this research will increase productivity of bananas through enhancing the survival and post-transplant performance of tissue cultured plantlets. This will uplift the position of bananas as important food security crop in Kenya and the rest of the World.

REFERENCES

Azcón, R., Go´mez, M., and Tobar, R. 1992. Effects of nitrogen source on growth, nutrition, photosynthetic rate and nitrogen metabolism of mycorrhizal and phosphorus-fertilized plants of *Lactuca sativa* L. *New Phytologist*. 121:227-234.

Azcón, R., Go´mez, M., and Tobar, R. 1996. Physiological and nutritional responses by *Lactuca sativa* L. to nitrogen sources and mycorrhizal fungi under drought conditions. *Biology and Fertility of Soils* 22:156-161.

Azcón-Aguilar, C., Cantos M., Troncoso, A., and Barea, J.M. 1997. Beneficial effect of arbuscular mycorrhizas on acclimatization of micropropagated cassava plantlets. *Scientia Horticulturae* 72:63-71.

Berta, G., Fusconi, A., and Trotta, A. 1993. VA mycorrhizal infection and the morphology and function of root systems. *Environmental and Experimental Botany* 33:159-173.

Berta, G., Trotta, A.F., Hooke, J., Munro, R., Atkinson, D., Giovanetti, M., Marini, S., Loreti, F., Tisserant, B., Gianinazzi-Pearson, V., and Gianinazzi, S. 1995. The effects of arbuscular mycorrhizal infection on plant growth, root system morphology and soluble protein content in *Prunus cerasifera* L. *Tree Physiology* 15:281-293.

Bolan, N.S. 1991. A critical review of the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil* 134:189-207.

References

Boshart, J. 2004. Study of the Arbuscular Mycorrhizal Fungi in Musa spp. Dissertationes de agricultura. Katholieke Universiteit Leuven 7:112-221.

Bouamri, R., Dalpe, Y., Serrhini, M.N., and Bennani, A. 2006. Arbuscular Mycorrhizal Fungi species associated with rhizosphere of *Phoenix dactylifera* L. in Morocco. *African Journal of Biotechnology* 5:510-516.

Brazanti, B., Gianinazzi-Pearson, V., and Gianinazzi, S. 1992. Influence of phosphate fertilization on the growth and nutrient status of micropropagated apple infected with endomycorrhizal fungi during the weaning stage. *Agronomie* 12:841-845.

Cornejo, P., Borie, F., Rubio, R., and Azcon, R. 2006. Influence of nitrogen source on the viability, functionality and persistence of *Glomus etunicatum* fungal propagules in an Andisol. *Applied Soil Ecology* 35: 423-431.

Cruz, C., Green, J.J., Watson, A.C., Wilson, F., and Martins-Loucao, M.A. 2003. Functional aspects of root architecture and mycorrhizal inoculation with respect to nutrient uptake capacity. *Mycorrhiza* 14:177-184.

Cuenca, G., and Azcon, R. 1994. Effects of ammonium and nitrate on the growth of vesicular–arbuscular mycorrhizal *Eiythrina poeppigiana* O.I. cook seedlings. *Biology and Fertility of Soils* 18, 249-254.

Cui, M., and Caldwell, M.M. 1996a. Facilitation of plant phosphate acquisition by arbuscular mycorrhizas from enriched soil patches. I. Roots and hyphae exploiting the same soil volume. *New Phytologist* 133:453-460.

Cui, M., and Caldwell, M.M. 1996b. Facilitation of plant phosphate acquisition by arbuscular mycorrhizas from enriched soil patches. II. Hyphae exploiting root-free soil. *New Phytologist* 133:461-467.

Declerck, S., Devos, B., Delvaux, B., and Plenchette, C. 1994. Growth response of micropropagated banana plants to VAM inoculation. *Fruits* 49: 103-109.

Declerck, S., Plenchette, C., and Strullu, D. 1995. Mycorrhizal dependency of banana cultivar (*Musa acuminata*, AAA group). *Plant and Soil* 176: 183-187.

Dexter, A.R. 1987. Compression of soil around roots. *Plant and Soil* 97:401-406.

Díaz-Zorita, M., Perfect, E., and Grove, J.H. 2002. Disruptive methods for assessing soil structure. *Soil and Tillage Research* 64: 3-22.

Douds, D.D.Jr., Galvez, L., Bécard, G., and Kapulnik, Y. 1998. Regulation of arbuscular mycorrhizal development by plant host and fungus species in alfafa. *New Phytologist* 138 : 27-35.

Duponnois, R., Plenchette, C., Thioulouse, J., and Cadet, P. 2001. The mycorrhizal soil infectivity and arbuscular mycorrhizal fungal spore communities in different aged fallows in Senegal. *Applied soil ecology* 17: 239-251.

Elsen, A., Declerck, S., and DeWaele, D. 2001. Effects of *Glomus intraradices* on the reproduction of the burrowing nematode (*Radopholus similis*) in dixenic culture. *Mycorrhiza* 11: 49-51.

References

Elsen, A. 2002. Study of the interaction between Arbuscular Mycorrhizal Fungi and plant parasitic nematodes in Musa spp. Dissertationes de agricultura. Katholieke Universiteit Leuven 12:109-119.

Elsen, A., Baimey, H., Swennen, R., and DeWaele, D. 2003. Relative mycorrhizal dependency and mycorrhiza-nematode interaction in banana cultivars (Musa spp.) differing in nematode susceptibility. *Plant and soil* 256: 303-313.

Food and Agricultural Organization. 2001. *In*: Lusty, C., and Smale, M. (eds) Assessing the social and economic impact of improved banana varieties in East Africa. Proceedings of an interdisciplinary research design workshop jointly organized by the international Network for the Improvement of banana and Plantain (INIBAP) and the International Food Policy Research Institute (IFPRI) at the Equatoria Hotel Kampala.

FAOSTAT. 2004. The FAOSTAT ProdSTAT module on crops contains detailed agricultural Production data [Online]. Availed by Food and Agricultural Organisation.
(http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567).

FAOSTAT. 2005. FAO Statistical Databases & Data-sets on production of major food security crops in ASIA and Sub-Saharan Africa. Primary accessed on 15th May, 2009. http://faostat.fao.org/faostat/form?collection=Production.Crops.

Fitter, A.H. 2000. Functioning of Vesicular Arbuscular Mycorrhiza under field conditions. *New Phytologist*, 99:257-265.

References

Flores-Aylas, W.W., Saggin-Júnior, O.J., Siqueira, J.O., and Davide, A.C. 2003. Effects of *Glomus etunicatum* and phosphorus on initial growth of woody species at direct seeding. *Pesquisa Agropecuária Brasileira*, Brasília, 38:257-266.

Fogain, R., and Njifenjou, S. 2002. Effect of a mycorrhizal Glomus sp. on growth of plantain and on the development of *Radopholus similis* under controlled conditions. *African Plant Protection* 8:1-4.

Fortuna, P., Citernesi, S., Morini, S., Giovannetti, M., and Loreti, F. 1992. Infectivity and effectiveness of different species of arbuscular mycorrhizal fungi in micropropagated plants of Mr S 2/5 plum rootstock. *Agronomie* 12:825-829.

Frison, E., and Putter, C. 1989. Technical Guidelines for the safe movement of Musa Germplasm. FAO Rome/International Board for plants Genetic Resources, Rome.

Fuchs, B., and Haselwandter, K. 2004. Red list plants: colonization by Arbuscular Mycorrhizal Fungi and dark septate endophytes. *Mycorrhiza* 14: 277-281.

Gemma, J., and Koske, R. 1989. A modified procedure for staining roots to detect VAMycorrhizas. *Mycological Research* 92: 486-505.

Guillemin, J.P., Gianinazzi, S., and Trouvelot, A. 1992. Screening of arbuscular endomycorrhizal fungi for establishment of micropropagated pineapple plants. *Agronomie* 12: 831-836.

References

Guillemin, J.P., Gianinazzi, S., Gianinazzi-Pearson, V., and Marchal, J. 1995. Influence des endomycorhizes à arbuscules sur la croissance et la nutrition minérale de vitroplants d'ananas dans un sol à forte salinité. *Fruits* 50: 333-341.

Granger, R.L., Plenchette, C., and Fortin, J.A. 1983. Effect of a vesicular arbuscular (VA) endomycorrhizal fungus (*Glomus epigaeum*) on the growth and leaf mineral content of two apple clones propagated *in vitro*. *Canadian Journal of Plant Science* 63:551-555.

Harrison, M.J., Dewbre, G.R., and Liu, J.Y. 2002. A phosphate transporter from Medicago truncatula involved in the acquisiton of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* 14:2413-2429.

Hewitt, E.J. 1966. Sand and water culture methods used in the study of plant nutrition. Commonwealth Agricultural Bureau, Farnham Royal, UK 4:187-237.

Horn, R., and Dexter, A.R. 1989. Dynamics of soil aggregation in desert loess. *Soil and Tillage Research* 13: 253-266.

Horn, R., Taubner, H., Wuttke, M., and Baumgartl, T. 1994. Soil physical properties related to soil structure. *Soil and Tillage Research* 30: 187-216.

References

International Institute for Tropical Agriculture. 1995. In: Lusty C, Smale M (eds) Assessing the social and economic impact of improved banana varieties in East Africa. Proceedings of an interdisciplinary research design workshop jointly organized by the international Network for the Improvement of banana and Plantain (INIBAP) and the International Food Policy Research Institute (IFPRI) at the Equatoria Hotel Kampala. 7 - 11 November 2002.

IITA. 1998. International Institute of Tropical Agriculture (IITA) annual report. IITA, IBADAN NIGERIA.

Ingleby, K. 2007. Mycorrhizal training manual. Assessment of mycorrhizal diversity in soils and roots, and nursery inoculation to improve the survival and growth of seedlings. Centre for Ecology and Hydrology (CEH), Bush estate, PENICUIK Midlothian, United Kingdom.

Ingleby, K., and Mason, P. 1973. Mycorrhizal training manual. Production of arbuscular mycorrhizal inoculum in the glasshouse and nursery. Institute of Terrestrial Ecology, UK.

International Network for the Improvement of Banana and Plantain. 1991. Regional Network for Eastern Africa. INIBAP, Montpellier, France.

INIBAP. 2001. Regional Network for Eastern Africa Annual report.2001. INIBAP, Montpellier, France.

Jackson, M. L. 1958. Soil chemical analysis. Prentice Hall, New Dehli, *In*: Kormanik, P.P., Schultz, R.C., and Bryan, W.C. 1982. The influence of vesicular-arbuscular mycorrhizae on growth and development of eight hardwood tree species. *Forest Science* 28:531-539.

References

Jaizme-Vega, M., and Azcón, R. 1991. Effect of vesicular arbuscular mycorrhizal fungi on pineapple (*Ananas comosus* (L) Merr.) in the Canary Island. *Fruits* 46: 110-118.

Jaizme-Vega, M., and Azcón, R. 1995. Response of some tropical and sub tropical cultures to endomycorrhizal fungi. *Mycorrhiza* 5: 213-217.

Jaizme-Vega, M., Penoury, T., Pinochet, J., and Jaumot, M. 1997. Interactions between the root-knot nematode Meloidogyne incognita and Glomus mosseae in banana. *Plant and Soil* 196: 27-35.

Jaizme-Vega, M., Tenoury, P., Pinochet, J., and Jaumot, M. 1998. Effects of arbuscular mycorrhizal fungi on severity of root rot of bananas. *Mycorrhiza* 6: 221-229.

Jaizme-Vega, M., Rodríguez-Romero, A., and Piñero-Guerra, M. 2002a. Effect of Arbuscular Mycorrhizal Fungi (AMF) and other rhizosphere microorganisms on development of the banana root system. *Mycological Research* 98: 96-107.

Jaizme-Vega, M., Esquivel, P., Delamo, M., Tenoury-Dominguez, P., and Romero, A.R. 2002b. Effects of mycorrhization on the development of two cultivars of micropropagated banana. *InfoMusa* 11: 25-28.

Jaizme-Vega, M.C., Rodriguez-Romero, A.S., Marin-Hermoso, C., and Declerck, S. 2003. Growth of micropropagated bananas colonized by root-organ culture produced arbuscular mycorrhizal fungi entrapped in Ca-alginate beads. *Plant and Soil* 254:329-335.

Janos, P.D. 2007. Plants responsiveness to mycorrhizas differs from dependence upon mycorrhizas. *Mycorrhiza* 17: 75-91.

Jones, D.L., Hodge, A., and Kuzyakov, Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist* 163: 459-480.

Kenya Agricultural Research Institute. 2004. Banana handout. National Horticultural Research Station, Thika.

Karugaba, A., and Kimaru, G. 1999. Banana Production in Uganda-An essential food and cash crop. Technical Handbook No. 18: 8-15.

Kjeldahl, A.K. 1994. Standard Operating Procedure for Total Kjeldahl Nitrogen (Lachat Method). Grace Analytical Lab 536 South Clark Street 10th Floor Chicago, IL 60605 April 15, 1994 Revision 2.

Kormanik, P.P., and McGraw, A.C. 1982. Quantification of Vesicular-Arbuscular Mycorrhizae in Plant Roots. In Methods and Principles of Mycorrhizae Research. Schenck, N.C. (ed). The American Phytopathological Society, pp. 36-37.

Kothari, S.K., Marschner, H., and Romheld, V. 1991. Contribution of the VA mycorrhizal hyphaes in acquisition of phosphorus and zinc by maize grown in a calcareous soil. *Plant and Soi*l 131:177-185.

Krik-Orian, A. 1990. Baseline tissue and cell cultures studies for use in Banana improvement schemes. pages 127-133, *In*: Fusarium wilt of banana. Ploetz, R.C. (ed). APS Press, American Phytopathological Society, St. Paul, Minnesota.

References

Lesueur, D., Ingleby, K., Odee, D., Chamberlain, J., Wilson, J., Manga, T., Sarrailh, J., and Pottinger, A. 2001. Improvement of forage production in Calliandra calothyrsus: methodology for the identification of an effective inoculum containing Rhizobium strains and arbuscular mycorrhizal isolates. *Journal of Biotechnology* 91: 269-282.

Liu, A., Hamel, C., Hamilton, R.I., and Smith, D.L. 2000. Mycorrhizae formation and nutrient uptake of new corn (*Zea mays* L.) hybrids with extreme canopy and leaf architecture as influenced by soil N and P levels. *Plant and soil* 221: 157-166.

Lusty, C., and Smale, M. 2002. Assessing the social and economic impact of improved banana varieties in East Africa. Proceedings of an interdisciplinary research design workshop jointly organized by the international Network for the Improvement of banana and Plantain (INIBAP) and the International Food Policy Research Institute (IFPRI) at the Equatoria Hotel Kampala. 7 - 11 November 2002.

Mathur, N., and Vyas, A. 1995. In vitro production of *Glomus deserticola* in association with *Ziziphus nummularia*. *Plant Cell Reproduction* 14:735–737.

Miller, R.M., and Jastrow, J.D. 2000. Mycorrhizal fungi influence soil structure. *In*: Kapulnik Y, Douds DD, eds. *Arbuscular mycorrhizas: molecular biology and physiology*. Dordrecht, the Netherlands: Kluwer Academic, 3-18.

Miller, S.P. 2000. Arbuscular Mycorrhizal Colonization of Semi-aquatic grasses along a wide hydrologic gradient. *New Phytologist* 145:145-155.

References

Millner, P.D., Mulbry, W.W., and Reynolds, S.L. 2001. Taxon-specific oligonucleotide primers for detection of *Glomus etunicatum*. *Mycorrhiza* 10:259-265.

Ministry of Agriculture (MoA). 1997. In: Banana handout. Kenya Agricultural Research Institute, Thika.

Monteiro, S.E.M., Matos, R.M.B., Paula, M.A., and Guerra, J.G.M. 1991. Micorrizas vesículo-arbusculares em bananeiras: aclimatação e transplante de mudas micropropagadas. *In*: Reunião Brasileira sobre micorriza, 4, Resumo Mendes: Embrapa-CNPBS/UFRRJ, p 163.

Muok, B., and Ishii, T. 2005. Effect of Arbuscular Mycorrhizal Fungi on Tree Growth and Nutrient Uptake of *Sclerocarya birrea* under Water Stress, Salt Stress and Flooding. *J. Japan. Society of Horticultural Science* 75: 26-31.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco culture. *Physiology of Plant* 15: 473-479.

Mwashasha, R. 2005. Evaluation of nine tissue cultured banana cultivars for arbuscular mycorrhizae dependency. MSc thesis, Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Nowak, J. 1998. Benefits of in vitro biotization of Plant tissue cultures with microbial inoculants. *In vitro Cell Development in Plants* 34: 122-130.

Paszkowski, U., Kroken, S., Roux, C., and Briggs, S.P. 2002. Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. Proc. Natl. Acad. Sci. USA 99: 13324-13329.

Pereira, J.O., Carneiro-Vieira, M.L., and Azevedo, J.L. 1999. Endophytic fungi from *Musa acuminata* and their reintroduction into axenic plants. *World Journal of Microbiology and Biotechnology* 15:37-40.

Plenchette, C. 2000. Receptiveness of some tropical soils from banana fields in Martinique to the arbuscular fungus *Glomus intraradices*. *Applied soil ecology* 15: 253-260.

Qaim, M. 1999. A Socioeconomic Outlook on Tissue Culture Technology in Kenyan Banana Production *Biotechnology and Development Monitor*, No. 40, p. 18-22.

Quilambo, O.A. 2003. The vesicular-arbuscular mycorrhizal symbiosis. *African Journal of Biotechnology* 2: 539-546.

Reyes, R., Parra, M., Garcia, L., Rodriguez, R., Martinez, L., and Gonzalez, M. 1995. The influence of mycorrhizae and phosphate solubilizing bacteria on the growth and development of banana in vitro plants. *InfoMusa* 4: 9-10.

Rillig, M. 2004. Arbuscular Mycorrhizae fungi and terrestrial ecosystem processes. *Ecology letters* 7: 740-754.

Rillig, M.C., Wright, S.F., and Eviner, V. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant and Soil* 238: 325-333.

Rizzardi, V. 1990. Effects of inoculation with vesicular-arbuscular-mycorrhizal fungi on the growth of micropropagated *Musa acuminata* clone 'Grand Naine'. *Revista di Agricoltura Subtropicale e Tropical* 84:473-484.

Robinson, J.C. 1996. Bananas and plantains. Crop production science in horticulture series. CAB International, UK. 238pp.

Robinson, J.C. 2000. Banana productivity-the impact of agronomic practices. *Acta Horticulturae*. (ISHS) 540:247-258. http://www.actahort.org/books/540/540_28.htm accessed on 10th May, 2010.

Rurangwa, E. 2010. The Influence of Arbuscular Mycorrhizal Fungi on Nursery Inoculated Tissue Cultured Banana and Initial Field Performance in Rwanda. MSc thesis, Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology.

Rutto, K.L., Mizutani, F., and Kadoya, K. 2002. Effect of rootzone flooding on mycorrhizal and non-mycorrhizal peach (*Prunus persica* Batsch) seedlings. *Scientia Horticulturae* 94: 285-295.

Sánchez-Díaz, M., and Honrubia, M. 1994. Water relations and alleviation of drought stress in mycorrhizal plants. Pages 167–178, *In*: S. Gianinazzi and H. Schüepp, eds. Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhäuser Verlag, Boston, MA.

Sastry, M.S.R., Sharma, A.K., and Johri, B.N. 2000. Effect of an AM consortium and pseudomonas on the growth and nutrient uptake of *Eucalyptus hybrid*. *Mycorrhiza* 10:55-61.

Schroeder, M.S., and Janos, D.P. 2005. Plant growth, phosphorus nutrition, and root morphological responses to arbuscular mycorrhizas, phosphorus fertilization and intraspecific density. *Mycorrhiza* 15: 203-216.

Six, J., Bossuyt, H., Degryze, S., and Denef, K. 2004. A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research* 79: 7-31.

Six, J., Feller, C., Denef, K., Ogle, S.M., de Moraes, J.C., and Albrecht, A. 2002. Soil organic matter, biota and aggregation in temperate and tropical soils-effects of no-tillage. *Agronomie* 22: 755-775.

Smith, F.A., and Smith, S.E. 1997. Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. *New Phytologist* 137:373-388.

Smith, S., and Read, D. 1997. Mycorrhizal symbiosis. Academic Press, Inc San Diego California.

Smith, S.E., Robson, A.D., and Abbott, L.K. 1992. The involvement of mycorrhizas in assessment of genetically dependent efficiency of nutrient uptake and use. *Plant and Soil* 146:169-179.

Statistical Analysis Software (SAS). 2003. Business Analytics Software and Services. Statistical Analysis Software Institute, USA.

Stribley, D.P., Tinker, P.B., and Rayner, J.H. 1980. Relation of internal phosphorus concentration and plant weight in plants infected by vesicular-arbuscular mycorrhiza. *New Phytologist* 86:261-266.

References

Strosse, H., Vandenhouwe, B., and Panis, K. 2004. Banana cell and tissue culture. Review paper. Laboratory of Tropical Crop Improvement, Catholic University, Belgium.

Subramanian, K.S., and Charest, C. 1999. Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought stressed and well-watered conditions. *Mycorrhiza* 9: 69-75.

Swennen, R., and Vulysteke, D. 2004. Bananas. pp. 530-552 in Raemaekers, R.H. (ed). Crop Production in Tropical Africa. Directorate General for International Co-operation (DGIC) Brussels, Belgium.

Tobar, R.M., Azcon, R., and Barea, J.M. 1994. The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscular mycorrhizae. *Mycorrhiza* 4:105-108.

Tsuchida, K., and Nonaka, M. 2002. Effect of the indigenous arbuscular mycorrhizal fungi on growth of the grass in grassland. *Japanese Journal of Soil Science and Plant Nutrition* 73:485-491.

Umesh, K., Krishnappa, K., and Bagyara, J.D. 1988. In: Rizzardi, V. Effect of inoculation with vesicular-arbuscular mycorhizal fungi on the growth of micropropagated *Musa acuminata* clone 'Grand Nain'. *Revista di Agricoltura Subtropicale e Tropical* 84: 473-484.

VanDyk, J. 2005. Banana Black Sigatoka Disease. *Plant Pathology* 67:97-103.

References

Vanlauwe, B., Sanginga, N., Jefwa, J., Kahangi, E., Rutto, L.K., Odee, D., Gichuki, C., Kibugu, E., Mubiru, D., Ssali, H., Van Asten, P., Okech, S., Gold, C., and Elsen, A. 2005. Exploration of Integrated Soil fertility management for banana production and marketing in Uganda and Kenya: Arbuscular Mycorrhizal Fungi help establishment and production of tissue culture banana – Final report phase I –January 2004 – February 2005.

Vigo, C., Norman, J. R., and Hooker, J. E. 2000. Biocontrol of the pathogen Phytophthora parasitica by arbuscular mycorrhizal fungi is a consequence of effects on infection loci. *Plant Pathology* 49:509-514.

Wambugu, F.M., and Kiome, R.M. 2001. The Benefits of Biotechnology for Small-Scale Banana Producers in Kenya. *ISAAA Briefs No.22*:1-34.

Wamocho, L., Michieka, R., Yamashita, K., and Ishii, T. 2000. Charcoal Application and VAM Inoculation Promote Vine Growth, Yield and Quality of Passion Fruits in Kenya. International Symposium on Temperate Fruit Growing in the Tropics and Subtropics. *Acta Horticulturae* 565:110

Yano-Melo, A., Saggin, O., Lima-Filho, J., and Melo, N. 1999. Effect of arbuscular mycorrhizal fungi on the acclimatization of micropropagated banana plantlets. *Mycorrhiza* 9: 119-123.

APPENDICES

Appendix 1: Recipe for Modified Hewitt nutrient solution

Stock solution					**Final solution**	
		g/l	g/1.5l	ml/l	ml/15l	ml/20l
Stock 1	KNO_3	40.44	60.66	10	150	200
Stock 2	$CaNO_3.4H_20$	47.23	70.85	20	300	400
Stock 3	$MgSO_4.7H_2O$	36.97	55.46	10	150	200
	a KH_2OPO_4	0.272	0.408			
	b KH_2OPO_4	0.027	0.041			
Stock 4	Na EDTA-Fe	4.21	6.315	10	150	200
Stock 5	$MnSO_4.4H_2O$	2.23	3.345	1	15	20
	H_3BO_3	3.09	4.635			
	$ZnSO_4.2H_2O$	0.268	0.432			
	$CuSO_4.5H_2O$	0.250	0.375			

Source: Hewitt, 1966.

Appendix 2: Kormanik and McGraw (1982) methodology for estimating AMF colonization through visual observation under magnification of 400X of a compound microscope (Nikon 59533, Japan).

Categories	Estimated percent colonisation
1	0-5
2	6-26
3	26-50
4	51-75
5	76-100

Key;

1- Assigned to roots with small colonisation sites widely scattered along the root;

2- Roots with larger colonisation sites more uniformly distributed through the colonised roots, but rarely coalescing;

3- Roots which were almost solidly colonised but about half of the root sample was not colonised;

4- Total length of root sample lacking mycorrhizae structures was greater than 25% of the total root length; and

5- Total length of root sample lacking mycorrhizae structures was less than 25% of the total root length.

Source: Kormanik and McGraw, 1982.

Appendix3: The average weather data during the study period (March-August, 2007).

	Feb	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**
Temperature								
Max ^{o}C	25	26	25	24	21	21	24	24
Min ^{o}C	12	13	14	14	13	12	15	15
Humidity %	61	77	81	75	70	71	76	64