ENHANCING COWPEA PRODUCTION THROUGH ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION AND WIDE INTERSPECIFIC CROSSES

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DECLARATION

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

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This work is dedicated to my late father, Erastus Oruru, who believed in me and sacrificed all he had to see my excellence and success all through my academic life. His memories will forever be cherished.

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ACRONYMS AND ABBREVIATIONS

AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
CAbMV	Cowpea Aphid-borne Mosaic Virus
CEC	Cation Exchange Capacity
CRD	Completely Randomized Design
CYMV	Cowpea Yellow Mosaic Virus
FAO	Food and Agriculture Organization
HSD	Honest Significance Difference
ICIPE	International Center for Insect Physiology and Ecology
pH	Potential Hydrogen
SPSS	Statistical Package for the Social Sciences
SSA	Sub-Saharan Africa

ABSTRACT

Cowpea is a multipurpose legume crop that serves as human food, livestock fodder, and income source and is widely produced in sub-Saharan Africa. Soil fertility and attack by insect pests and diseases are significant limitations to its production. Although farm inputs such as phosphate-rich fertilizers and pesticides can solve the mentioned constraints, they are costly for resource-poor farmers. This study aimed at determining the effect of arbuscular mycorrhizal fungi inoculation on AMF root colonization and growth of cowpea. Additionally, it aimed at developing an insect-pest resistant hybrid by crossing the cultivated cowpea (*Vigna unguiculata*) with a wild (*Vigna vexillata*) accession. Pot experiments were set up in a completely randomized design using a wild cowpea species (Ni935) and three cultivated cultivars (Katumani 80, Kunde Mboga and KenKunde 1). There were two treatments; mycorrhizal treatment using a commercial inoculum comprising of four mycorrhizal species, *Rhizophagus irregularis, Funneliformis mosseae, Glomus aggregatum* and *Glomus etunicatum* and non-mycorrhizal treatment (control). The pots were maintained in a greenhouse for 30 days prior to harvesting. Data on percentage root mycorrhizal colonization, root and shoot dry weights, nodule number and nodule dry weight were recorded. The dried shoots were also analyzed for N, P and K content. Hybridization experiment was done by crossing the cultivated cowpea cultivar (sp 219) with five different wild accessions (Ni935, Ni936, 263, V268 and AC305) to form immature hybrid pods. Data on pod retention frequencies was recorded. All the data collected from the greenhouse experiment was tested for homogeneity of variance then analyzed by two-way ANOVA and Pearson correlation. Results showed a statistically significant effect of genotype and AMF inoculation on percentage root AMF colonization ($p<0.001$) as well as on other growth parameters. The cultivated cultivars were more susceptible to AMF colonization and had higher root and shoot dry matter content and nodulation compared to the wild species. There was a positive correlation between AMF colonization and the levels of shoot P and N. Shoot P and N nutrition was also higher in the cultivated cultivars than the wild species. Hybridization experiment revealed differences in pod retention among different accessions of *Vigna vexillata*. However, an insect-pest resistant hybrid was not generated, possibly due to post-zygotic barriers related to interspecific crossing. This study has demonstrated the importance of AMF inoculation in improving cowpea's performance. Moreover, it has shown that the cultivated cultivars are still more responsive to mycorrhizal inoculation than the wild species. This is contrary to previous studies that have shown that modern breeding programs may result to suppression of mycorrhizal colonization. Therefore, there is the need to screen different cowpea cultivars and other crops for mycorrhizal symbiosis.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Cowpea (*Vigna unguiculata* (L).Walp) is a tropical grain legume widely grown in sub-Saharan Africa (SSA), Asia, parts of the United States and Southern Europe (Singh *et al*., 1997). Most cowpea does well in Africa, with Niger and Nigeria accounting for 66% of the world cowpea production. Between 2010 and 2014, Nigeria produced an average of 3.5 tonnes of cowpea followed by Niger with approximately 1.6 tonnes (faostart.org; updated in Aug, 2015). Other major producers in the Sahel region include Senegal, Ghana, Mali, Burkina Faso, Tanzania and Cameroon.

Saidi and Itulya (2010) have noted that it is one of the highly appreciated species of African leafy vegetable. It is of great importance to livelihoods of millions of people in West and Central Africa's semi-arid regions. In addition to being the most important grain legume crop in sub-Saharan Africa, cowpea is mainly grown by smallholders in the hot, drought-prone savannas and very arid Sahelian agro-ecological zones where it is often intercropped with sorghum and pearl millet (Dugje *et al*., 2009). In Kenya, cowpea does well in warmer climatic regions such as the Coast and Eastern provinces. The legume is at times grown under intercropping systems with cassava and/or maize.

Cowpea being a protein-rich grain is a suitable complement to starchy tuber crops and staple cereals. It serves as livestock fodder, improves the soil via nitrogen fixation, and benefits households by bringing in cash and diversifying income sources. Vital household income is

also generated through the sale of cowpea leaves and stems for animal feed during the dry season. Despite the great significance that cowpea has in regions such as the sub-Saharan Africa, the yields are significantly low. Plant growth is affected by low phosphorous availability in many soils as a result of P fixation by Fe, Ca and Al, which leads to formation of inorganic phosphates that are insoluble in soil (Ibijbijen *et al*., 1996).

Additionally, cowpea growth and productivity are lowered by prevalence of various diseases and insect attacks. There is at least one major insect pest at every stage of cowpea's life cycle, which could cause serious damage and negatively impact the yield. The legume is also prone to a number of viral diseases such as cowpea yellow mosaic virus (CYMV), cowpea aphid borne mosaic virus (CAbMV), as well as storage pests such as bruchids (*Collosobruchus maculatus)* (Gomathinayagam *et al*., 1998). Several plants possess different and specific inducible defense mechanisms for protection against insect attacks, allowing them to acquire nutrients more efficiently under stress conditions. Despite having such mechanisms, the major cowpea insect pests such as the legume pod borer, are still uncontrolled.

Most farmers rely on costly commercial farm inputs such as insecticides and phosphate-rich fertilizers to address the mentioned constraints. There is the need to come up with alternatives for minimizing over-reliance on such commercial farm inputs. Host-plant resistance is the most economical and environmentally friendly way of controlling insect pests (Sharma and Ortiz, 2002). Introducing insect pest resistance genes into cowpea should result in the availability of varieties that can be grown by farmers in sub-Saharan Africa with

minimal use of chemical farm inputs. This will also reduce cowpea production costs, hence increasing the profit margin for farmers. Because of the potentially immense benefits of growing insect-resistance cowpea varieties, efforts should be directed towards the search for and transfer of the desired genes from wild cowpea species to the cultivated cowpea. Although several attempts have been made in the past to cross *Vigna vexillata* and *V. unguiculata,* no viable hybrid has been obtained, suggesting a strong cross incompatibility between the two species (Fatokun, 2002). Hence, further studies should be conducted on effective ways of overcoming this interspecies incompatibility in order to get a viable hybrid with the desired genes.

According to Cardoso and Kuyper (2006), biotic processes like symbioses have the potential of improving agricultural sustainability with less dependence on non-renewable inputs such as artificial fertilizers. Such symbiotic processes include arbuscular mycorrhizal fungi (AMF), a group of beneficial soil microbiota that forms symbiotic associations with roots of higher plants enhancing plant nutritional uptake and resistance to biotic and abiotic stresses (Smith and Read, 1997). Although AMF are considered essential primarily for phosphorous uptake, they can also increase plant acquisition of other minerals such as Zn, N, Cu and Fe (Cavagnaro, 2008). Therefore, these symbiotic associations are increasingly gaining popularity as a significant factor of sustainable agro-ecosystems. AMF are especially important for plant nutrition in organic and low input farming systems because these systems do not utilize synthetic fertilizers and fungicides, which can tremendously reduce levels of root colonization by AMF (Cavagnaro *et al*., 2011).

The nature of interaction between AMF and their hosts vary depending on the host plant, as well as the cultivar type (Estaún and Calvet, 2010). Studies conducted on cereals have confirmed that different cereal cultivars respond differently to AMF colonization (Castellanos-Morales *et al*., 2011). The age of the cultivar may also determine the extent of fungal colonization and dependency. This is according to a study carried out by (Hetrick *et al*., 2003), where results indicated that old wheat cultivars had a higher degree of root colonization and were more responsive to AMF colonization compared to modern cultivars. To our knowledge, no studies have been done to investigate how different cowpea cultivars respond to AMF inoculation.

1.2 Problem Statement and Justification

Cowpea is a legume that offers the potential for food security to the poor as it can be grown in dry areas where most staples, particularly cereals and root tubers, do not grow effectively. In comparison to cereals, cowpea is a cheap source of proteins and amino acids (Elhardallou *et al*., 2015). The legume's production among smallholder farmers in SSA is affected by insect pest attacks and deficiency of soils in nutrients, especially nitrogen and phosphorous. Although several commercial insecticides and fertilizers are available for control of pests and provision of essential nutrients; they are costly for resource-poor farmers. Excessive use of pesticides currently may also be toxic. All pesticides must be toxic in order to be effective against the pest they are intended to control. Hence, they are potentially hazardous to humans, animals, other organisms and the environment.

It is therefore necessary to explore other alternatives such as AMF, which are bio-enhancers that have the potential of solving the issues of soil fertility and cost concerns. One way of circumventing the issue of costly commercial inputs is the use of microbial inoculants such as arbuscular mycorrhizal fungi that are beneficial plant symbionts (Mohammadi *et al*., 2011). Effective arbuscular mycorrhizal fungal strains can be used as bio-enhancers in sustainable plant production systems (Parniske, 2008). Their use is also cost effective, environmental-friendly and more sustainable as it leads to generation of healthy crops.

An alternative to pesticides is the use of pest-resistance cultivars that are less toxic. Currently, the primary gene pool lacks durable and adequate levels of resistance to post-flowering pests (Gomathinayagam *et al*., 1998). *Vigna vexillata*, a wild cowpea species distinguished by pubescence in stems, pods and leaves, suffers less damage by insect pests. Since reliable sources of resistance have not yet been unearthed in cowpea, it would be desirable to transfer insect resistance traits from *Vigna vexillata* to the cultivated cowpea to control insect pests and consequently increase productivity.

Attempts to hybridize cowpea with its wild relative have always failed due to post-zygotic barriers. Some of the techniques thought to have the potential to overcome these barriers include use of mixed pollen, embryo rescue, application of hormones, among others. Unfortunately, most of these attempts have been unsuccessful (Fatokun, 2002). This study will entail wide crosses between *Vigna unguiculata* and *Vigna vexillata* followed by an attempt to rescue immature embryos through *in vitro* embryo culture.

1.3 Research Hypotheses

i) Arbuscular mycorrhizal fungi inoculation has the ability to improve AMF root colonization and productivity of the wild cowpea species and the cultivated cowpea cultivars.

ii) The susceptibility to AMF colonization differs between the wild species and the cultivated cowpea cultivars.

iii) Arbuscular mycorrhizal fungi inoculation improves shoot nutrition of the major elements (N, P and K) in the wild cowpea species and the cultivated cowpea cultivars.

iv) Hybridization between *Vigna unguiculata* and *Vigna vexillata* through *in vitro* embryo culture is an effective way of conferring resistance against insect pests.

1.4. Objectives of the Study

1.4.1 General Objective

The overall aim of this study is to determine the effect of arbuscular mycorrhizal inoculation and interspecific hybridization in enhancing cowpea productivity and resistance to insect pests.

1.4.2 Specific Objectives

i) To determine the effect of AMF root colonization in both the wild species and cultivated cowpea cultivars and compare their effect on different growth parameters.

ii) To compare the susceptibility of the wild cowpea species, *Vigna vexillata* versus a three cultivated cultivars to AMF colonization.

iii) To determine the effect of mycorrhizal inoculation on shoot nutrition of the major elements (N, P and K) in both the wild cowpea species and the cultivated cultivars.

iv) To compare the pod retention frequencies of different wild cowpea accessions and develop an insect pest-resistant hybrid between *Vigna vexillata* and *Vigna unguiculata* through *In-vitro* embryo culture.

1.5 Significance of the Study and Anticipated Output

This study entails inoculation of a wild cowpea species and three cultivated cowpea cultivars with a commercial arbuscular mycorrhizal fungal inoculum. Incorporating AMF in agricultural systems has a potential to promote sustainable agriculture since it will improve crop production without deteriorating natural resources and affecting agricultural quality. The inocula will also improve crop growth with less use of farm inputs like high-cost chemical fertilizers, which will lead to a reduction in the cost of cowpea production. Essentially, cowpea production will become more attractive to the generally resource-poor farmers in African savanna zones. AMF play a key role in agricultural ecosystems by enhancing nutrient uptake (mainly P and N) and improving soil aggregate stabilization, representing a resource for sustainable soil management. The use of AMF as bio-fertilizers is not only cost-effective, but it will also minimize environmental pollution. Plant quality and safety will be enhanced. This work is also significant because it aims towards incorporating pest- resistance genes into cowpea, which would result in the generation of varieties that farmers in SSA can cultivate with minimal chemical use.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cowpea Biology and Ecology

Cowpea (*Vigna unguiculata*) is a self-pollinating species that belongs to the Fabaceae family and has a diploid genome (2n=2x=22) with a size of 1C=620 Mbp (Chen *et al*., 2007). Grown in the semi-arid regions of Africa, Asia, United States, Europe, Central and South America, cowpea serves as human food and livestock fodder (Singh and Ajeigbe, 2003). According to FAO, approximately 6.2 million tons of cowpea was produced across the globe on about 11.3 million hectares in 2013 (faostart.org; updated in Aug, 2015). Sub-Saharan Africa accounts for 70 % of the total global production (FAO, 2013). Cowpea contains numerous vitamins and minerals and is capable of withstanding drought and growing well in a wide range of soils. Subsequently, since it is a legume, it can replenish low fertility soils through biological nitrogen fixation. Small-scale farmers in developing countries cultivate cowpea with other crops because it tolerates shade. Additionally, it grows and covers the ground quickly, thus preventing soil erosion (Dugje *et al*., 2009).

2.2 Cowpea Distribution and Importance in Sub-Saharan Africa

Cowpea is mainly distributed in middle/low altitude and dry regions of central and western zones of SSA. Nigeria and Niger are the leading cowpea producers in Africa, with 70% of the total continental production. Some of the notable cowpea producers in Eastern Africa include Tanzania, Kenya, Mozambique, Uganda, and Malawi (Faostat. fao.org).

Cowpea is evidently a crop with numerous uses. Since the earliest practice of agriculture, it has been consumed by humans and ascribed nutritional and medicinal roles. Cowpea is not only drought-tolerant forage, but also an edible pulse. It can be utilized as cover crop, green manure, erosion control as well as in nitrogen fixation. The young pods and young leaves are edible vegetables that can also be used as fodder (Timko and Singh, 2008). Seeds can be consumed green or dried. In more than 18 African countries, the tender leaves of young cowpea are harvested and consumed as leafy vegetables (Okonya and Maass, 2014). The leaves are also sold in dry and fresh forms in numerous African markets.

It has been noted that cowpea, particularly in sub-Saharan Africa, is mainly grown by smallholder farmers who play a significant role in contributing to global food production. Both rural and urban food consumers in developing nations heavily rely on the efficiency of local smallholder farmers to meet their subsistence needs. These famers are therefore gross domestic food and nutrient providers and play a crucial role in the world's effort to improve nutritional and food security (Dioula *et al*., 2013). It is thus necessary for efforts to be geared towards increasing the productivity of food crops grown by smallholders and cowpea is a good example of such a crop.

2.3 Some Cowpea Varieties Grown in Kenya

2.3.1 Katumani 80

This is a dual-purpose cowpea variety that is suitable for both leaf and grain production. One of the key characteristic of this variety is a semi-spreading habit. It also has elongated leaves with unique silvery midrib. The flowers are purple blue while the corollas have an ivory

white pigment. Immature seeds are green and at maturity they turn white brown with interspersed faint red brown spots. This variety is resistant to aphids and moderately tolerant to pod borers, thrips and leafhopper. Additionally, it is moderately tolerant to mosaic virus and fungal diseases. Potential yields range from 320-720 kg/acre or 800-1800 kg/ha (Karanja *et al*., 2008).

2.3.2 KenKunde 1 (KK1)

This dual-purpose cowpea variety takes 75-90 days to mature. One of the special attributes associated with this variety is drought tolerance. It also does well in a wide range of soils (Karanja *et al*., 2008).

2.3.3 Kunde Mboga

This variety, which ideally does well in warm climates, takes 30-45 days to mature. It is a local vegetable with soft dark green leaves. On investment, it has a very high return. Its harvesting period is very long. A major advantage or special attribute of this variety is the fact that it aids in restoring soil fertility (Karanja *et al*., 2008).

2.3.4 Machakos 66

This is a bushy semi-spreading variety grown for both grain and leaves. Its leaves and midribs are dark green while the flowers are purple with a white corolla. When young, the pods are green and then turn bright red during grain filling and brown purple when dry. Flowering takes 55-60 days while maturing takes 80-90 days (Karanja *et al*., 2008).

2.3.5 KVU 27-1

This is a dual-purpose variety suitable for grain and leaf production with a semi-spreading habit and indeterminate flowering pattern. It has pointed leaves and purple blue flowers while its grains are dark red in color. Potential yields of this variety range from 320-720 kg/acre. It has a moderate tolerant to thrips and aphids, leaf hoppers, and pod borers. It is also moderately resistant to mosaic virus and foliar fungal disease (Karanja *et al*., 2008).

2.4 Overview of *Vigna vexillata*

This is a wild cowpea species that is a twining vine, widely distributed in Africa, Australia and Asia. It produces large, thickened tubers that are edible. *Vigna vexillata* also serves as green manure, pasture cover crop and erosion control plant (Leu *et al*., 2012). There is a close relationship between *Vigna vexillata* and the cultivated cowpea (*Vigna unguiculata)* in that; both species have similar flowers and pods. The major differences are in keel and stipule shapes. In *Vigna vexillata*, the keel shape leads to nortotribic pollination while in *Vigna unguiculata*, it leads to sternotribic pollination (Damayanti *et al*., 2010).

One of the agronomic characters of interest in *Vigna vexillata* is that its seeds are resistant to cowpea weevil, one of the major cowpea pests (Damayanti *et al*., 2010). Therefore, even though successful crossing of both species has not yet been done, attempts should be made to transfer these resistance genes to cowpea by hybridization or genetic transformation.

2.5 Challenges Facing Cowpea Production

The total annual cowpea grain production in the world is valued at approximately 5.7 Million tonnes (faostat.org: updated on 7th Feb 2014). Therefore, raising the average yield per hectare will increase annual global production and hence the revenue. Low soil fertility is one of the challenges facing cowpea production, especially in sub-Saharan Africa. Most smallholder farming systems are faced by the constraint of soil fertility management. Soils have minimal concentrations of nutrients such as phosphorous and nitrogen. Small-scale farmers have for several decades removed large quantities of nutrients from their soils without using enough quantities of fertilizers to replenish the soil. This has led to an average annual depletion rate of 22 kg of Nitrogen, 2.5 kg of phosphorous and 15 kg of potassium per hectare of cultivated land in 37 African countries over the last 30 years (Koohafkan and Altieri, 2010). The traditional method of overcoming nutrient depletion is the use of chemical fertilizers, which are unfortunately too costly for the majority of smallholder farmers.

There are several insect pests that have been known to attack cowpea (Gungula and Garjila, 2005). Some of the flowering and post-flowering insect pests include flower thrips, blister beetles, maruca pod borer, and pod-sucking bugs (Oyewale and Bamaiyi, 2013). These pests severely attack the crop at every developmental stage, which makes it imperative to use the tolerant varieties and insecticide sprays. When cowpea fields are infested by post-flowering insect pests causing damage to gain yield, farmers, particularly those in arid and semi-arid regions, opt to harvest the fodder in order to generate some income. It is however, clear that farmers get more financial benefit from cowpea grain than from fodder. If grain yield is to be obtained, farmers are obliged to spray cowpea field with insecticides two to three times. In

the recent years, progress has been made through genetic improvement whereby disease and pest resistance genes have been incorporated into new cowpea varies (Dzemo *et al*., 2010). Some traditional varieties have further been improved by introducing into them simple inherited resistance genes. Such progress has enabled farmers to obtain high grain yield using fewer insecticide sprays. Unfortunately, no cowpea line has been found to posses adequate levels of resistance to the pod sucking bugs (*Clavigralla tomentosicollis, Riptortus dentipes* and *Anoplocnemis curvipes*), all of which are post-flowering pests (Oyewale and Bamaiyi, 2013).

There has been an increasing interest in searching for eco-friendly and sustainable agricultural practices that could help in overcoming the mentioned challenges (Malusá *et al*., 2012). Such practices include the use of biofertilizers and biopesticides containing microbes to improve plant growth. In addition to enhancing plant growth, they also help in sustaining the environment and increasing crop productivity. Smallholder farmers could adopt such practices to serve as a cheap and efficient alternative to the costly chemical farm inputs. There are different types of efficient microbes in the rhizospheric soils, such as AMF that have beneficial effects on plant productivity.

2.6 An Overview of Arbuscular Mycorrhizal Fungi

AMF are beneficial soil biota that belongs to the phylum Glomeromycota and that form symbiotic associations with majority of wild and cultivated plant species. They are microscopic filamentous fungi that colonize the roots and their rhizosphere simultaneously, and spread out in form of ramified filaments. Arbuscular mycorrhiza have been in existence

for more than 400 million years (Bainard *et al*., 2011) and are among the most widespread terrestrial symbioses, formed by 70-90% of land plant species (Barrow *et al*., 2008). The general life cycle of AMF begins with spore germination and hyphal growth in response to signaling compounds released by a plant root. The fungus grows into the roots to form hyphae as well as tree-shaped sub-cellular structures known as arbuscules, which serve as the main site of nutrient exchange between the plant and fungal symbiotic partners (Smith *et al*., 2011). Similarly, the fungus grows out into the soil to form a branched mycelium that plays the role of exploring the soil to take up nutrients. This external mycelium forms spores thereby completing the life cycle.

AMF are biotrophic endophytes that depend on the host plant for the supply of photosynthetic carbohydrates. In return, they not only stimulate plant growth, but also improve the physical quality of their soil environment and protect them against soil-borne pathogens. These fungi form extensive hyphal networks in the top soil layer onto which most plants of an ecosystem are connected (Avio *et al*., 2006).

2.7 AMF and Cowpea Production

Farming systems, especially smallholder farming systems that are the main cowpea producers are identified by low input cropping systems where the natural activities of microbes contributed to improved nutrient supply and biocontrol of plant pathogens, thus maintaining crop health and production. Symbiotic fungi such as AMF form a primary component of microbial populations that form symbiotic associations with higher plants, thus influencing the plants' growth and productivity (Johansson *et al*., 2004). AMF are

multifunctional in nature and may play a fundamental role in dissolution, weathering and cycling of mineral nutrients (Wallander, 2006), carbon cycling, nutrient mobilization from organic substrates and mediation of plant responses to different environmental stresses such as soil salinity, heavy metal toxicity, drought, heat stress, plant pathogens and soil acidification (Azcón and Barea, 2010).

In mycorrhizal symbiosis, the fungus complements the role of plant's root hair and acts as an extension of the root system (Muchovej, 2004). Mycorrhizal colonization increases the absorption surface area, exposes greater soil area and increases the life-span of absorbing roots. In this way, soluble nutrients are better utilized and retained because of reduced reaction with soil colloids or leaching losses (Selveraj and Chellappan, 2006).

AMF have also been found to increase nodulation and atmospheric nitrogen fixation potential in legumes such as cowpea (Turk *et al*., 2008). This is because AMF improves phosphorous uptake by the plant, which in turn would avail more energy for nitrogen fixation by rhizobia. Mycorrhizal colonized roots are highly unlikely to be colonized by other microbes, and their susceptibility to soil-borne pathogens such as phytopathogenic fungi or nematodes is lowered (Selveraj and Chellappan, 2006).

Mycorrhizal fungi have been shown to aid in detoxification of an environment with heavy metal concentration, thus, allowing plant growth (Muchovej, 2004). The real value of these symbionts is that they form a link between plants and heterogeneously distributed nutrients needed for growth. Hence, they allow the flow of energy-rich compounds needed for nutrient

mobilization while at the same time providing a means through which the mobilized products are transported back to their hosts (Azcón-Aguilar and Barea, 2015).

Differences in the way plants respond to AMF can be observed not only among plant species, but also cultivar types. For example, a study carried out by Tarawaya (2003), showed that improved, high yielding wheat cultivars responded less to AMF compared to landrace cultivars. Similar findings were obtained by (Zhu *et al*., 2003) in barley, where an improved barley cultivar was less responsive compared to a landrace barley. Steinkellner *et al*. (2012), conducted a study to compare the interaction of wild-type, old and modern tomato cultivars with arbuscular mycorrhizal fungus, *Glomus mosseae.* According to their results, old tomato cultivars showed both the lowest and highest levels of AMF colonization. Modern cultivars showed high levels of arbuscular mycorrhizal root colonization. It is clear that sometimes the age of a cultivar or even crop improvement can reduce the response of crops to AM fungi. Since very few studies have been done on the response of cowpea to AMF, there is a possibility that different cultivars differing in their genetic makeup or improvement respond differently to AMF inoculation.

2.8 Crop Genotype and AMF Symbiosis

Several forms of mycorrhizal associations have been found in 336 plant families, representing 99% of flowering plants with more than 10,000 plants having literature records of mycorrhizal associations (Brundrett, 2009). The AMF symbiosis is the most widespread plant symbiosis, with different combinations of host plant and AMF resulting in differential effects on gene expression, cellular functions, root morphology and nutrient status

(Fedderman *et al*., 2010). There is genetic variability in the AMF colonization capacity of various genotypes of host species (Manjarrez *et al*., 2009). The knowledge of physiology and genetics of colonization process will be fundamental in designing screening procedures and molecular markers to breed genotypes for more efficient AMF symbiosis. Although AMF are less host-specific than rhizobia, their colonization rate and other host benefits are genotype-dependent (Marschner and Rengel, 2010). For instance, studies done to investigate the effect of AMF inoculation on different wheat cultivars showed that mycorrhizal responsiveness in terms of P uptake was lower in modern wheat genotypes in comparison to older cultivars (Zhu *et al*., 2001).

A study of root colonization of 255 maize genotypes, including inbred lines, hybrids and landraces originating from different locations then grown in a field for two consecutive years was carried out by (An *et al*., 2010). Although the results indicated that all the genotypes expressed colonization, there were genetic variations with respect to AMF colonization. When assessing mycorrhizal symbiosis variation in both cereal and legume crops, some of the plants evaluated are the older ones domesticated for food by humans. These plants are often selected in open fields where mycorrhizal are present, and it is therefore expected that mycorrhization trait has not been lost. Some experimental data have in fact shown that the modern cultivars are still optimizing this symbiosis to increase P uptake efficiency ability, among other desirable aspects (Hildermann *et al*., 2010). The knowledge of existing genetic differences with respect to various cultivars response to AMF symbiosis is a powerful breeding resource that can result in the selection of desirable traits for the optimization of this symbiosis for agricultural use.

2.9 Tripartite Symbiosis by AMF, Rhizobia and Legume Plants

Nitrogen and phosphorous are the primary nutrients needed for plant growth. Although both nutrients can be supplied by manure or fertilizers, leguminous plants are capable of fixing N from the atmosphere via symbiosis with host-specific gram-negative bacteria known as rhizobia. Two of the most important plant symbionts that affect the plants' capacity to acquire nutrients are AMF and rhizobia. AMF benefits the host through mobilization of phosphorus from non-labile sources while rhizobia fix nitrogen (van der Heijden *et al*., 2006). As much as 97 % of the total plant N can be accounted for by symbiotic dinitrogen (N_2) fixation. Such a contribution minimizes the necessity for mineral N fertilizer and, even if the grain legume's yield is not enhanced, any increase in N_2 may result in greater protein content of the seed. Symbiotic nitrogen fixation is therefore seen as an environmentally friendly way of supplying nitrogen to plant (Azcón-Aguilar and Barea, 2015).

Legumes can host both AMF and nitrogen-fixing bacteria at the same time. However, the two symbioses are rarely studied together because of the obligate biotrophy of AMF. Although at the initial glance the symbioses of plants with rhizobia and AMF seem to have little in common as the phenotypic impacts differ, the interaction between the three symbiotic partners leads to new phenomena. This interaction was first studied in 1944 when it was reported that several legumes did not form nodules in autoclaved soil unless they were mycorrhizal (Azcón and Barea, 2010). Other studies that followed years later showed that the presence of mycorrhiza stimulated nodulation and growth of numerous legumes (Xavier and Germida, 2002). Some of the additional effects produced by the interaction include greater number and dry weight of nodules, enhanced symbiotic nitrogen fixation and higher nitrogen

content (Shockley *et al*., 2004). An assumption that has been made is that the beneficial effect of N_2 fixation by AMF colonization is due to increased P supply to the nodules by the symbiotic fungal partner.

2.10 Interspecific Crosses to Control Insect Pests

Host-plant resistance is the most environmentally friendly and economical way of controlling insect pests. Introduction of genes for resistance to different insect pests should result in the availability of varieties that can be grown by smallholder farmers with minimal use of chemical farm inputs (Sharma and Ortiz, 2002). A phylogenetic study that was conducted involving different *Vigna* species and based on Restriction Fragment Length Polymorphism (RFLP) markers revealed that among species that showed high levels of resistance to insect pests, *Vigna vexillata* is the closest to cowpea (*Vigna unguiculata)* (Fatokun, 1991). The various lines of *Vigna vexillata* showed high resistance levels to flower thrips, pod sucking bugs, *Striga gesnerioides, Maruca nitrate*, and bruchids among other pests.

Interspecific crosses between *Vigna vexillata* and cowpea are thus attractive and worth pursuing because of the possession of the mentioned traits (Damayanti *et al*., 2010). Attempted crosses between the two species have been carried out with the aim of conferring resistance to insect pests from *Vigna vexillata* to the cultivated cowpea (*Vigna unguiculata).* For most of the attempted crosses, no viable hybrid seed was obtained, which suggests a strong cross incompatibility between the two species (Fatokun, 1991). However, Gomathinayagam *et al*. (1998), claimed that they succeeded in getting F_1 plants but they did not attempt any back-cross with their parents. Failure of interspecific hybridization between

the wild and cultivated cultivars is mainly caused by reduced fertilization caused by pollen-pistill incompatibility and the collapse of fertilized ovules. Low fertilization is as a result of abnormal growth behavior of the pollen tube following interspecific crossing.

2.11 Possible Ways of Overcoming Interspecies Incompatibility

Developments and improvements in cell and tissue culture procedures have greatly contributed to the progress attained in interspecies gene exchange in many crops.

2.11.1 *In-vitro* Embryo Culture

Young interspecific hybrid embryos have been rescued prior to their abortion using *in vitro* culture methods. This is especially important in cases where the cause of incompatibility occurs after fertilization, for instance, endosperm abortion and subsequent starvation of the embryo. Fatokun (1991), observed that there was a need to rescue embryos of the cross between cowpea and a wild relative, *Vigna unguiculata* subspecies *pubescens*, otherwise the embryos that resulted from the cross collapsed prior to full development. Fatokun (2002) further found out that the endosperm and embryo resulting from the cross between *Vigna vexillata* and cowpea collapsed within five and eight days after fertilization. Embryo development especially during the early phases depends on the presence of a well-formed endosperm, which is the main source of nourishment for the embryo. It is also essential for a harmonious relationship to exist between the embryo and endosperm tissue if the former is to undergo development process.

Successful rescue of interspecific hybrid embryos that are at the globular phase has been difficult to achieve in many plants. Embryo rescue is more successful as the embryo gets older. Usually, the development of embryos into plants occurs more readily once they are past the globular developmental stage and beyond (Barone *et al*., 1992). Based on interspecific crossing attempts that have been previously made, it is evident that fertilization occurs when cowpea pollen are placed on the stigma of *Vigna vexillata.* Embryo rescue technique has been used to enhance interspecific hybridization between *Vigna mungo* and *Vigna umbellata* (Bharathi *et al*., 2006).

2.11.2 Crossing Numerous Accessions of both Species

Reports obtained from previous wide crossing experiments have indicated that hybrids between some accessions are more productive than others. This is mainly because particular species' accessions form better combinations with some individuals of another species (Rebernig *et al*., 2015). For example, crosses done between tobacco (*Nicotiana tobacum*) and different *Nicotiana repanda* cultivars resulted to finding that only one combination formed F_1 hybrids (Liu and Marubashi, 2014). A number of *Tripsacum dactyloides* was also tested in combination with corn and it was found that only one of the accessions was effective in the transfer of genetic material to maize (Eubanks, 1997). In attempted crosses between cowpea and *Vigna vexillata*¸ some pods are retained on *Vigna vexillata* when emasculated flowers are pollinated with cowpea but none were retained in reciprocal crosses (Fatokun, 2002). It can therefore, be presumed that different *Vigna vexillata* lines will respond differently, for instance, in the frequency of pod retention, when different cowpea accessions are used to pollinate the flowers.

2.11.3 Use of Mixed Pollen

Although cowpea pollen grains produce tubes when placed on the sigma of *V. vexillata*, the frequency of tube production is extremely low. Also, there is malformation in some of the developed pollen tube, which consequently fail to penetrate the style fast enough to reach the ovule in order to effect fertilization. A few pollen grains of *Vigna vexillata* plants deliberately placed on the stigma along with some of cowpea pollen led to development of pods with few normal sized seeds. However, none of the seeds resulted from interspecific hybridization but rather they were from self fertilization (Lelou and Van Damme, 2006). The mixed pollen technique has been successfully used to enable interspecies crosses in passion fruit (genus *Passiflora)* (Cerqueira-Silva *et al*., 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

Interspecific hybridization experiments were carried out at International Center of Insect Physiology and Ecology (ICIPE) while the greenhouse experiment was conducted at Kenyatta University.

3.2 Arbuscular Mycorrhizal Fungal isolates

Myco Apply Custom Super concentrate Powder, comprising of four mycorrhizal species, *Rhizophagus irregularis, Funneliformis mosseae, Glomus aggregatum* and *Glomus etunicatum* was used as the inoculum. The super concentrate powder contains 220,200 mycorrhizal propagules per gram.

3.3 Soil Samples

The soil used for the greenhouse experiment was sampled from four farms in Runyenjes sub-county, Embu County, a major cowpea producing region in Kenya. Agriculture is the main economic driver of this county with more than 70% of the population being smallholder farmers. Cowpea is among the major food crops produced in the county, although its production is much lower than staples such as maize. Ten sub-samples of about 900g were collected from each of the four farms by removing soil core from 15-20cm depth with soil auger. They were thoroughly mixed to obtain a homogenous composite sample. The experimental soil was analyzed for different chemical properties using the methods of

Jalaluddin and Anwar (1991), Nelson and Sommer (1982), Hussain (1989) and Olsen and Sommers (1982).

3.4 Greenhouse Experiment

Two experiments were laid out in a completely randomized design (CRD). One of the experiments was done using sterilized soil while the other one was done using non-sterilized soil. The sterilized soil experiment was done to investigate the sole effect of the commercial AMF inoculum while the experiment that utilized non-sterilized soil represented a field scenario to investigate the combined effect of commercial and native AMF. For each experiment, there were two treatments (control and mycorrhizal-inoculated) using a wild cowpea species and three cultivated cowpea cultivars. The wild cultivar used was Ni935, which was obtained from the coastal Kenya. The cultivated cultivars used were Katumani 80, KenKunde 1 and Kunde Mboga, which were purchased from Simlaw Seeds, Kenya. These cultivars were chosen because they are not only locally available, but also mostly grown and by smallholder farmers in Kenya. Each treatment was replicated 4 times with 2 plants in each pot (2 treatments x 4 varieties of cowpea x 4 replicates).

The soil was mixed with sand in the ratio of 2:1 (soil/sand) resulting in a sandy loam textured soil. Sterilization was done overnight in an oven preheated to 100 ^{0}C. Mycorrhizal inoculation was done by mixing the inoculum with soil at a depth of 3 to 6 cm prior to sowing as per the manufacturer's instruction. All pots were also supplied with a filtrate obtained by sieving an aliquot of non-sterilized soil through a 40-µm sieve to provide the substrate with an equivalent soil microbiota (Njeru *et al*., 2014). Two cowpea seeds were

planted in each pot. The pot size for this experiment was 1 kg while watering was done immediately after planting the seeds and then at two-day interval following germination. After 40 days, the plants were harvested and nodule number determined. The dry weights of shoots, roots and nodules were determined by drying samples at 65^0C till a constant weight was attained.

3.4.1 Staining for Mycorrhizal Colonization

About 1 g of roots from each treatment was thoroughly washed and placed in falcon tubes and then cleared using 10% KOH. They were heated in 80^0C water bath for 10-15 minutes. The roots were washed and 2% HCl added and allowed to stand for 5-10 minutes. Staining was then done by adding 0.05% trypan blue in lactic acid and heating in 80^0C water bath for 10-15 minutes. To destain the roots, 10% lactic acid was added to the samples (Phillips and Hayman, 1970).

3.4.2 Measuring % Mycorrhizal Colonization

The destained roots were spread out evenly in the Petri dish. Under a dissecting microscope, the vertical and horizontal lines of the grid were scanned. The total number of intersection of roots and grid lines as well as the number of intersections with mycorrhizal-infected roots were recorded. Percentage mycorrhizal colonization was calculated using the formula shown below (Giovannetti & Mosse, 1980):

%MycCol = (Total No. of intersections with mycorrhizal-infected roots/ Total No. of Intersections between root and gridline) X 100

3.4.3 Determination of Shoot P, N and K

Shoot samples from each treatment were dried followed by ashing. About 1 gram of the ground samples from each treatment was digested in 1ml of conc. H_2SO_4 at 300^o C prior to nutrient analysis. Total nitrogen using Kjedahl method by measuring the amount of ammonium ions produced. The NH_4^+- N created during the digestion procedure was determined by making the digest strongly alkaline, with sodium hydroxide and collecting the volatilized ammonia into a boric acid indicator solution by steam distillation. The NH_3 distilled is titrated into H_3BO_3 solution using standard 0.01 M HCl or 0.005M H_2SO_4. The formula used to calculate percentage nitrogen is shown below (Bremner, 1982):

%N = (T-B) x *N* x 1.401/g sample

Where, T = mL of sample titrated, B = mL of blank titrated, *N* = acid normality.

The amount of phosphorous was determined by colorimetric method. An acidified solution of ammonium molybdate containing ascorbic acid and antimony was added to the digested plant tissue samples. The phosphate in the plant tissues reacts with the acidified ammonium molybdate to form ammonium molydiphosphate complex. A blue colored solution was formed following the reduction of the ammonium molydiphosphate complex by ascorbic acid. The blue color intensity was proportional to the amount of molybdophosphorous present. The amount of light absorbed by the solution at 660nm was measured with a spectrophotometer. The instrument reading was read as the concentration of P in parts per million (Olsen and Sommers, 1982).

Following sulfuric/perchloric acid digestion, the readily exchangeable, water soluble K was determined in neutral NH_4 acetate extractant. The aim of NH_4 ion was to provide a sharp rapid separation of K from exchange complex. The tissue extract was atomized in the flame where atoms of the element are excited, emitting radiations of characteristics wavelength. The radiation emitted by the K atoms is passed through the filter which falls on photocell emitting electrons, that is, the electric current, which is measured on the flame photometer's galvanometer. The electric current generated is proportional to the K concentration in the extract (Page *et al*., 1982).

3.5 Hybridization Experiment

3.5.1 Interspecific Crossing

Crosses were made between five *Vigna* vexillata accessions, V263, V268, AC301, Ni935 and Ni936, and *Vigna unguiculata* accession 219 and 524B. Hybridization was conducted by hand pollination in the morning, using newly-open flowers that had been emasculated before sunset on the day before. Manual pollination was done by placing pollen of cowpea on the stigma of *Vigna vexillata*. Success of crossing and setting of pods was recorded.

3.5.2 Culturing of Hybrid Embryos

A total of 143 immature hybrid embryos and a few selfed embryos of 10-12 days old at the heart shaped stage were carefully excised under a laminar flow hood using a stereo microscope. The excised embryos were cultured on Murashige and Skoog media supplemented with 2% sucrose. The cultures were incubated in darkness at 25^0 C for the first

two weeks and then when the first regeneration indications appeared, they were transferred to a culture room under a 16-hours light period and light intensity of 3000 Lux. The embryos were transferred to fresh media every three weeks. In the second true leaf stage, the regenerated plants were transferred into micropots with soil mixture and watered. Young true leaves of the regenerated plants were used for isoenzyme analysis using starch gel electrophoresis.

3.6 Data analysis

All data were tested for homogeneity of variance by Bartlett test before analyses. The % data were arcsine ($\sqrt{x}$) transformed, while other data was log (x+1) transformed wherever necessary to fulfill the assumptions of ANOVA. The data reported in tables and graphs were back transformed. Data from greenhouse experiment were analyzed by two-way ANOVA as a completely randomized design. Pearson correlation coefficient was used to determine the relationship between shoot nutrition and mycorrhizal colonization. Wherever applicable, post hoc test was performed using Tukey's HSD test ($P < 0.05$). All statistical analyses were performed with the SPSS (version 20.0).

CHAPTER FOUR

RESULTS

4.1 Soil Content Analysis

The soil used for the greenhouse experiment was acidic with a pH of 4.4. The electrical conductivity and levels of C, and K were low (Table 4.1). The cation exchange capacity (CEC) is a useful soil fertility indicator since it shows the ability of the soil to supply three important plant nutrients; Ca, Mg and K. In the experimental soil, the CEC was more than 10 Cmol/kg (Table 4.1), indicating that the soil was suitable for plant production. The levels of phosphorous and Nitrogen were within the limits required range for normal plant growth.

Table 4. 1: Chemical properties of soil used for the greenhouse experiment

Soil Chemical Properties	
Water	5.20
pH (0.01 m $CaCl_2$)	4.40
EC_{25}^{0C}	0.20
C (%)	1.82
N (%)	0.15
K (%)	0.50
Na (Cmol/Kg)	TRACE
Ca (Cmol/Kg)	6.50
Mg (Cmol/Kg)	6.00
CEC (CamoL/Kg)	13.40
P (ppm)	14.40

4.2 Root Mycorrhizal Colonization and Growth Parameters in Sterilized Soil

Two way ANOVA data showed that root AMF colonization was significantly affected by both the genotype ($p < 0.001$) and AMF inoculation ($p < 0.001$) with modern cultivars having higher root colonization compared to the wild species (Table 4.2). Moreover, significant differences in AMF colonization were observed within the modern cultivars. Katumani 80 and Kunde Mboga had higher level of root AMF colonization compared to KenKunde 1, while non-inoculated plants were not colonized. There was a significant ($p < 0.001$) genotype × AMF interaction on root AMF colonization (Table 4.2).

The nodule number varied significantly ($p = 0.022$) in all the cowpea genotypes. The Katumani 80 cultivar had the highest mean nodule number while the wild species had the lowest mean nodule number. The nodule numbers for Kunde Mboga and KenKun1 did not differ statistically (Table 4.2). The effect of AMF inoculation on nodule number was very significant ($p<0.001$), with inoculated plants having a higher number of nodules compared to the non-inoculated plants (Table 4.2).

Additionally, the effect of genotype × AMF interaction on nodule number was significant ($p=0.055$) (Table 4.1). The highest increase in nodulation was observed in the wild cultivar (57.91%) followed by KenKunde 1 (52.51%) while Katumani 80 had the lowest percentage increase in nodulation (27.84%) (Table 4.3).

Correspondingly, the nodule dry weight was significantly affected by the genotype ($p=0.001$). While the nodule dry weight of Katumani 80 and Kunde Mboga cultivars did not

differ statistically, the wild species had the lowest nodule dry weight (Table 4.1). The effect of AMF inoculation on nodule dry weight was also significant ($p<0.001$), with inoculated plants having higher nodule dry weight compared to the non-inoculated plants (Table 4.2). The effect of genotype × AMF interaction on nodule dry weight of cowpea was not significant (Table 4.2).

The root and shoot dry weights varied significantly ($p<0.001$) in all the cowpea cultivars. Katumani 80 cultivar had the highest root and shoot dry weights while the wild species had the lowest root and shoot dry weights. Similarly, the root and shoot dry weights varied significantly ($p<0.001$) between the AMF inoculated and the non-inoculated cowpea. The root and shoot dry weights of inoculated plants were higher than that of their non-inoculated counterparts (Table 4.2).

There was a very strong significant interaction ($p<0.001$) between the genotype and AMF inoculation in determining both the root and shoot dry weights of cowpea (Table 4.2). The wild cultivar had the highest increase in root dry weight (56.70%) followed closely by KenKunde 1 (56.33%) and Kunde Mboga (56.11%) while Katumani 80 had the lowest percentage increase in root dry weight (46.99%) (Table 4.3). Similarly, Kunde Mboga had the highest increase in shoot dry weight (58.25%) followed by KenKunde 1 (56.97%) and the wild cultivar (56.82%) while Katumani 80 had the lowest percentage increase in shoot dry weight (47.00%) (Table 4.3).

4.3 Susceptibility of the Cowpea Cultivars to AMF Colonization in Sterilized Soil

There was a very strong significant difference in AMF colonization ($p < 0.001$) in all the four cultivars (Table 4.2). The results indicated that the cultivated cultivars were more susceptible to AMF colonization compared to the wild species. There was significant genetic variability with respect to colonization even within the modern or cultivated cultivars. Katumani 80 had the highest susceptibility and hence, the highest response to AMF inoculation, followed by Kunde Mboga cultivar. KenKunde had the least percentage of root mycorrhizal colonization among the modern cultivars (Table 4.2).

Table 4. 2: ANOVA results for the effects of cowpea genotype, AMF inoculation and their interaction on root mycorrhizal colonization, nodule number, nodule dry weight, root dry weight and shoot dry weight of cowpea grown in sterilized soil.

	%MC	NN	NDW (mg plant^{-1})	RDW (mg plant^{-1})	SDW (mg plant^{-1})
Cowpea genotype					
K	21.28 ± 8.04a	21.63 ± 1.50a	11.83 ± 2.83a	175.70 ± 20.48a	975.00 ± 113.59a
KM	19.53 ± 7.38b	19.13 ± 2.42ab	10.48 ± 2.66a	151.09 ± 22.85b	831.61 ± 129.28b
KK	18.48 ± 6.99c	19.75 ± 2.70ab	9.73 ± 2.49ab	140.76 ± 21.10c	780.88 ± 117.33c
Wild (Ni935)	14.61 ± 5.53d	17.00 ± 2.80b	8.00 ± 2.08b	113.54 ± 16.96d	627.35 ± 94.11d
AMF inoculation					
M	36.95 ± 1.28	25.13 ± 0.81a	16.51 ± 0.74a	199.03 ± 6.83a	1104.18 ± 37.93a
NM (Control)	0	13.63 ± 0.86b	3.51 ± 0.21b	91.51 ± 4.99b	503.24 ± 27.75b
P values of the main factors and interaction					
Genotype	< 0.001	0.022	0.001	< 0.001	< 0.001
AMF In	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Genotype x AMF In	< 0.001	0.055	0.114	< 0.001	< 0.001

Values followed by the same letter in a column within each treatment are not significantly different at $p<0.05$ (Tukey's HSD test)
Key: %MC- Mycorrhizal colonization percentage, NN- Nodule number, NDW-Nodule dry weight, RDW-Root dry weight, SDW-Shoot dry weight, K-Katumani 80, KM-Kunde mboga, KK-KenKunde1, M-Mycorrhizal, NM-Non-mycorrhizal AMF In- Arbuscular mycorrhizal fungi inoculation

Table 4. 3: The effect of interaction between genotype and AMF inoculation on mycorrhizal colonization, nodule number, , root dry weight and shoot dry weight of cowpea grown using sterilized soil with the mean standard errors shown in parenthesis.

Cultivar	Treatment	%MC	NN	RDW (mg plant^{-1})	SDW (mg plant^{-1})
K	M	42.56 (0.24)	25.00 (1.47)	229.88 (1.01)	1275.50 (2.15)
	NM	0	18.25 (0.85)	121.53 (0.86)	674.50 (2.68)
KM	M	39.06 (0.11)	25.00 (1.58)	211.45 (1.25)	1173.65 (1.91)
	NM	0	13.25 (1.38)	90.73 (2.61)	489.58 (1.21)
KK	M	36.96 (0.58)	26.75 (1.11)	196.45 (2.47)	1091.25 (4.28)
	NM	0	12.75 (0.48)	85.08 (1.79)	470.50 (0.89)
Wild	M	29.21 (0.58)	23.75 (2.39)	158.35 (1.62)	876.33 (2.54)
	NM	0	10.25 (0.75)	68.73 (0.53)	378.38 (1.09)

Key: %MC- Mycorrhizal colonization percentage, NN- Number of nodules, NDW-Nodule dry weight, RDW-Root dry weight, SDW-Shoot dry weight, K-Katumani 80, KM-Kunde Mboga, KK-KenKunde1, M-Mycorrhizal, NM-Non-mycorrhizal (control), AMF In- Arbuscular mycorrhizal fungi inoculation.

4.4 Shoot P, N, and K Nutrition of Cowpea Using Sterilized Soil

The effect of cowpea genotype on the level of shoot nitrogen was statistically significant ($p<0.001$). The Katumani 80 cultivar had the highest percentage of shoot nitrogen while the wild species had the lowest percentage. The effect of AMF inoculation on shoot nitrogen was also significant ($p<0.001$). Mycorrhizal plants had a higher level of shoot total Nitrogen than the non-inoculated plants (Table 4.4). Additionally, the effect of genotype × AMF interaction on the level of shoot nitrogen was significant ($p<0.001$) (Table 4.4). The highest percentage increase in shoot N was recorded in the wild cultivar (57.30%) followed by Kunde Mboga (57.5%) while Katumani 80 had the lowest percentage increase in the level of shoot N (46.63 %) (Table 4.5).

Shoot potassium differed significantly ($p<0.001$) in all the cowpea cultivars. Kunde Mboga cultivar had the highest level of shoot potassium. Shoot potassium levels in KenKunde1 and the wild species were statistically similar (Table 4.4). There was a very strong significant difference ($p<0.001$) in shoot potassium levels between the AMF inoculated and non-inoculated plants. Interestingly, the non-mycorrhizal plants had higher potassium levels compared to the mycorrhizal-inoculated plants (Table 4.4). There was a very strong significant interaction ($p<0.001$) between the genotype and AMF inoculation in determining the level of shoot potassium (Table 4.4). Contrary to the trend observed in shoot nitrogen, the level of shoot potassium in non-inoculated plants was higher than that of the inoculated plants. KenKunde 1 cultivar recorded the highest percentage decrease in shoot K (13.60%) followed by Kunde Mboga (1.57%) and the wild cultivar (0.49%), while Katumani 80 had the lowest percentage decrease (0.08%) in the level of shoot K following inoculation with AMF (Table 4.5).

The effect of cowpea genotype on the level of shoot phosphorous was statistically significant ($p<0.001$). Katumani 80 cultivar had the highest shoot Phosphorous content while the wild species had the lowest shoot Phosphorous level. Also, the effect of AMF inoculation on shoot phosphorous was significant ($p<0.001$), with Mycorrhizal inoculated plants having a higher shoot Phosphorous content compared to the non-inoculated ones (Table 4.4).

Additionally, the effect of interaction between genotype and AMF inoculation on shoot phosphorous was also significant ($p<0.001$) (Table 4.4). Kunde Mboga cultivar had the highest percentage increase in shoot P (48.21%) followed by KenKunde 1 (47.85%) and the

wild cultivar (46.84%). Katumani 80 had the lowest percentage increase (40.47%) in shoot P following inoculation of cowpea with AMF using sterilized soil (Table 4.5).

Table 4. 4: Means and probability values from ANOVA of cowpea genotype, AMF inoculation and interactions on shoot N, K and P nutrition of cowpea grown using sterilized soil

%N		K (ppm)	P (ppm)
Cowpea genotype			
K	2.94 ± 0.35a	2870.13 ± 13.56b	1304.50 ± 125.21a
KM	2.50 ± 0.39b	2995.50 ± 8.95a	1091.00 ± 130.96b
KK	2.37 ± 0.35c	2795.00 ± 76.84c	1004.25 ± 119.47c
Wild	1.89 ± 0.28d	2775.75 ± 3.17c	830.13 ± 96.10d
AMF inoculation			
M	3.33 ± 0.12a	2797.75 ± 36.38b	1369.38 ± 51.60a
NM	1.52 ± 0.08b	2920.44 ± 24.69a	745.56 ± 37.42b
P values of the main factors and interaction			
Genotype	< 0.001	< 0.001	< 0.001
AMF In	< 0.001	< 0.001	< 0.001
Genotype x AMF In	< 0.001	< 0.001	< 0.001

Values followed by the same letter in a column within each treatment are not significantly different at $p<0.05$ (Tukey's HSD test

Key: %N-Percentage K-Katumani 80, KM-Kunde mboga, KK-KenKunde1, M-Mycorrhizal, NM-Non-mycorrhizal (control), AMF In-Arbuscular mycorrhizal fungi inoculation.

Table 4. 5: The effect of interaction between genotype and AMF inoculation on shoot N, K and P nutrition of cowpea grown using sterilized soil. The mean standard errors are presented in parentheses**.**

Cultivar	Treatment	%N	K(ppm)	P (ppm)
K	M	3.85 (0.01)	2857.00 (27.06)	1635.75 (3.01)
	NM	2.02 (0.04)	2883.25 (3.20)	973.25 (2.29)
KM	M	3.53 (0.00)	2972.25 (1.75)	1437.25 (9.60)
	NM	1.48 (0.02)	3018.75 (3.17)	744.75 (4.52)
KK	M	3.30 (0.02)	2591.75 (1.84)	1320.25 (4.87)
	NM	1.43 (0.03)	2998.25 (3.59)	688.25 (3.54)
Wild	M	2.64 (0.03)	2770.00 (2.35)	1084.25 (5.86)
	NM	1.14 (0.00)	2781.50 (4.41)	576.00 (3.49)

Key: K-Katumani 80, KM-Kunde Mboga, KK-KenKunde1, M-Mycorrhizal, NM-Non-mycorrhizal (control), AMF In- Arbuscular mycorrhizal fungi inoculation.

4.5 Correlation between AMF Colonization and Shoot Nutrition in Sterilized Soil

There was a strong positive correlation between root AMF colonization and shoot nitrogen (r^2 = 0.9854, p <0.001) (Table 4.6). An increase in percentage AMF corresponded to increased levels of shoot nitrogen and vice versa (Fig. 4.1). Similarly, there was a strong positive correlation between root AMF colonization and shoot phosphorous nutrition (r^2 = 0.9440, p <0.001) (Fig 4.2). A high percentage of AMF colonization corresponded to high levels of shoot phosphorous. On the contrary, the correlation between root AMF colonization and shoot potassium content was not significant (r^2 = 0.1071, p = 0.2159) (Table 4.6).

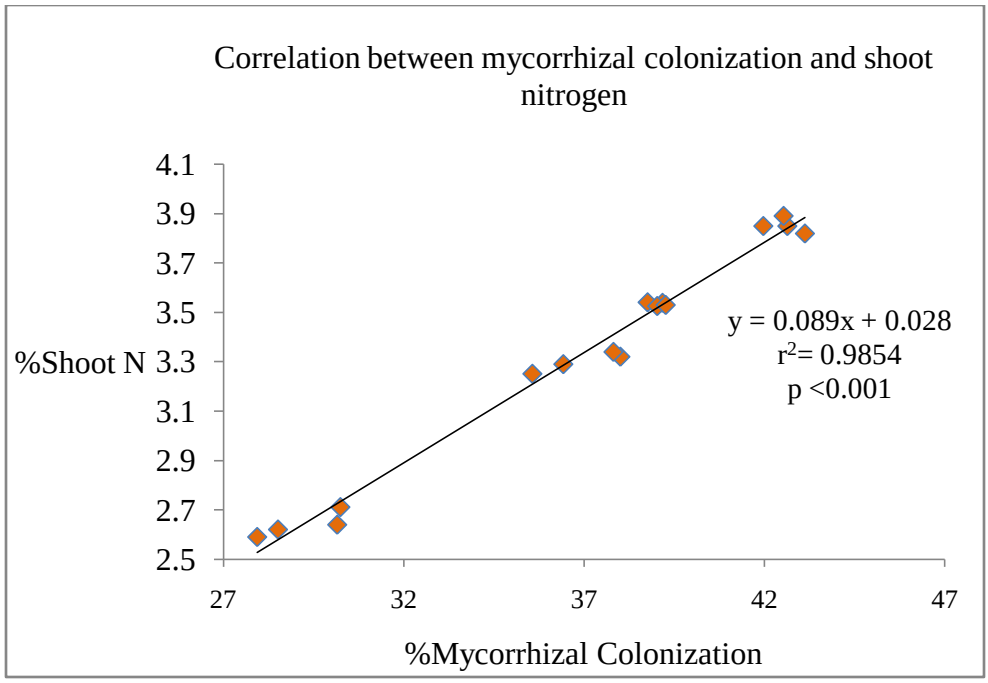

Fig 4. 1: Relationship between root mycorrhizal colonization and shoot nitrogen level following AMF inoculation of cowpea grown in sterilized soil.

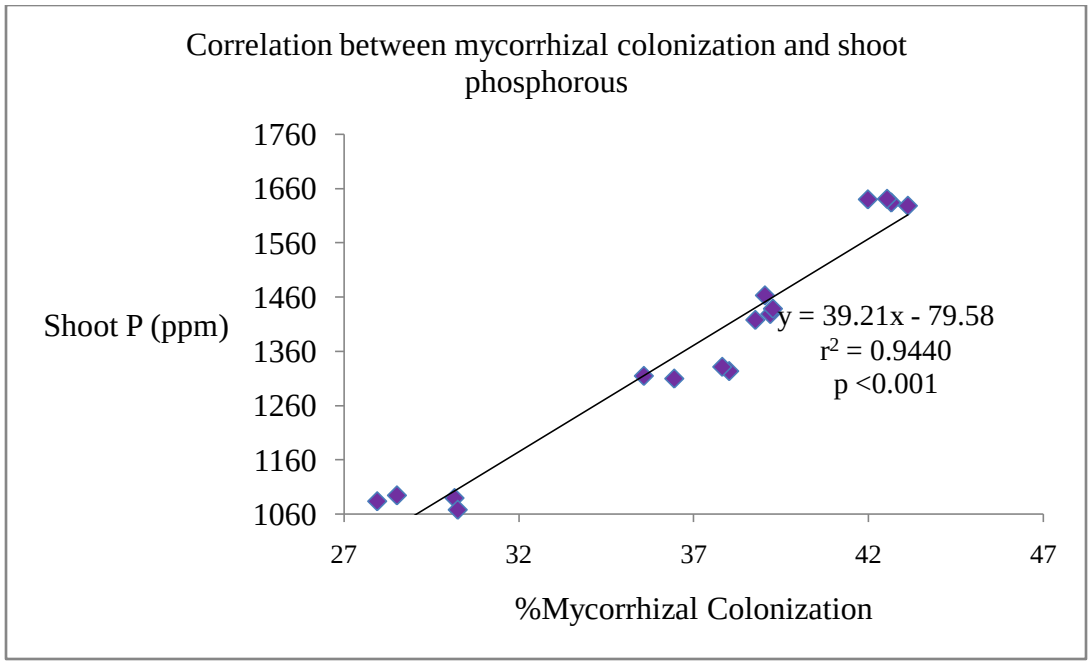

Fig 4. 2: Relationship between root mycorrhizal colonization and shoot phosphorous level following AMF inoculation of cowpea grown insterilized soil.

Table 4. 6: Correlation between mycorrhizal colonization, root dry weight shoot dry weight, nodule number, shoot %N, K and P in sterilized soil

		%MC	RDW	SDW	NN	NDW	%N	K (ppm)	P (ppm)
%MC		1							
RDW	Pearson Correlation	0.976**	1						
	Sig. (2-tailed)	<0.001							
SDW	Pearson Correlation	0.985**	0.993**	1					
	Sig. (2-tailed)	<0.001	<0.001						
NN	Pearson Correlation	0.137	0.114	0.130	1				
	Sig. (2-tailed)	0.612	0.675	0.632					
NDW	Pearson Correlation	0.669**	0.740**	0.731**	0.334	1			
	Sig. (2-tailed)	0.005	0.001	0.001	0.206				
%N	Pearson Correlation	0.993**	0.992**	0.996**	0.129	0.719**	1		
	Sig. (2-tailed)	<0.001	<0.001	<0.001	0.635	0.002			
K (ppm)	Pearson Correlation	0.327	0.381	0.378	-0.166	0.228	0.365	1	
	Sig. (2-tailed)	0.023	0.145	0.149	0.540	0.396	0.164		
P (ppm)	Pearson Correlation	0.972**	0.984**	0.987**	0.112**	0.733**	0.985**	0.406	1
	Sig. (2-tailed)	<0.001	<0.001	<0.001	0.679	0.001	<0.001	0.119	

**. Correlation is significant at the 0.01 level (2-tailed).

Key: MC-Mycorrhizal colonization, SDW-Shoot dry weight, NN-Nodule number, NDW-Nodule dry weight

4.6 Root Mycorrhizal Colonization and Growth Parameters Using Non-sterilized Soil

Based on two-way ANOVA, cowpea genotype had a significant effect on percentage root mycorrhizal colonization ($p<0.001$). Katumani 80 had the highest percentage mycorrhizal colonization and the wild species had the lowest (Table 4.7). Mycorrhizal inoculation also had a significant effect on percentage mycorrhizal colonization ($p<0.001$). Inoculated cultivars had a higher percentage of mycorrhizal colonization compared to the non-inoculated cultivars (Table 4.7).

Additionally, the effect of interaction between genotype and AMF inoculation on percentage mycorrhizal colonization was very significant ($p<0.001$) (Table 4.7). The highest percentage increase in root AMF colonization was observed in Kunde Mboga (48.93%) followed by KenKunde 1 (46.84%) and the wild cultivar (42.70%). Katumani 80 had the lowest percentage increase (40.77%) in root AMF colonization following inoculation of cowpea with AMF using non-sterilized soil (Table 4.8).

The nodule number differed significantly ($p<0.001$) in all the four cowpea cultivars. Katumani 80 cultivar had the highest mean nodule number while the wild species had the lowest mean nodule number. The nodule numbers in Kunde Mboga and KenKunde1 were statistically the same (Table 4.7). There was a very strong significant difference ($p<0.001$) in nodule number between mycorrhizal inoculated cowpea and the non-inoculated cowpea cultivars. Mycorrhizal cowpea had a higher mean nodule number than non-mycorrhizal cowpea (Table 4.7).

Additionally, the effect of genotype × AMF interaction on nodule number was significant ($p=0.039$). KenKunde 1 had the highest increase in nodule number (40.82%) followed by the wild cultivar (31.45%). Kunde Mboga had a 24.76% increase while Katumani 80 had the lowest increase (6.60%) in nodule number following inoculation of cowpea with AMF using non-sterilized soil (Table 4.8).

The nodule dry weight differed significantly ($p<0.001$) in all the cowpea genotypes. Katumani 80 cultivar had the highest mean nodule dry weight while the wild species had the lowest (Table 4.6). The significant difference in nodule number was very strong ($p<0.001$) between the mycorrhizal inoculated cowpea and the non-inoculated cowpea. The inoculated plants had a higher mean nodule dry weight compared to the non-inoculated plants (Table 4.7).

The effect of interaction between genotype and AMF inoculation on nodule dry weight was statistically significant ($p=0.001$). KenKunde 1 had the highest increase in nodule dry weight (64.26%) followed by Kunde Mboga (63.73%) and Katumani 80 (55.37%). The wild cultivar had the lowest percentage increase (53.39%) following inoculation of cowpea with AMF using non-sterilized soil (Table 4.8).

In all the cowpea genotypes, the mean root and shoot dry weights differed significantly ($p<0.001$). Katumani 80 genotype had the highest means of both root and shoot dry weights while the wild species had the lowest. Similarly, there was a very strong significant

difference ($p<0.001$) in both root and shoot dry weights between mycorrhizal inoculated cowpea and the non-inoculated ones. Mycorrhizal cowpea had higher mean root and shoot dry weights compared to the non-mycorrhizal cowpea (Table 4.7).

The effect of genotype × AMF interaction on root and shoot dry weights was also statistically significant ($p<0.001$). Kunde Mboga cultivar had the highest increase in root dry weight (49.92%) followed by KenKunde 1 (43.07%) while the wild cultivar had the lowest increase in root dry weight (32.94). Similarly, Kunde Mboga had the highest increase in shoot dry weight (49.89%) followed by KenKunde 1 (47.74%) and Katumani 80 (41.30%). The wild cultivar had the lowest increase in shoot dry weight (33.66%) following inoculation of cowpea with AMF using non-sterilized soil (Table 4.8)

4.7 Susceptibility of the Cowpea Cultivars to AMF Colonization in Non-sterilized Soil

Following inoculation of AMF in cowpea grown on non-sterilized soil, results indicated a very strong significance difference ($p <0.001$) in root colonization by AMF in all the cowpea cultivars (Table 4.7). The cultivated cultivars had a higher percentage of AMF colonization compared to the wild species. This shows that the cultivated cultivars were more susceptible to AMF colonization in comparison to the wild species. There were genetic variations even within the modern cultivars, with Katumani 80 having the highest susceptibility followed by Kunde Mboga cultivar and KenKunde1 being the least colonized (Table 4.7).

Table 4. 7: ANOVA results for the effects of cowpea genotype, AMF inoculation and their interaction on root mycorrhizal colonization, nodule number, nodule dry weight, and root dry weight and shoot dry weight using non-sterilized soil.

	%MC	NN	NDW (mg plant^{-1})	RDW (mg plant^{-1})	SDW (mg plant^{-1})
Cowpea genotype					
K	37.51 ± 3.79a	23.00 ± 1.67a	16.55 ± 2.67a	201.39 ± 20.15a	1121.48 ± 110.35a
KM	33.94 ± 4.35b	21.50 ± 1.49ab	15.47 ± 2.87ab	182.43 ± 23.07b	1011.49 ± 127.35b
KK	28.95 ± 3.45c	22.25 ± 2.36ab	13.56 ± 2.50b	145.40 ± 15.19c	878.31 ± 104.85c
Wild	20.96 ± 2.24d	17.88 ± 1.71b	9.96 ± 1.49c	119.09 ± 9.25d	660.65 ± 50.74d
AMF inoculation					
M	39.42 ± 2.10a	24.56 ± 0.99a	20.04 ± 1.07a	206.00 ± 12.03a	1178.08 ± 62.16a
NM	21.26 ± 1.20b	17.75 ± 1.06b	7.73 ± 0.45b	118.15 ± 5.20b	657.88 ± 28.96b
P values of the main factors and interaction					
Genotype	< 0.001	0.027	< 0.001	< 0.001	< 0.001
AMF In	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Genotype * AMF In	< 0.001	0.039	0.001	< 0.001	< 0.001

Values followed by the same letter in a column within each treatment are not significantly different at $p<0.05$ (Tukey's HSD test)
Key: %MC- Mycorrhizal colonization percentage, NN- Number of nodules, NDW-Nodule dry weight, RDW-Root dry weight, SDW-Shoot dry weight, K-Katumani 80, KM-Kunde Mboga, KK-KenKunde1, M-Mycorrhizal, NM-Non-mycorrhizal (control), AMF In- Arbuscular mycorrhizal fungi inoculation.

Table 4. 8: The effect of interaction between genotype and AMF inoculation on mycorrhizal colonization, nodule number, nodule dry weight, root dry weight and shoot dry weight of cowpea with the mean standard errors shown in parenthesis.

Cultivar	Treatment	%MC	NN	NDW (mg)	RDW (mg)	SDW (mg)
K	M	47.49 (0.29)	23.75 (2.63)	23.48 (0.45)	254.55 (2.15)	1413.43(1.47)
	NM	27.53 (0.77)	22.25 (2.39)	9.63 (1.05)	148.23 (2.50)	829.53 (1.01)
KM	M	45.38 (0.46)	24.75 (1.25)	23.03 (0.38)	243.43 (1.41)	1348.40 (1.90)
	NM	22.51 (0.90)	18.25 (1.31)	7.93 (0.56)	121.43 (1.19)	674.58 (2.48)
KK	M	38.01(0.54)	28.25 (0.95)	20.00(1.13)	182.90 (11.47)	1155.68 (1.56)
	NM	19.89(0.64)	16.25 (1.03)	7.13 (0.42)	107.90 (2.76)	600.95 (3.84)
Wild	M	26.80 (0.60)	21.50 (1.55)	13.68 (0.93)	143.13 (2.60)	794.83 (2.46)
	NM	15.11 (0.59)	14.25 (1.55)	6.25 (0.56)	95.05 (2.67)	526.48(2.41)

Key: %MC- Mycorrhizal colonization percentage, NN- Number of nodules, NDW-Nodule dry weight, RDW-Root dry weight, SDW-Shoot dry weight, K-Katumani 80, KM-Kunde Mboga, KK-KenKunde1, M-Mycorrhizal, NM-Non-mycorrhizal (control), AMF In- Arbuscular mycorrhizal fungi inoculation.

4.8 Shoot Nutrition Using Non-sterilized Soil

Shoot nitrogen differed significantly ($p<0.001$) in all the cowpea genotypes. Katumani 80 had the highest mean percentage of total shoot N while the wild species had the lowest (Table 4.9). The level of N between mycorrhizal-inoculated cowpea and non-mycorrhizal cowpea differed significantly ($p<0.001$). The mean percentage of total N was higher in mycorrhizal inoculated plants in comparison to the non-inoculated plants (Table 4.9).

The effect of genotype × AMF interaction on the level of shoot nitrogen was also significant ($p<0.001$). Kunde Mboga had the highest level of shoot N (50.25%) followed by KenKunde 1 (47.29%) and Katumani 80 (40.00%). The wild cultivar had the lowest increase in shoot N (33.61%) following inoculation of cowpea with AMF using non-sterilized soil (Table 4.10).

There was a very strong significant difference ($p<0.001$) in shoot K in all the cowpea cultivars. The wild species had the highest mean of shoot K while Katumani 80 had the lowest mean of shoot K (Table 4.8). Shoot K content between the mycorrhizal inoculated and non-inoculated cowpea differed significantly ($p<0.001$). Mycorrhizal inoculated cowpea had a higher content of shoot K compared to the non-inoculated (Table 4.9).

The genotype × AMF interaction had a significant effect ($p<0.001$) on shoot potassium in cowpea. The highest percentage increase in shoot K was recorded in Katumani 80 (3.84%) followed by Kunde Mboga (2.69%) and KenKunde 1 (1.21%). Contrary results were observed in the wild cultivar where there was a 3.69% decrease in shoot K following inoculation of cowpea with AMF using non-sterilized soil (Table 4.10).

Shoot P level differed significantly ($p<0.001$) in all the cowpea genotypes. Katumani 80 had the highest level of shoot phosphorous while the wild species had the lowest (Table 4.9). There was also a very significant difference ($p<0.001$) in shoot P level between mycorrhizal-inoculated and the non-inoculated cowpea, with the inoculated plants having a higher level of shoot phosphorous compared to the non-inoculated plants (Table 4.9).

The effect genotype × AMF interaction on the level of shoot phosphorous was very significant ($p<0.001$). The highest percentage increase in shoot P was observed in Kunde Mboga (44.07%) followed by KenKunde 1 (43.18%) and Katumani 80 (34.03%). The wild

cultivar had the lowest percentage increase (28.75%) in shoot P following inoculation of cowpea with AMF using non-sterilized soil (Table 4.10).

Table 4. 9: ANOVA results for the effects of cowpea genotype, AMF inoculation and their interaction on shoot N, K and P of cowpea grown in non-sterilized soil.

	%N	K (ppm)	P (ppm)
Cowpea genotype			
K	3.38 ± 0.32a	2540.00 ±19.15c	1487.50 ± 115.39a
KM	3.04 ± 0.38b	2612.88 ± 8.07b	1288.38 ± 138.34b
KK	2.66 ± 0.31c	2418.88 ± 6.59d	1117.13 ± 116.50c
Wild	2.01 ± 0.15d	2827.50 ± 20.26a	888.125 ± 55.97d
AMF inoculation			
M	3.55 ± 0.18a	2608.13 ± 31.26a	1477.06 ± 74.00a
NM	2.00 ± 0.09b	2591.50 ± 46.53b	913.50 ± 43.48b
P values of the main factors and interaction			
Genotype	< 0.001	< 0.001	< 0.001
AMF In	< 0.001	< 0.001	< 0.001
Genotype x AMF In	< 0.001	< 0.001	< 0.001

Values followed by the same letter in a column within each treatment are not significantly different at $p<0.05$ (Tukey's HSD test).

Key: K-Katumani 80, KM-Kunde mboga, KK-KenKunde1, M-Mycorrhizal, NM-Non-mycorrhizal (control), AMF In- Arbuscular mycorrhizal fungi inoculation.

Table 4. 10: The effect of interaction between genotype and AMF inoculation on shoot N, K and P nutrition of cowpea grown using non-sterilized soil. The mean standard errors are presented in parentheses

Cultivar	Treatment	%N	K(ppm)	P (ppm)
K	M	4.23 (0.02)	2590.50 (1.55)	1792.75 (3.15)
	NM	2.53 (0.02)	2489.50 (2.84)	1182.25 (2.56)
KM	M	4.05 (0.03)	2663.25 (4.21)	1654.25 (5.79)
	NM	2.03 (0.00)	2592.50 (3.10)	922.50 (5.81)
KK	M	3.49 (0.02)	2434.75 (2.95)	1425.25 (5.14)
	NM	1.83 (0.02)	2403.00 (5.12)	809.00 (3.70)
Wild	M	2.42 (0.02)	2774.00 (2.20)	1036.00 (5.87)
	NM	1.61 (0.01)	2881.00 (1.68)	740.25 (2.10)

Key: K-Katumani 80, KM-Kunde Mboga, KK-KenKunde1, M-Mycorrhizal, NM-Non-mycorrhizal (control), AMF In- Arbuscular mycorrhizal fungi inoculation.

4.9 Correlation between root AMF colonization and shoot nutrition in non-sterilized soil

There was a very strong positive correlation (r^2 = 0.9860, p <0.001) between root AMF colonization and shoot nitrogen in cowpea cultivars inoculated and grown using non-sterilized soil (Fig 4.3; Table 4.11). Increasing levels of root AMF colonization corresponded to high levels of shoot nitrogen (Fig 4.3). A similar scenario was also recorded for shoot phosphorous, which had a strong positive correlation with root AMF colonization (r^2 = 0.9759, p <0.001) (Fig 4.4). A contrary result was obtained for shoot potassium. There was a no correlation between root AMF colonization and shoot potassium levels (r^2 = -0.0610, p = 0.173) (Table 4.11).

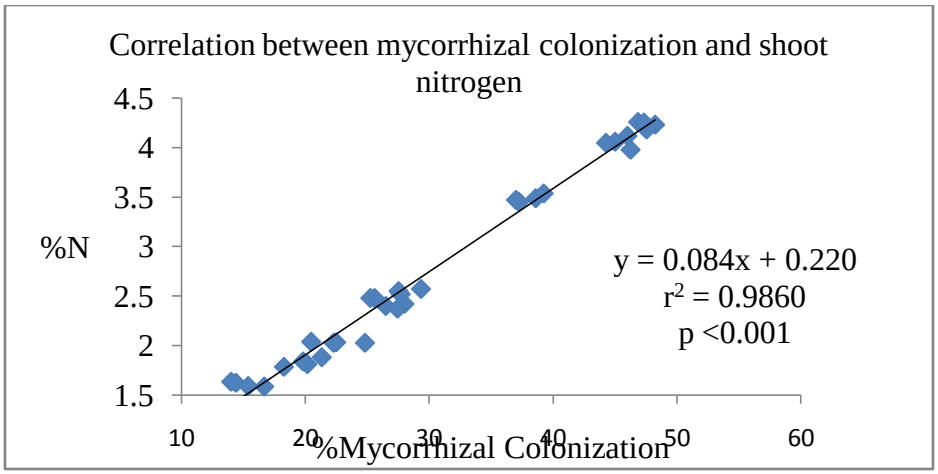

Fig 4. 3: Relationship between root mycorrhizal colonization and shoot nitrogen level following AMF inoculation of cowpea grown in non-sterilized soil

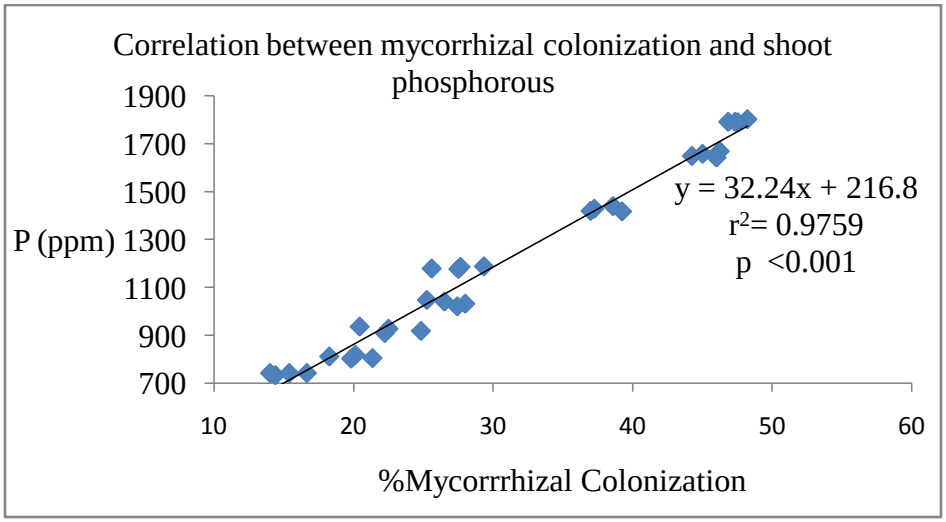

Fig 4. 4: Relationship between root mycorrhizal colonization and shoot phosphorous level following AMF inoculation of cowpea grown in non-sterilized soil

Table 4. 11: Correlation between root AMF colonization, root dry weight, shoot dry weight, nodule number, nodule dry weight, % N, K and P in non-sterilized soil.

		%MC	RDW	SDW	NN	NDW	%N	K (ppm)	P (ppm)
%MC		1							
RDW	Pearson Correlation	0.975**	1						
	Sig. (2-tailed)	<0.001							
SDW	Pearson Correlation	0.993**	0.980**	1					
	Sig. (2-tailed)	<0.001	<0.001						
NN	Pearson Correlation	0.684**	0.630**	0.695**	1				
	Sig. (2-tailed)	<0.001	<0.001	<0.001					
NDW	Pearson Correlation	0.955**	0.934**	0.963**	0.752**	1			
	Sig. (2-tailed)	<0.001	<0.001	<0.001	<0.001				
%N	Pearson Correlation	0.993**	0.979**	.999**	0.693**	0.963**	1		
	Sig. (2-tailed)	<0.001	<0.001	<0.001	<0.001	<0.001			
K (ppm)	Pearson Correlation	-0.247	-0.161	-0.221	-0.326	-0.134	-0.222	1	
	Sig. (2-tailed)	0.173	0.380	0.225	0.069	0.463	0.221		
P (ppm)	Pearson Correlation	0.988**	0.978**	0.994**	0.688**	0.941**	0.993**	-0.231	1
	Sig. (2-tailed)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.203	

**. Correlation is significant at the 0.01 level (2-tailed)

Key: MC- Mycorrhizal colonization, SDW-Shoot dry weight, NN- Nodule number, NDW- Nodule dry weight.

4.10 Pod retention frequencies among different *Vigna vexillata* and *Vigna unguiculata* Combinations

The cowpea accession line 219 combined with *Vigna vexillata* line Ni935 better than line 524B. The combination Ni935 X 219 resulted to formation of hybrid pods that were retained for more days compared to the combination Ni935 X524B (Table 4.12). Although most of the pods formed from Ni935 X 219 fell within 1 and 6 days after pollination (DAP), eight pods were retained up to 12 DAP. On the other hand, none of the pods formed by the combination Ni953 X 524B reached 12 days. Most of the pods resulting from this combination withered and dropped between 1 and 5 days. More than 50% of the pods dropped 2 DAP (Table 4.11). Ni935 X 219 was a better combination with respect to pod retention compared to Ni953 X 524B combination (Fig 4.5).

The details of pod retention of cowpea line 219 crossed with different lines of wild species *V. vexillata*¸ with *V. vexillata* as the pollen donor were also determined (Table 4.13). Differences in pod retention were evident among different *vexillata* lines. Ni935 had the highest pod retention frequency, with more than 50% of the pods being retained for more than 7 days, while v263 had the lowest pod retention frequency (Table 4.13). All the pods formed from crossing Ni935 and v263 withered prematurely within 10 days after pollination (DAP). Only crosses with Ni935 and Ni936 *Vexillata* cultivars retained pods up to 12 DAP (Table 4.13).

Table 4. 12: A comparison of pod retention frequencies between *Vigna vexillata* (Ni 935) and two cowpea lines (219 and 524B).

No. of days at dropping	No. of pods (Ni 935 x219)	No. of pods (Ni 935 x524B)
1	1	2
2	14	61
3	39	66
4	44	47
5	29	24
6	5	0
7	3	0
8	5	0
9	13	0
10	27	0
11	12	0
12	8	0
Total cross attempts	200	200

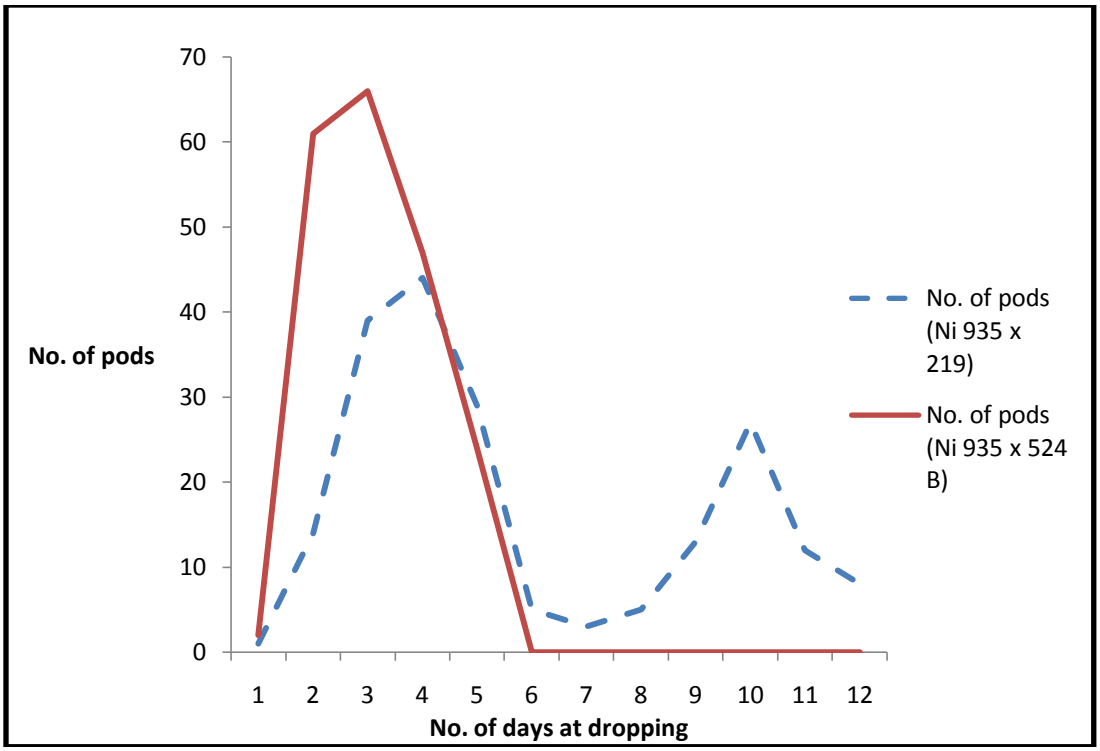

Fig 4. 5: The relationship between cowpea lines 219 and 524B with respect to pod retention when manually pollinated with *Vigna vexillata* line Ni 935

Table 4. 13: Pod retention frequencies between *V.unguiculata* (sp 219) and different *V. vexillata* lines. A total of 200 attempts were made for each combination.

	No. of pods retained				
DAP	Ni935 x 219	V263 x 219	Ni936 x 219	V268 x 219	AC305 x 219
1-2	15 (7.5%)	76 (38%)	49 (24.5%)	54 (27%)	24 (12%)
3-4	8 (4%)	85 (42.5%)	47 (23.5%)	90 (45%)	97 (48.5%)
5-6	34 (17%)	23 (11.5%)	42 (21%)	20 (10%)	44 (22%)
7-8	83 (41.5%)	8 (4%)	33 (16.5%)	8 (4%)	10 (5%)
9-10	40 (20%)	8 (4%)	24 (12%)	21 (10.5%)	21 (10.5%)
11-12	20 (10%)	0 (0%)	5 (2.5%)	4 (2%)	0 (0%)

Key; DAP-days after pollination

4.11 Embryo rescue

Following manual pollination using the cultivated cowpea as the pollen donor and the wild cowpea lines as pollen recipients, pods were generated (plate 4.1 and 4.2). While majority of the pods withered and fell down within 9 days, 10 to 12 day old pods were harvested and embryos excised prior to culturing them in artificial media. Ni935 x 219 had the highest number of immature embryos while V263 x 219 had the lowest number of immature embryos. All the embryos cultured following self pollination of the six cowpea accessions used in this study were rescued successfully (Table 4.14). These served as the controls, to show that the MS media used for culturing contained all the nutrients necessary to support endosperm growth and germination. Their successful rescue also showed that all the accessions used were viable. Plate 4.3 and 4.4 clearly illustrate 12 day old and 10 day old embryos resulting from self-pollination of Ni935 cowpea accession and that were successfully rescued in artificial media. Unfortunately, none of the embryos resulted from interspecific crossing was successfully rescued (Table 4.14). Hence, no interspecific hybrid was generated.

Plate 4. 1: Interspecific cross (Ni935 x 219)

Plate 4. 2: Interspecific cross (Ni935 x 219)

Plate 4. 3: Selfed embryo cultured in MS media

Table 4. 14: Embryos rescued following self pollination and interspecific pollination between the cultivated cowpea (sp 219) versus different *V. vexillata* lines

Cross combination	No. of pods	No. of embryos cultures	Successful rescues
Ni935 x 219	60	410	0
V263 x 219	8	42	0
Ni936 x 219	29	147	0
V268 x 219	25	152	0
AC305 x 219	21	103	0
219 selfed	10	50	50
Ni935 selfed	10	50	50
V263 selfed	10	50	50
Ni936 selfed	10	50	50
V268 selfed	10	50	50
AC305 selfed	10	50	50

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Effect of AMF Inoculation on Root Colonization

AMF symbiosis, which plays a fundamental role in plant nutrient uptake, starts with root colonization by AMF. Previous studies have shown that the level of AMF colonization differs among plant genotypes (Zhu *et al*., 2001; Tawaraya, 2003). This is consistent with the findings of this study where root mycorrhizal colonization of cowpea was significantly affected by the genotype ($p<0.001$). The modern cultivars showed a higher AMF colonization in comparison to the wild species. Additionally, there was variability within the modern cultivars, with Katumani 80 having the highest percentage of AMF-colonized root length and KenKunde1 having the lowest colonization as shown in Table 4.2. The results of the present study were consistent with the findings of a study carried out by (Zhu *et al*., 2001), which showed that modern cultivars had higher colonization than the older cultivars. In an extensive comparison of several lines of maize, modern hybrids showed a significantly higher percentage mycorrhizal colonization than older landraces and inbred lines (An *et al*., 2010). A study of mutants of soybean and *Lotus japonicas* revealed accelerated AMF colonization and increased arbuscule formation compared to wild type plants (Solaiman and Senoo, 2005). This may be due to differences the extent of development of fine roots.

In contrast, other studies have shown higher AMF colonization in wild species. For instance, studies conducted by (Yücel *et al*., 2009), led to the finding that old wheat cultivars relied

more on mycorrhizal symbiosis than the modern wheat cultivars. Hence, old wheat cultivars had higher AMF colonization and infection than the modern cultivars. While they concluded that breeding could be effective in developing new varieties for reduced input agriculture (Sawers *et al*., 2010), this may not be case for the current study using cowpea. According to Steinkellner *et al*. (2012), though tomato cultivars differed in their susceptibility to AMF colonization, these differences were not dependent on the cultivar age. These findings are contrary to those obtained from the current study, where AMF colonization was dependent on the type of cultivar.

Genotypic variation to AMF responses may arise from differences in the degree of fine root sdevelopment (Lebrón *et al*., 2012). In this case, the wild cowpea cultivar had lower hyphal length compared to the modern cultivars, thereby allocating less photosynthate to AMF, which might limit their ability to grow into the soil, and absorb nutrients. The differences could also be a reflection of the inherent traits of the cultivars, there rhizosphere, or differences in soil nutrients' availability.

The plant genotype determines the effect of AMF by influencing AMF development and consequently the AMF populations flourishing in the soil. Some AMF that are good colonizers on other species may even be denied access in some species (Sanders *et al*., 2003) and fail to reproduce. This differential colonization accounts for the genotypic differences observed with respect to parameters such as nodulation, dry matter and nutrition. Hence, treatments with high AMF root colonization had higher levels of different parameters such as dry matter and nodulation.

5.1.2 Effect of AMF Colonization on Nodulation

In the current study, AMF inoculation increased the nodule number and nodule dry weight in all the cowpea genotypes. The inoculated plants had a higher nodule number and dry weight compared to the non-inoculated plants. This is in line with studies that have shown that different AMF species are able to increase nodulation and N fixation. Tajini *et al*. (2012), found that common bean (*Phaseolus vulgaris* L.) plants inoculated with *Glomus intraradices* and *Rhizobium* had higher number of nodules and higher nodule dry weight than non-inoculated plants. This suggested that combined inoculation of plants with both AMF and rhizobia increases the phosphorous use efficiency for symbiotic nitrogen fixation. Our results are also consistent to those obtained by Goicoechea *et al*. (2004) on *Anthylis cytisoides* L., a drought-tolerant legume that can form symbiosis with both mycorrhizal and rhizobial microsymbionts.

The study demonstrated that AMF inoculation increased nodulation and nitrogen fixation, which in turn improved N nutrition. Similarly, a study done by Huang *et al*. (2014), on white clover (*Trifolium repens*) found a significant correlation between percentage AMF colonization and number of nodules, and these findings are in line with those obtained from the current study. Contrary findings were obtained in alfalfa (Catford *et al*., 2003), whereby colonization of roots with AMF systematically inhibited further mycorrhization as well as nodule formation.

Larimer *et al*. (2014) investigated the synergistic effects of AMF and rhizobia on growth and nodulation of a prairie legume, *Armorpha canescens.* Strong synergistic effects between both

symbionts were found on plant biomass production and nodulation, which were dependent on nutrient level. AMF infection increased root nodule number and mass while rhizobia inoculation decreased AMF hyphal root colonization. Larimer *et al.* (2014), further noted that the relative benefits of each combination of symbionts were determined by the phosphorous level in the soil. Phosphorous is the key nutrient utilized by legumes for nodulation process. In the current study, AMF inoculation resulted in increased phosphorous uptake, and this could account for increased nodulation.

5.1.3 Effect of AMF Colonization on Dry Matter

The current study showed that the shoot and root dry matter increased in all the cowpea varieties following inoculation with AMF. This suggests that mycorrhizal inoculation has a positive effect on plant height, leaves and roots, which consequently results to increased dry weight. These results are in line with the findings of (Sharif *et al*., 2009), which showed that the root and shoot dry matter of wheat increased after inoculation with AMF. The dry matter differences between mycorrhizal and non-mycorrhizal plants were due to beneficial effects derived from mycorrhizal association.

Al-Karaki *et al*. (1998) studied the effects of AMF inoculation on two wheat genotypes and the findings revealed that that AMF inoculated genotypes had higher root and shoot dry matter than non-inoculated plants. Similar findings were found by Jan *et al*. (2014), indicating a positive correlation between root dry weights and shoot P in wheat samples inoculated with AMF, results that are consistent with those obtained from the current study. According to Mandou *et al*. (2015), AMF inoculation increases the shoot and root dry matter

of micropropagated banana plantlets, which may enhance photosynthesis rate and nutrient uptake. It has been noted that enhanced growth effects on mycorrhizal plants are due to improved water relations resulting from enhanced P nutrition (Mandou *et al*., 2015).

5.1.4 Effect of AMF Colonization on Shoot Nutrition

The results of the current study showed a significant difference in the effects of treatment and varieties in the uptake of nutrients (P and N) in all the cowpea genotypes ($p<0.001$). Mycorrhizal plants had higher levels of shoot phosphorous and nitrogen compared to non-mycorrhizal plants. These results are consistent with those obtained by (Yaseen *et al*., 2011), who found out that nutrient uptake in two cowpea varieties inoculated with AMF was higher than that in non-inoculated plants. The results of this study are also in line with those of (Ghazala, 2005; Singh and Gogoi, 2012 and Sharma *et al*., 2013), who reported that mycorrhizal plants had higher nutrient uptake compared to non-mycorrhizal plants.

Other studies that have shown enhanced P nutrition following AMF inoculation have been done on cowpea (Yaseen *et al*., 2011), maize (Antunes *et al*., 2009; Miransari, 2011), tomato (Cavagnaro *et al*., 2006; Cavagnaro and Martin, 2010; Abdel Latef and Chaoxing, 2011), and cucumber (Ortas, 2010). Although phosphorous is critical for plant growth and makes up about 0.2% of dry mass, it is one of the most difficult nutrients for plants to acquire (Habibzadeh, 2015). It may be present in relatively large amounts in soil, but much of it is poorly available because of the very low solubility of phosphates of aluminum, iron and calcium, or very low mobility (Ryan *et al*., 2005). The increase in phosphorous uptake is one of the most dramatic effects of mycorrhizal infection on the host plant (Bai *et al*., 2008), and

this is because mycorrhizal fungi have the ability to absorb phosphate from soil and transfer it to the host plant. These results are further supported by the statement that AMF improve uptake of immobile nutrients such as P and Zn (Balakrishnan and Subramanian, 2012). The extensive AMF hyphal network alters the physiochemical properties of soil and directly or indirectly contributes to the release of phosphates from inorganic complexes that have low solubility (Finlay, 2008).

The AMF cytoplasm may serve as a host to bacterial endophytes, particularly, plant growth promoting rhizobacteria. Although, there is a need to clarify the role of endophytes living within AMF spores, some evidence has shown that they could be involved in nutrient exchange between the partners (Varennes and Goss, 2007). Tripartite symbiosis, which incorporates nitrogen fixation by rhizobia, could explain why there was increased N uptake by AMF inoculated plants in the current study. Through this symbiosis, the legume-rhizobia crop fixes nitrogen and provides adequate carbon to AMF which in turn provides nutritional benefits, especially P, a key nutrient for N_2 fixation. The plant N acquisition may also have been enhanced by increased exploration of the soil volume by mycorrhizal hyphae. Enhanced N uptake through AMF symbiosis has further been shown in maize (*Zea mays* L.) (Miransari, 2011), melon plant (*Cucumis melo* L.) (Martínez-Medina *et al*., 2011) and Long pepper (*Piper longum* L.) (Singh and Gogoi, 2012). Nonetheless, contradictory results have been reported by Reynolds *et al*. (2005), who found that AMF did not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen supply.

Although limited studies have been done to investigate the role of AMF in K uptake, a few studies have reported increased uptake of the nutrient. Studies done on tomato (*Lycopersicon*

esculentum) (Abdel Latef and Chaoxing, 2011), maize (*Zea mays*) (Miransari, 2011) and melon plant (*Cucumis melo* L.) (Martínez-Medina *et al*., 2011) showed that K uptake was enhanced following AMF inoculation. These results are contrary to those from the current study where there was no significant relationship between K uptake and AMF colonization.

5.1.5 Effect of Genotype on the Success of Cross Pollination

The wide interspecific crossing in the current study showed that *Vigna vexillata* accession Ni 935 combined better with *Vigna unguiculata* accession 219 in comparison to accession 524B. Further crossing of accession 219 with different *Vigna vexillata* accessions revealed differences with respect to pod retention frequency. Combination Ni935 X 219 had the highest pod retention frequency since it led to formation of pods that were retained for the longest days. The results of this study are consistent with previous interspecific hybridization studies that have shown that certain cultivars and accessions of species are better combiners to recover interspecific hybrids than other cross combinations (Pickersgill, 1983).

Similarly, a study conducted by Gomathinayagam, *et al*. (1998) using cowpea cultivar Co6 with the wild species *V. vexillata* revealed significant differences in pod retention among direct and reciprocal crosses. In line with our study, pod retention was extended up to 12 days. Differences in pod retention have also been reported in four species of *Vigna* food legumes (Chen *et al*., 1983).

5.1.6 Embryo Culture

In the present study, most of the interspecific pods collapsed within 5-8 days after pollination, in line with the findings obtained by Barone *et al*. (1992). The authors noted that the interspecific embryo showed a slower growth compared to a selfed one, and the growth stops at 5-8 days (globular stage). The cause of embryo abortion and subsequent pod collapse seems to be caused by failure of endosperm development. In the present study, no interspecific hybrid was formed following embryo rescue. These results are consistent with those obtained by Gomathinayagam *et al*. (1998), where embryos died following their rescue using MS media.

Barone *et al*. (1992) identified two factors that led to the failure of interspecific hybridization between *Vigna vexillata* and *Vigna unguiculata*. Firstly, there is pollen-pistil incompatibility, which results to reduction in fertilization and the collapse of fertilized ovules. The second factor is the existence of a pre-zygotic barrier after interspecific pollination, which is characterized by abnormal growth behavior of the pollen tube after interspecific crossing.

5.2 Conclusions

In this study, AMF might improve cowpea production by enhancing uptake of nutrients, particularly P and N. AMF inoculation had a positive effect on different cowpea growth parameters including nodule number, nodule dry weight and root and shoot dry weights. Thus, the study further revealed that modern cultivars were more responsive to AMF inoculation than the wild species since they had higher root colonization, dry matter, and shoot P and N than the wild species.

The current study has shown that the modern cultivars were more responsive to AMF inoculation than the wild species. For this reason, they had higher root AMF colonization and higher dry matter than the old cultivar. This is contrary to previous studies, which have shown that modern breeding programs may suppress AMF colonization. This is probably because modern plant cultivars are usually grown in nutrient-rich environments, which have been thought to negatively affect AMF colonization.

Although modern breeding may suppress AMF colonization since plant cultivars are usually bred in nutrient-rich environment, this is not the case in our study. Hence, there is the need to screen different cowpea cultivars for AMF symbiosis, which should be extended to other crops. Considering the differences revealed in terms of responses of different plant genotypes to AMF, as demonstrated in this other studies, future studies should elucidate appropriate genotype-AMF combinations in order to obtain the optimal benefit from mycorrhizal symbiosis.

The present results have also shown that wide interspecific crosses between cowpea and its wild relative *Vigna unguiculata* is still a challenge. Some cross combinations are still better than others with respect to pod retention, although an insect pest-resistant hybrid was not generated.

5.3 Recommendations

- The current findings push for the need to screen different cowpea cultivars for AMF symbiosis. The screening in question should also be extended to other crops.
- Although shoot nutrition was generally enhanced following AMF inoculation, it is still unclear whether this was extended to the leaves and grains. It is thus, recommended to do studies to examine the quality of vegetables and grains.
- The hybridization experiment in the current study targeted 9-12 day old embryos, which are at a heart-shaped stage. Although culturing younger embryos necessitates addition of complex nutrients, future studies should still attempt to culture younger embryos at globular stage.

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