IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION ON SOYBEAN (*Glycine max*) PRODUCTION IN THE SEMI-DECIDUOUS FOREST ZONE OF GHANA

By

Malamine Thioub

May, 2018

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND

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(BSc Natural Sciences; MPhil. Phytopharmacy and Plant Protection)

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DOCTOR OF PHILOSOPHY IN SOIL SCIENCE

May, 2018

DECLARATION

I, Malamine Thioub, hereby declare that this submission is my own work towards the PhD degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any degree of the University, except where due acknowledgement has been made in the text.

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(Head of Department) Signature Date

DEDICATION

I gratefully dedicate this dissertation to my father Samba Thioub, my mother Thiané Wade, Prof. Libasse Diop and Prof. Kandioura Noba of Cheikh Anta Diop University of Dakar.

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ABSTRACT

Phosphorus (P) is an important but limiting major nutrient in crop production. Its availability in the soil and consequently for plant nutrition remains a major challenge. The overall aim of this study therefore was to assess the potential of four arbuscular mycorrhizal fungi (AMF) isolates in enhancing mineral P fertilizer use efficiency in soybean and their beneficial residual effects on succeeding maize crop. Two pot experiments were conducted under greenhouse conditions using sterile river sand and a non-sterile soil on which two soybean lines (TGx 1989-48 FN and TGx 1989-75 FN) were evaluated. The soybean lines were inoculated with the four mycorrhizal isolates (*Glomus etunicatum*, *G. fasciculatum*, *G. mosseae* and *Rhizophagus irregularis*) as a preliminary screening study to select the top 50% symbionts. A factorial arrangement using a completely randomized design was used. Furthermore, a two-year field experiment was also conducted following the screening study during the minor and major cropping seasons of 2016 and 2017 respectively to evaluate the potential of combined mycorrhizal inoculation and P mineral fertilizer in enhancing soil P availability, soybean growth and yield and economic profitability. The two aforementioned soybean lines used in the pot experiments were separately inoculated with the top 50% mycorrhizal isolates (*G. mosseae* and *R. irregularis*). An uninoculated control treatment was included. Triple superphosphate (TSP) was applied at three levels (0, 15 and 30 kg P ha^{-1}). A split-split plot design with three replications was used; soybean lines as the main-plot factor, TSP rates as the sub-plot factor while the AMF isolates constituted the sub-sub plot factor. In a separate experiment, an early maturing maize variety (Omankwa) was planted following the minor cropping season of 2017 to evaluate the residual effects of the imposed

treatments on root length colonization, N and P uptake, shoot biomass and grain yields.

The greenhouse study revealed that *R. irregularis* and *G. mosseae* improved P uptake, plant height, stem girth and shoot biomass yield of soybean relative to *G. etunicatum* and *G. fasciculatum.* In the field experiment, inoculation with the selected AMF isolates combined with 15 kg P ha^{-1} resulted in the highest soybean root length colonization, P use efficiency, shoot and grain P uptake, plant height, shoot biomass and grain yields. Furthermore, AMF + 15 kg P ha^{-1} reduced soil microbial biomass P by stimulating P release by soil microbes and thus increased soil available P. Again, *G. mosseae* showed higher performance in soybean root length colonization and P uptake, which reflected in the better growth and yield improvement than *R. irregularis*. The economic analysis indicated that inoculation using the selected mycorrhizal isolates combined with 15 kg P ha^{-1} had the highest net benefit and marginal rates of return and is therefore economically more profitable for soybean production. *Glomus mosseae*, underfield conditions persisted even after two cropping years. Maize root length colonization was higher in plots previously inoculated with AMF, which reflected in highest maize shoot N and P uptake, grain P uptake, shoot biomass and grain yields relative to the control. This study established that, the appropriate management of AMF inoculation is a potential to reducing the use of inorganic P

fertilizers.

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CHAPTER ONE

1.0 INTRODUCTION

Major metabolic processes in plants such as photosynthesis, energy transfer, biosynthesis of macromolecules, respiration and signal transduction involve phosphorus (Khan *et al.*, 2010). Consequently, the mineral nutrition of plants mostly depends on the soil content of the compounds that can be absorbed as a soluble phosphate (Ehteshami, 2011). Phosphorus is therefore an important nutrient that plays a crucial role in soil fertility management for optimal growth and productivity of plants. Despite its importance, phosphorus is among the most deficient macronutrients in agricultural soils and its natural reserves are reducing at a higher rate due to intensive nutrient mining (Wahid *et al.*, 2016). Estimates show that soil phosphorus (P) reserves will be depleted by the end of 2050 particularly at the subtropical and tropical regions of the earth (Balemi and Negisho, 2012). Moreover, P is present in soils in both inorganic and organic forms. Due to complex formations with cations such as iron and aluminium, P is mostly unavailable to plants (Sharma *et al.*, 2013). Regular addition of P fertilizers is therefore necessary in phosphorus deficient soils to maintain a sufficient level of the element for crop sustainability (Naseer and Muhammad, 2014; da Costa *et al.*, 2015).

Despite the fact that phosphorus supply is highly needed to ensure food security for the world's increasing population, the huge and regular addition of phosphorus fertilizers increases the cost of production and environmental risks because of its high energy requirement for distribution, transportation and processing (Pizzeghello *et al.*, 2011). In addition, due to the world's population estimated to exceed nine billion by

2050, yield increase is forecasted to exceed the current global capacity to produce food, which therefore, emphasizes the necessity to establish or revive eco-friendly technologies (Rodriguez and Sanders, 2014).

Grown on large scale because of its ability to adapt to different climatic and soil conditions (Cely *et al.*, 2016), soybean (*Glycine max* L.) occupies the third position among the grain legumes, in terms of production and utilization in Ghana, after groundnut and cowpea (SRID, 2011). However, a major abiotic constraint hindering its production and leading to lower yields is rhizosphere nutrients availability, which is often a limiting factor (Garcia *et al.*, 2016). Precisely, He *et al.* (2017) reported that soybean yield is limited by low P availability.

An energy source for the transformation of dinitrogen into ammonia and bacterial growth is required during the nitrogen fixation process in legumes and P supplies the mechanism for energy accumulation in the form of adenosine triphosphate and the transfer of that source of energy (Murrell *et al.*, 1999). Plants have consequently evolved various strategies, including beneficial interactions with soil microorganisms that increase either phosphorus availability in soil or phosphorus uptake capacity (Bapaume and Reinhardt, 2012; Rai *et al.*, 2013). Various phosphate solubilizing fungi and bacteria with varied potentials, to solubilize inorganic P have been found in the root zone of plants (Jain *et al.*, 2012). Among the microbial components involved in soil bio-functioning are arbuscular mycorrhizal fungi (AMF) regarded as major elements of the plant/soil interface (Duponnois *et al.*, 2012).

In the context of sustainable agriculture, although conventional agriculture plays an important role in meeting the food needs of an increasing human population, farmers are forced by the global economic crisis to reduce the application of P fertilizers by using mycorrhizal inocula (Bhardwaj *et al.*, 2014; Berruti *et al.*, 2016). In addition to

the possible pollution of underground water reservoirs, the continuous application of high levels of inorganic fertilizers to soil drastically alters the interaction between microbial communities and plants (Berruti *et al.*, 2014). On the other hand, AMF increase soil inorganic nutrients uptake, particularly phosphorus (Neumann and George, 2010). The roots of cultivated plants are generally colonized by AMF and when establishing symbiosis, the proliferation of mycelial hyphae into the soil increases the prospecting volume with respect to plant mineral resources. These hyphae can alter the complex forms of phosphorus and transfer the bioavailable phosphorus to the plant (Duponnois *et al.*, 2012). In the mycorrhizal symbiosis, partner selection is regarded as a key factor in stabilising the mutualism since the benefits are not free of charge, and one-sided for plants only (Bücking and Kafle, 2015). Arbuscular mycorrhizal fungi may use 4 to 16% of recently fixed photosynthates to maintain their growth, activity and reserves, but may supply in turn 100% of the plant nutrient requirements (Kaschuk, 2009). Both host plants and AMF have been reported to preferentially allocate nutrients to higher quality partners (Werner and Kiers, 2015).

In Ghana, small-scale soybean producers use little or no inorganic fertilizers and take advantage of biological nitrogen fixation to supplement its high nitrogen requirement (Mbanya, 2011). However, there is an increasingly urgent need to create awareness among farmers on the potential benefit of AMF in soybean and other crops production to supply supplementary phosphorus which limits crop yields. Although TwumAmpofo (2008) showed the importance of some AMF for legume tree growth and nutrition, a few researches have focused on AMF in Ghana, especially on cultivated crops.

Research oriented towards the development of high quality inocula with long shelf life, high proportions of infective propagules and formulations easy to handle, apply and

store should be carried out. Priority should be given to understanding the environments, crop species and mycorrhizal species interaction in order to select the most promising combinations; and assessing the efficiency of mycorrhizal inoculation under field conditions (Rouphael *et al.*, 2015). Nevertheless, applied research focused on defining the best inoculum formulation strategies and robust inoculation practices have first to be implemented even though the establishment of viable microbial populations that can persist over multiple seasons is one major problem (Verbruggen *et al.*, 2013). In addition, the role of AMF in the mobilization of microbial biomass pool of nutrients and the residual effects of mycorrhizal inoculation on succeeding crops deserve urgent research attention.

This research aims at evaluating the influence of some AMF isolates in enhancing mineral phosphorus fertilizer use efficiency of soybean and their potential benefit on succeeding maize crops. Based on the null hypothesis that inoculation with AMF will not enhance phosphorus uptake, phosphorus use efficiency, growth and yield of soybean and succeeding maize crop when combined with low P fertilization than high P, the following specific objectives were set to:

i. assess the symbiotic potential of four AMF isolates to improve the growth and yield of soybean, ii. investigate the effect of AMF inoculation and different P rates on nutrient uptake, growth and yield of soybean, iii. ascertain the available phosphorus and microbial biomass phosphorus status of the soil in relation to their response to AMF inoculation, iv. estimate the economic feasibility of AMF inoculation in increasing soybean yield and reducing phosphorus fertilizer application,

v. determine the residual effect of the arbuscular mycorrhizal inoculation on succeeding maize crops.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Economic importance of soybean

Cultivated on an estimated area of 6% of the world's arable land, soybean is one of the most important crops worldwide; its grains are consumed as vegetable oil and protein meal (Hartman *et al.*, 2011). Since the 1970s, the area of soybean production had the highest increase compared with other major crops (Hartman *et al.*, 2011). The world annual production of soybeans was 241,732,260 metric tonnes for a cultivated area of 105,477,217 ha in 2012. In Africa, production reached 2,125,078 metric tonnes with a cultivated area of 1,826,709 ha in 2012 (FAOSTAT, 2017). According to the Ministry of Food and Agriculture (MoFA) of Ghana, 151,709 metric tonnes of soybeans were produced in 2012, of which 128,953 metric tonnes were available for human consumption, from a yield of 1.9 metric tonnes per ha (SRID, 2013).

Soybean, like other leguminous crops, is capable of fixing atmospheric nitrogen for its own use and for the benefit of the succeeding crop in rotation as a residual effect. Its use in rotation with cereals results in drastic reduction in striga seed bank in soils (Kroschel and Sauerborn, 1988; Alabi, *et al.*, 1994; Berner *et al.*, 1994; Denwar and Ofori, 2003) and then reducing the devastating effect of the witchweed on such cereals.

Soybean (*Glycine max* (L.) Merill) utilization is rising in Ghana because of its numerous potential such as the supply of high quality protein that rank it even better than cowpea (CSRI-SARI, 2014). Rich in minerals particularly phosphorus, calcium, vitamins and iron (Messina, 1999; Nti *et al.*, 2005), soybean has a high quality oil without cholesterol and its protein is likewise of high quality and comparable to those from animal sources such as milk, egg and meat (Carroll *et al.*, 1978). In the animal feed industry in Ghana, soybean meal/cake is a preferred protein source. It is currently used to prepare weaning foods for babies to prevent kwashiorkor in children (AsafoAdjei *et al.*, 2005), to fortify various traditional foods such as kenkey, banku,

sauces, stew and gari to improve their nutritional levels without changing their cooking time or taste (Asafo-Adjei *et al.*, 2005).

2.2 Phosphorus deficiency as limiting factor to legume nitrogen fixation and yield

Scarcity of other resources, such as P, is the main cause of restrictions on plant growth due to the fact that cultivated legumes can symbiotically reduce atmospheric dinitrogen (N_2) and make it available to plants (Tinker and Nye, 2000). Phosphorus plays a crucial role in the symbiotic nitrogen (N) fixation process of legumes by improving shoot and root growth (the ability of a plant to fix N is reduced by restricted root development). Phosphorus allows to increase the size and number of nodules and the amount of nitrogen assimilated per unit weight of nodules, and shorten the time required for nodules to become active and benefit the host legume. Finally, P plays a key role in increasing the total amount and percentage of N in the harvested portion of the host legume and increasing the density of rhizobia in the rhizosphere (Murrell *et al.*, 1999).

The high phosphorus concentration in nodules expresses the high demand of legumes for phosphorus nutrition (Rotaru, 2010). Phosphorus is involved as an energy source when 16 molecules of adenosine triphosphate are converted to adenosine diphosphate as each molecule of N_2 is reduced to NH_3. The adenosine triphosphate is generated during photosynthesis of plants, when light energy is transformed and stored in the form of adenosine triphosphate (Murrell *et al.*, 1999).

In spite of its paramount importance, phosphorus scarcity largely limits legume production because nitrogen nutrition of legume mainly depends on the rhizobial symbiosis, especially in acidic and calcareous soils. Thus, in several low-input systems, low soil phosphorus availability is a major constraint to legume productivity (Drevon *et al.*, 2015).

2.3 Phosphorus and plants

2.3.1 Functions of phosphorus in plants

Phosphorus plays a vital role in virtually all plant metabolic processes involving photosynthesis, energy transfer and signal transduction, crop quality, resistance to plant diseases and nitrogen fixation in legumes (Khan *et al.*, 2014). Any other nutrient cannot perform its functions, and for optimum growth and reproduction, an adequate supply of phosphorus is required. Classified as a major nutrient, P is frequently deficient in agricultural soils and required by crops in relatively large amounts. The total phosphorus concentration in agricultural crops generally varies from 0.1 to 0.5% (Murrell *et al.*, 1999).

Phosphorus is essential in various physiological processes of plants, notably in carbon metabolism, membrane formation and photosynthesis (Wu *et al.*, 2005). Its deficiency affects seed development, normal crop maturity (Anand *et al.*, 2016) and root architecture because it plays a key role in root elongation and proliferation (Borch *et al.*, 1999; Williamson, 2001). An appropriate addition of P during early stages of plant growth is crucial for synthesizing the primordia of plant reproductive organs (Sharma *et al.*, 2013). Phosphorus deficiency negatively affects grain yields (Anand *et al.*, 2016) because a major part of phosphorus absorbed by plants is amassed in grains as phytase (Richardson, 1994).

2.3.2 Uptake and transport of phosphorus

Phosphorus penetrates the plant through the outermost layers of root cells, root tips, and root hairs. Phosphorus uptake is also facilitated by the symbiotic association between mycorrhizal fungi and the roots of many crops. Phosphorus is mainly absorbed as the primary orthophosphate ion ($H_2PO_4^-$), but may also be taken up as

secondary orthophosphate (HPO_4^{2-}) and the latter form increases with the increase in soil pH (Murrell *et al.*, 1999).

Once inside root, P may either be stored in the root or transported to the upper portions of the plant. It is incorporated, through various chemical reactions, into organic compounds including RNA and DNA (nucleic acids), phospholipids, enzymes, phosphoproteins, energy-rich phosphate compounds such as adenosine triphosphate and sugar phosphates. Phosphorus is moved throughout the plant in these organic forms and also the inorganic phosphate ion, where it is available for further reactions (Murrell *et al.*, 1999).

2.3.3 Phosphorus use efficiency and plant growth

Phosphorus use efficiency can be defined as the capability of plants of producing their dry matter in a soil limiting in phosphorus, considering yield parameters (Buso and Bliss, 1988). The term nutrient efficiency ratio, calculated as the reciprocal of the nutrient concentration in the whole plant, has been used by some scientists (Gourley *et al.*, 1994) and the term nutrient uptake efficiency has also been used by others (Buso and Bliss, 1988). The latter is defined as the specific uptake per unit root length or the total uptake per plant (Marschner, 1995). It was hypothesized by Nuruzzaman *et al.* (2005) that phosphorus acquisition efficiency in plant species varies with their rhizosphere chemistry and volume.

When a plant is deficient in phosphorus, one of the two factors that control plant growth is the phosphorus use efficiency (PUE) (Haynes *et al.*, 1991; Koide, 1991). The other factor is the rate of phosphorus absorption or phosphorus influx. The phosphorus use efficiency may vary according to tissue type. For instance, because stems require less phosphorus to produce a given dry weight than do seeds, they often have higher phosphorus use efficiency than seeds. Phosphorus use efficiency can also vary

depending on mycorrhizal colonization and soil available phosphorus (Haynes *et al.*, 1991; Koide, 1991), and among plant species (Christie and Moorby, 1975; Haynes *et al.*, 1991).

2.3.4 Factors affecting phosphorus use efficiency

The rate of phosphorus influx or phosphorus absorption into the roots is controlled by two factors: (i) the first factor is the amount of diverse nutrients available for plant, including nitrogen, phosphorus etc., it regulates phosphorus absorption by the plant and determines the ability of the root surface area to absorb phosphorus from the soil. The quantity of phosphorus utilized to produce root surface area in order to absorb more phosphorus from the soil increases with the increase of the internal phosphorus reserve of the plant (Bloom *et al.*, 1985). Therefore, among the nutrient elements which are under the control of the plant, phosphorus is the most critical. (ii) The second factor is the efficiency with which the phosphorus held by the plant is used to absorb phosphorus from the soil. However, because P is the limiting resource, instant benefit results from more efficient use of plant P (Rai *et al.*, 2013).

Phosphorus efficiency index (PEI) is defined as the efficiency with which the internal phosphorus reserve is used to absorb phosphorus from the soil (Koide *et al.*, 1999). Phosphorus absorption into roots is controlled by both plant phosphorus content and PEI, and accordingly, the plant growth rate is a function of plant phosphorus content, PUE and PEI (Rai *et al.*, 2013).

2.3.5 Current problems related to the use of mineral phosphorus fertilizers

Although it is usually present in abundance in soils, P is the second most limiting macronutrient in plant development after nitrogen. In fact, 75 to 90% of added phosphorus precipitate with metal–cation complexes and hence only a small amount of this phosphorus can be used by plants due to the fact that it is strongly bound in soils

(Sharma *et al.*, 2013). Furthermore, this element is sparingly mobile and the stock of inorganic P present in the immediate environment of plant roots are very quickly depleted by the them (Smith and Smith, 2011) (Figure 2.1). That is why today mineral large quantities of P fertilizers are applied to crops to ease off this lack in available P to meet the critical demand for crop productivity (Adhya *et al.*, 2015). However, it is estimated that only 15 to 30% of the intake is absorbed by plants and thereby the large addition of phosphates is relatively not effective (Cordell *et al.*, 2009).

The current mismanagement of inorganic chemical-based fertilizers is all the more regrettable because their use causes a serious threat to human health and environment (Bhardwaj *et al.*, 2014). In addition, the injudicious and repeated applications of chemical phosphorus fertilizers disturb microbial diversity, leading to the loss of soil fertility and consequently reduces yield of crops (Gyaneshwar *et al.*, 2002; Sharma *et al.*, 2013).

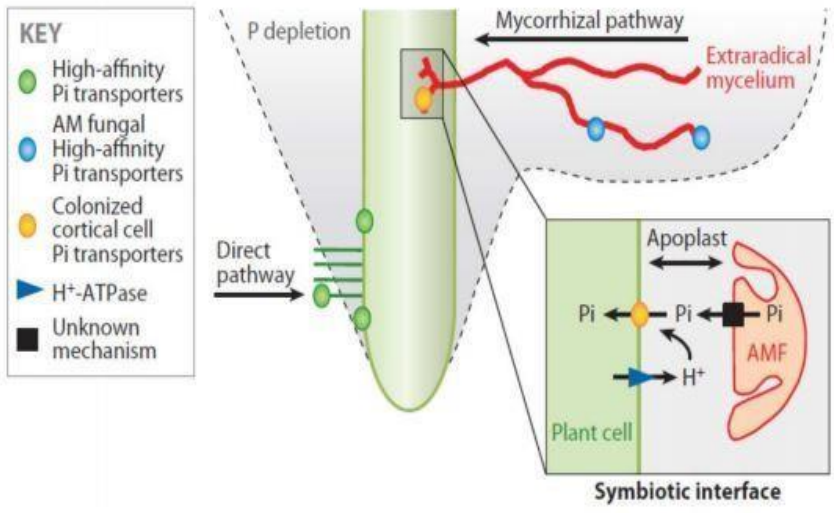

Figure 2.1. The different pathways of Pi acquisition in mycorrhizal plants (Smith and Smith, 2011)

The direct pathway (DP) is based on high affinity transporters located on the root hairs and in the epidermal area near the apex. This path leads to the formation of a zone depleted in Pi around the root.

The symbiotic pathway (MP = mycorrhizal pathway) involves the extraradical mycelium that captures the Pi present in the soil with high affinity transporters. The Pi is then transported through the hyphae of the fungus in the form of polyphosphates and is transferred to the roots at the level of the arbuscules by means of plant Pi transporters specifically expressed in the peri-arbuscular membrane (H^+ ATPases are also involved in the process of transfer of Pi). This two-way system (DP and MP) is also valid for nitrogen. In return, the fungus recovers sugars.

The long-term effect of application of phosphate fertilizers on microbial activities results in inhibition of substrate-induced respiration by microbial biomass carbon, actidione (bacterial activity) and streptomycin sulphate (fungal activity) (Bolan *et al.*, 1996). The addition of 94 kg of triple superphosphate per hectare has similarly shown an important decrease in microbial respiration and metabolic quotient (qCO_2) (Chandini and Dennis, 2002). In addition, the current system of plant production depends on fragile resources because the phosphorus brought into the fertilizers is mined from geological sediments which constitute limited natural mineral resources (Adhya *et al.*, 2015). Furthermore, as global demand constantly increases, phosphate reserves decline faster and after reaching a peak around 2030-2050, production is expected to decline (Gilbert, 2009).

Today scientists talk about the "phosphorus crisis", a crisis unfortunately still invisible to the majority of citizens but well known to farmers and plant biologists who study closely the regulatory channels and the multiple response mechanisms put in plants to cope with phosphate deficiency conditions. Today these processes are becoming well decrypted. They represent potential pathways for improving plant growing conditions, limiting the use of phosphate inputs (Balzergue, 2012).

2.3.6 Potential integrated management of excessive P fertilizers application

Despite the fact that P is a crucial macronutrient for development of plants, 95 to 99% of its amount found in the soil is in a insoluble form and thus cannot be utilized by plants (Vassileva *et al.*, 2000). This unavailability is because P either gets precipitated by free aluminium (Al^{3+}) and iron (Fe^{3+}) cations in the soil solution or is adsorbed on

the soil minerals (Sharma *et al.*, 2013). Soil P dynamics is characterized by biological and physicochemical processes. A large amount of P applied as fertilizers precipitates and enters into the immobile pools by reacting with Al^{3+} and Fe^{3+} in acidic and calcium (Ca^{2+}) in normal or calcareous soils (Gyaneshwar *et al.*, 2002; Hao *et al.*, 2000).

Although various amendments such as periodic incorporation of crop residues and application of organic manures are available for management of phosphorus in different soil, they are practically difficult and costlier. Therefore, even in the presence of high total soil phosphorus and regular application of phosphate fertilizers, the quantity of phosphorus available in the soil is determined by pH-dependent chemical fixation. The integrated phosphorus management includes a series of strategies such as manipulating soil and rhizosphere processes, improving phosphorus recycling efficiency and developing phosphorus efficient crops (Sharma *et al.*, 2013).

To change the scenario, the adaptation of approaches to convert a portion of unavailable phosphorus in soils into available form for plant absorption is needed. Among the various strategies, solubilization of the phosphorus bound in the soil using specific microorganisms is likely one of the most efficient approach. The use of microbial inoculants for improving soil fertility is common since last century, however rarely sufficient work has been reported on Phosphate Solubilizing Microorganisms (PSM) as compared to nitrogen fixation. Phosphate solubilizing microbes have the potential of making these phosphates available to the plants (Anand *et al.*, 2016).

Mediated phosphorus management using microbial inoculants is an cost effective and eco-friendly approach for sustainable agriculture (Sharma *et al.*, 2013). However, preparation of effective formulations after identifying the most suitable species and introduction of efficient agricultural management practices will be determinant in

increasing availability of soil phosphorus and improving phosphorus efficiency through microbial inoculation (Adhya *et al.*, 2015).

Arbuscular mycorrhizal fungi, notably *Funneliformis mosseae* (*G. mosseae*) and *Rhizophagus intraradices* (*R. irregularis*) are the most widely used in agriculture with the aim of enhancing phosphorus availability for crop plants (Krüger *et al.*, 2012). Acting as an extension of the root system, the extraradical mycelium of AMF increases soil nutrients uptake, especially phosphorus (Bücking and Kafle, 2015). In natural environments, one host plant is colonized by communities of AMF; and by simultaneously colonizing multiple host plants, fungal individuals play the role of interconnecting plants by common mycorrhizal networks. Plants and their AMF partners thereby form a complex network of many-to-many interactions (Fellbaum *et al.*, 2014). Therefore, consistent and extensive investigations in identifying and characterizing more P-solubilizing fungi are required for an ultimate application under field conditions (Khan *et al.*, 2010).

Over years, AMF and other PSM were thought to act individually and independently in soil but during the last recent years, they were found to simultaneously and collectively mobilize nutrient pools from the soil and AMF play a crucial and central role in that interaction (Taktek *et al.*, 2015).

2.4 Mycorrhizal fungi symbiosis with plants

2.4.1 The rhizosphere and its microbial community

Derived from the Greek "rhiza" meaning root and "sphere/sphaera" meaning circle of influence, the term rhizosphere was used for the first time by Hiltner (1904). The rhizosphere thus represents the field of influence of the root system, it includes all the roots as well as the zone of close soil influenced by these roots, either via root exudates

or via microorganisms interacting with the latter (Badri *et al.*, 2009). In the rhizosphere, roots live surrounded by many microorganisms forming a large reservoir of biological diversity. The set of microorganisms in a given environment is called microbiome. The importance of these microbial communities is increasingly studied in animals but also at the ecosystem scale (Berendsen *et al.*, 2012). The rhizosphere includes both beneficial and harmful organisms for plants. These organisms can be bacteria, viruses, fungi, oomycetes, insects, nematodes, but also parasitic plants or the roots of other neighbouring plants (Balzergue, 2012).

Among the various beneficial organisms for plants are mycorrhizal fungi. The word mycorrhiza comes from the Greek "mycos" for fungus and "rhiza" for root, so it defines an interaction between roots and fungi. The mycorrhizae can be morphologically and phylogenetically classified into two main groups: ectomycorrhizae where the fungus does not penetrate into root cells and endomycorrhizae where fungi enter the root cells to exchange with the plant. Other endo-root symbioses exist, in particular nitrogen-fixing symbioses making bacteria capable of assimilating atmospheric nitrogen (N), thus improving the N nutrition of the host plants (Balzergue, 2012).

The root zone can be divided up into five intergrading environments: bulk soil including senescent roots, thin layer of soil surrounding AMF hyphae called hyphosphere, soil between roots including AMF hyphae, root hair zone extending the rhizosphere up to some few centimetres away from the root surface and rhizosphere defined as the thin layer of soil surrounding roots (Hamel, 2004). Referring to the combined hyphosphere and rhizosphere zones of mycorrhizal plants, the term “mycorrhizosphere” was used by Linderman (1988).

2.4.2 Importance of mycorrhizal symbiosis with plant roots

The symbiotic relationship between soil fungi and plant roots, called mycorrhizal symbiosis, is among the predominant associations within terrestrial ecosystems (Smith and Read, 2008). Mycorrhizal fungi form symbiosis with nearly 95% of terrestrial plants, colonizing environments such as boreal, temperate and tropical forests, as well as tundras, grasslands and many cultivated lands (Read and Perez-Moreno, 2003).

During this interaction, the fungal hyphae networks, specialized in mineral absorption and soil organic nitrogen, act as an extension of the root system, increasing the uptake by the plant of essential nutrients such as phosphorus, nitrogen, sulphur and water (Smith and Read, 2008). Through this symbiosis, with the mutual fungi, plant species are able to acquire metabolic capacities allowing them to use ecological niches previously inaccessible (Read and Perez-Moreno, 2003). In addition, fungi protect their hosts against abiotic (metal pollution, drought) and biotic (pathogen infection) stresses (Smith and Read, 2008). In return, the fungi obtain sugars derived from photosynthesis. Up to 20% of the carbonated elements from terrestrial plants are allocated to AMF (Hogberg *et al.*, 2001). Thus, this symbiosis contributes significantly to the overall carbon cycle budget within ecosystems (Read and Perez-Moreno, 2003).

According to their symbiotic structures and the phylogenetic position of their partners, several types of mycorrhizae are defined. The two most common forms of mycorrhizal symbiosis are Arbuscular Mycorrhizal (AM) symbiosis and ectomycorrhizal (ECM) symbiosis. During the symbiosis of the ECM, the fungal partner colonizes the intercellular spaces of the roots (apoplast), whereas during the AM symbiosis, part of the hyphae develops inside the plant cells (Figure 2.2) (Smith and Read, 2008).

The AM symbiosis is probably the most widespread terrestrial symbiosis. Indeed, it is associated with more than 80% of current terrestrial plant species, mostly herbaceous

species, including important crops such as wheat, rice, maize and soybeans (Smith and Read, 2008). However, there are only about 160 species of fungi forming AM symbioses, all belonging to the phylum of Glomeromycota, a monophyletic clade, brother of Ascomycetes and Basidiomycetes (Figure 2.3-a) (Schüβler *et al.*, 2001). Fossil evidence demonstrates the existence of this symbiosis over 400 million years ago, coinciding with the appearance of the first terrestrial plants (Selosse and Le Tacon, 1998). It has therefore been suggested that arbuscular mycorrhizal fungi have played a crucial role in plant colonization of the terrestrial environment. This could explain the quasi-ubiquitous distribution of this arbuscular mycorrhizal symbiosis within ecosystems as in the plant kingdom (Tisserant, 2011).

The more recent ECM symbiosis (180 million years ago) (Lepage *et al.*, 1997) mainly involves woody species representing a relatively small number of plants (about 8000, 3% of seed plants). However, these species are the dominant species in temperate, boreal, some subtropical forests and Mediterranean forests, so that this symbiosis can be considered predominant within these ecosystems (Read and Perez-Moreno, 2003). ECM fungi, the number of species of which is estimated between 7000 and 10 000, do not form a phylogenetically distinct group, but have appeared within Basidiomycetes and Ascomycetes; among saprotrophic, non-mycorrhizal fungi. This suggests that the symbiotic lifestyle has emerged several times, during the evolution of these fungal lines. It would have evolved from brown rot in the Carboniferous (Hibbett and Matheny, 2009, Eastwood *et al.*, 2011).

The hyphae of ectomycorrhizal fungi aggregate around the root end to form the mantle and develop between the epidermal cells to form the Hartig net (Figure 2.2). The hyphae of arbuscular mycorrhizal fungi develop between and through plant cells and penetrate inside inner cortical cells to form arbuscules (Bonfante and Genre, 2010).

Mycorrhizal symbiosis plays a key role in carbon biosequestration, nutrient cycling, plant biodiversity, and the productivity of natural and agricultural ecosystems. The mycorrhizal mutual symbiotes are therefore of major importance as engines of ecological functions and evolutionary processes in terrestrial ecosystems (Tisserant, 2011).

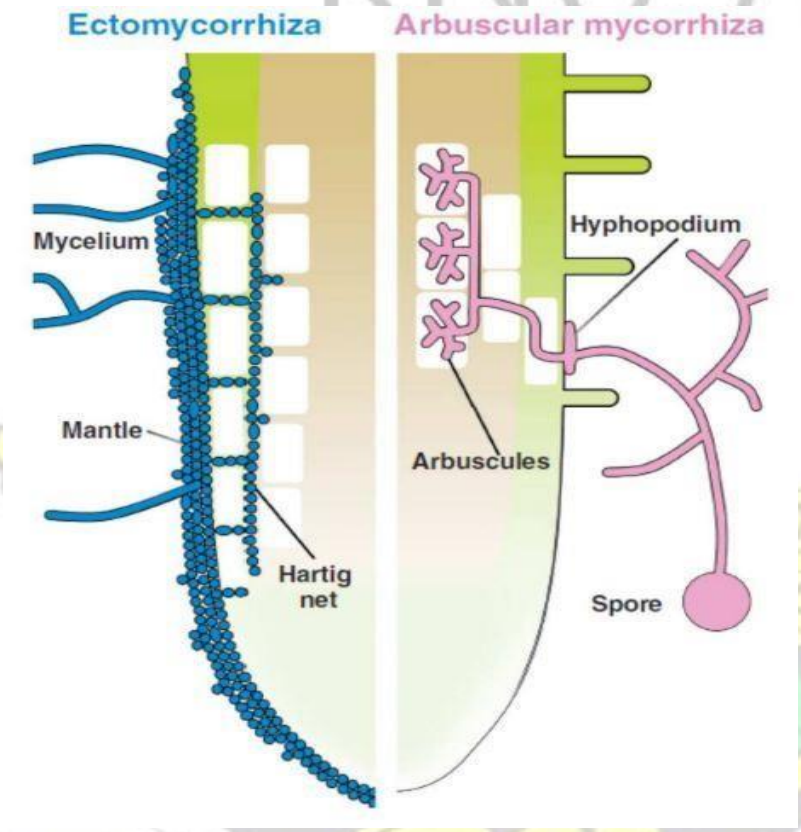

Figure 2.2. Root colonization structures of ectomycorrhizal symbiosis (blue) and arbuscular mycorrhizal symbiosis (pink) (Bonfante and Genre, 2010)

2.4.3 Classification of Glomeromycota

The Glomeromycota are composed of four orders: Glomerales (Glomus groups **a** and **b**), the Paraglomerales (Paraglomus), the Archeosporales (Archeospora, Ambispora and Geosiphon) and Diversisporales (Acaulospora, Gigaspora, Scutellospora, Pacispora and Diversispora) (Figure 2.3-b) (Classification according to Schüβler *et al.*, 2001). There are now about 150 species, an estimate that could be far below the reality

because of the difficulty of clearly delimiting species in these organisms (Smith and Read, 2008).

At the phylogenetic level, molecular analyses now make it possible to refine the classification of AM fungi. Recently, the scientific community has even changed the names of different mycorrhizal fungi, including the fungus model *Glomus intraradices* (DAOM197198), which was reclassified in 2009 as *Glomus irregularis* (Stockinger *et al.*, 2009). More recently, Walker and Schüßler proposed the name *Rhizophagus irregularis* (Schüßler and Walker, 2010; Krüger *et al.*, 2012).

2.4.4 Development of ectomycorrhizal symbiosis

The development of ECMs occurs through a series of well-characterized morphological events. Fungal hyphae emerge from soil spores or previously mycorrhizal roots and grow in the rhizosphere. The encounter and recognition between the two symbionts involve the exchange of chemical signals. Only the main lines of this signalling process are defined. In the immediate environment of the root, the morphology of the fungi changes, in particular with an increase in growth and an intense branching of the hyphae. Such a response is probably triggered by root exudates from the host, such as zeatin and rutin (Lagrange *et al.*, 2001).

2.4.5 Development of arbuscular mycorrhizal symbiosis

Arbuscular mycorrhizae are mandatory biotrophs that depend on their association with plant roots to complete their life cycle. Root colonization, vital to these fungi, follows a series of distinct stages. Arbuscular mycorrhizal fungi exist in the soil as spores, their development begins with the germination of spores from which emerge the hyphae which explore the soil searching for a host root. In the absence of a host, the growth of hyphae is limited by the amount of carbon stored in the spores in the form of lipids feeding hyphae (Bécard and Piché, 1989; Bago *et al.*, 2000). The hyphae then stop

growing and retract their cytoplasmic mass into the spore, the latter again enters into dormancy (Bécard *et al.*, 2004).

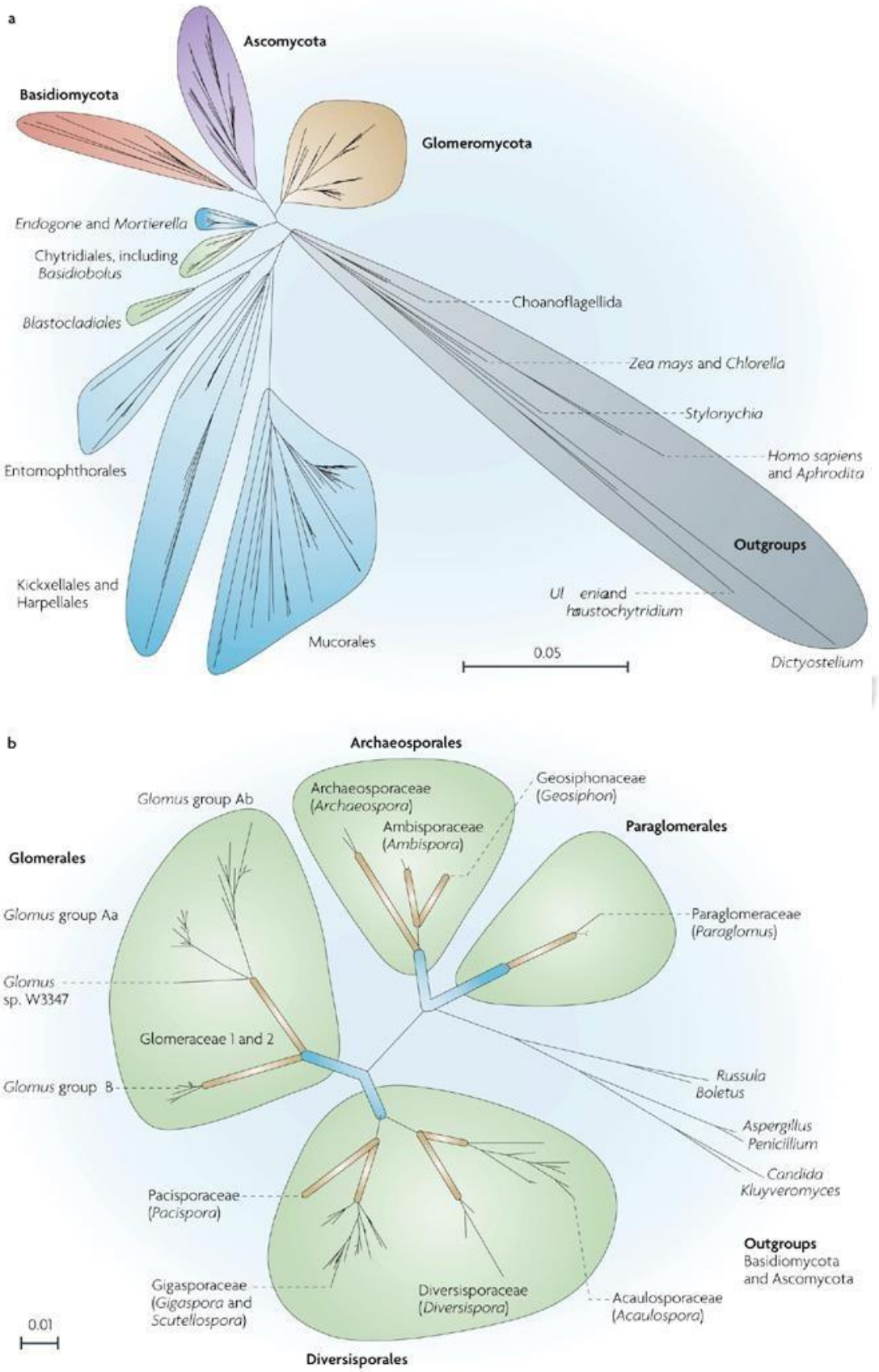

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Figure 2.3. Phylogeny of Glomeromycota (adapted from Parniske, 2008)

Successive cycles of spore germination can occur in AM fungi. The perception of the host plant by the mycelium is via root exudates diffused over a short distance and rapidly degraded, which have recently been identified as strigolactones (Akiyama *et al.*, 2005). These compounds induce a fungal pre-symbiotic reaction characterized by continuous hyphae growth, increased physiological and mitochondrial activity as well as abundant hyphal branching, which increases the chances of encounter with the host. In return, germinated spores produce diffusible signals, known as Myc factors, such as recently identified lipochitooligosaccharides, which are perceived by plant roots even in the absence of physical contact with the fungus (Maillet *et al.*, 2011). These signals, which stimulate root growth and branching, activate in the plant a signalling pathway common to that triggered by the symbiosis between rhizobial bacteria and leguminous plants (Figure 2.4) (Kistner *et al.*, 2005). Since the mycorrhizal symbiosis appeared before the nitrogen-fixing rhizobium symbiosis, the mycorrhizal signal transduction pathway may have been recruited by nitrogen-fixing bacteria (Kistner and Parniske, 2002).

The fungus forms an appressorium or hyphopodium on the surface of the root after the first physical contact between the hyphae and the plant, it colonizes through the appressorium the intercellular space of the root cortex (Genre *et al.*, 2005). The plant cell forms a "prepenetration apparatus" (PPA) which predetermines the path of growth of the hypha through the plant cell (Figure 2.5). It is only after the differentiation of this cytoplasmic bridge that the fungal hyphae penetrates into the host cell (Genre *et al.*, 2005).

The perception of signals derived from AMF and rhizobia bacteria triggers a signal transduction pathway that involves at least seven genes necessary for the correct

development of the two symbioses (SYM genes). Myc factors are recognized by the plant via a receptor still uncharacterized (Bonfante and Genre, 2010). The perception of these factors induces periodic and transient oscillations of the calcium concentration in the cytoplasm of the plant. A SYMRK membrane receptor detects the fungal signals and transmits them to the cytoplasm by phosphorylation of an unknown substrate. This signal is quickly transmitted to the nucleus. Two nucleoporins NUP85 and NUP133, as well as the two CASTOR and POLLUX proteins encoding potassium channels present in the nuclear envelope, are necessary for calcium oscillations (Parniske, 2008).

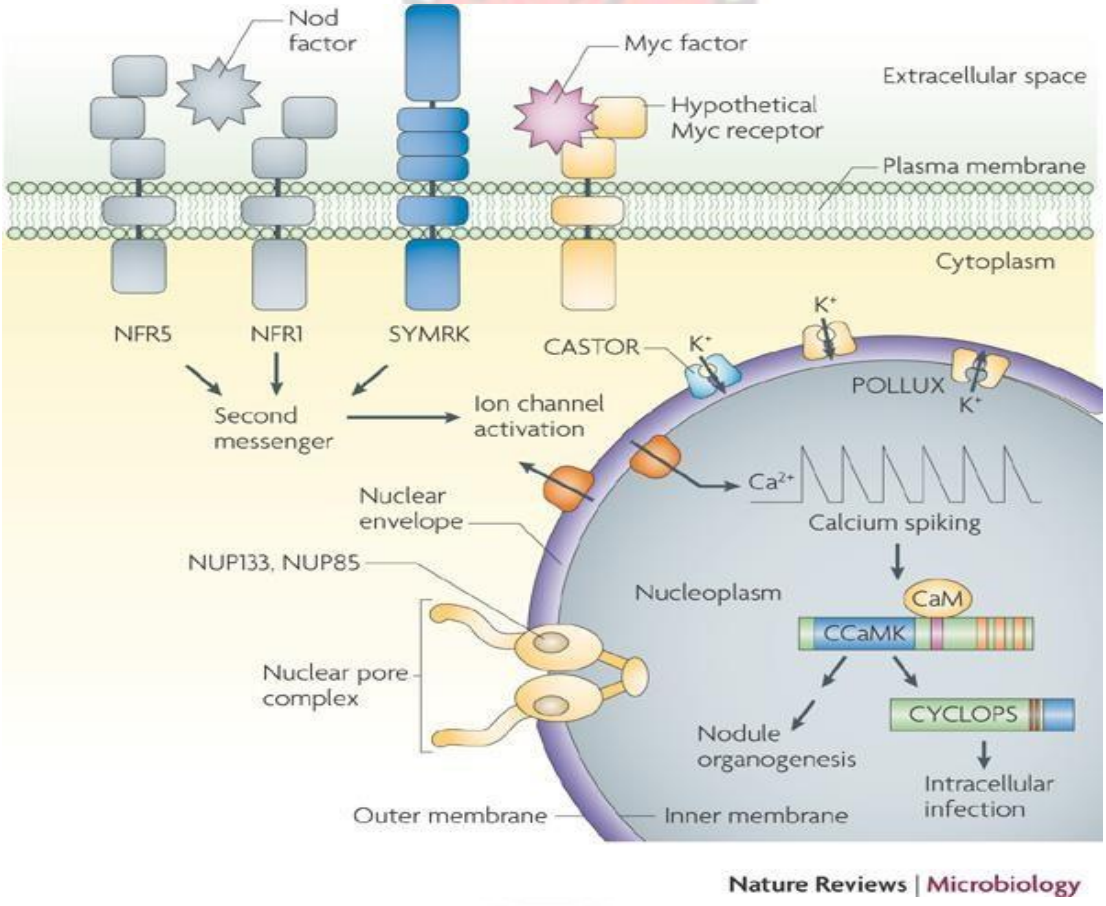


Figure 2.4. Common signalling pathway for rhizobial and arbuscular mycorrhizal symbioses (Parniske, 2008)

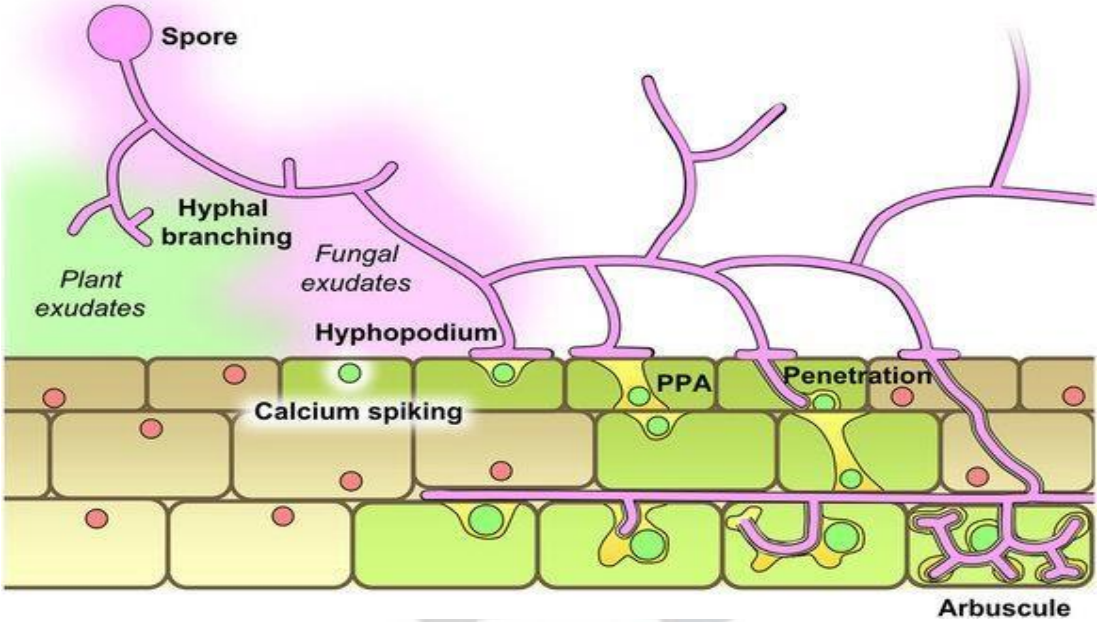


Figure 2.5. Different stages of colonization of AM fungi (adapted from Bonfante and Genre, 2010)

Then, the fungus passes through the outer cell layers, propagates longitudinally in the internal cortex and forms dichotomous branched hyphae within the cortical cells, called arbuscules (Figure 2.5). These elaborate structures remain separated from the cytoplasm of the plant cells by an extension of the plasma membrane of the host, the periarbuscular membrane. This membrane follows the contours of the hyphae branches, leading to an increase in the surface of the plasma membrane. Arbuscules are the main site for nutrient exchange between the fungus and the plant (Paszkowski, 2006; Bonfante and Genre, 2010), they are ephemeral structures with a lifespan estimated at 4 to 5 days. The host cell regains its former organization after the degradation fungal structures. Thus, the cortical cells can allow a new fungal penetration and the formation of arbuscules (Paszkowski, 2006; Bonfante and Genre, 2010).

The fungus also develops an extraradical mycelium that extends outside the root. This mycelium acquires the nutrients in the soil that will be transferred to the plant. The life cycle of arbuscular mycorrhizal fungi is completed by spores formation at the extraradical mycelium, which may enter into another colonization process (Tisserant, 2011).

2.4.6 The acquisition of phosphorus by the AM symbiotic pathway

2.4.6.1 Uptake of phosphate from the soil

The beneficial impact of AMF on P nutrition has been attributed to the exploration by the extraradical mycelium of large soil volumes from which orthophosphate (Pi) is foraged and conveyed to root cortical cells, bypassing plant direct pathway for phosphorus uptake (Jakobsen *et al.*, 1992; Jakobsen *et al.*, 2001); the small diameter of hyphae allowing the mycorrhizal fungus to creep into small soil cores in search of phosphorus and greater phosphorus absorption rates per surface unit (Jakobsen *et al.*, 1992; Li *et al.*, 2008); the ability of AMF to store phosphorus in the form of polyphosphates allowing them to maintain relatively low the internal orthophosphate concentration and permitting an efficient transfer of phosphorus from the extraradical mycelium to the intraradical mycelium (Hijikata *et al.*, 2010); and finally the production of organic acids and acid phosphatases facilitating the release of phosphorus from organic complexes (Ezawa *et al.*, 2005; Alvarez *et al.*, 2012).

Similarly as plants, mycorrhizal fungi also have two systems for phosphorus uptake: a high affinity system working against an electrochemical potential gradient, which absorbs orthophosphates from the soil though proton co-transport (Bucher, 2007; Javot *et al.*, 2007); and a low affinity system which encourages diffusion of orthophosphates across the plasma membrane of the fungi (Thomson *et al.*, 1990). In arbuscular mycorrhizal and ectomycorrhizal fungi, the high affinity phosphorus transporters

involved in the phosphorus absorption from the soil are expressed in the extraradical mycelium (Maldonado-Mendoza *et al.*, 2001; Tatry *et al.*, 2009).

Interestingly, *Hebeloma cylindrosporum* is an ECM fungus with two phosphorus transporters expressed in its extraradical mycelium, one of them (HcPT1) is upregulated under low phosphorus and the other transporter (HcPT2) up-regulated under high phosphorus supply conditions (Tatry *et al.*, 2009). The simultaneous expression of two fungal phosphorus transporters responding differently to the phosphorus level could enable the extraradical mycelium of the fungus to absorb phosphorus efficiently from the rhizosphere with its low orthophosphate concentrations or from nutrient hot spots (Bücking *et al.*, 2012).

2.4.6.2 Fungal phosphate metabolism

Orthophosphate (Pi) taken up by the extraradical mycelium can be incorporated into protein-phosphates, phospholipids, DNA- and RNA-; be conveyed into a storage pool of long- or short- chained polyphosphates; or replenish the cytoplasmic orthophosphate pools that are metabolically active (Beever and Burns, 1981). Inorganic polyphosphates are linear polymers with the orthophosphate residues connected by energy-rich phospho-anhydride bonds. Polyphosphates can be distinguished into two types in AMF: long chain polyphosphates with more than 20 orthophosphate residues and short chained polyphosphates with up to 20 orthophosphate residues. In average, the length of long chained polyphosphates in AMF has been estimated as 190-300 orthophosphates (Ezawa *et al.*, 1999; Ezawa *et al.*, 2004), and that of short chained polyphosphates as 11 to 20 orthophosphate residues (Shachar-Hill *et al.*, 1995; Viereck *et al.*, 2004).

Mycorrhizal fungi can rapidly store a considerable portion of their cellular phosphorus as polyphosphates (Hijikata *et al.*, 2010; Bücking and Heyser, 1999; Bücking and

Shachar-Hill, 2005). In the AMF symbiosis, polyphosphates are involved in:

(i) maintenance of low intracellular orthophosphate levels and phosphorus homeostasis in the hyphae. The efficiency with which phosphorus is taken up is increased with low orthophosphate levels in fungal hyphae. On the other hand, the osmotic stress at high internal phosphorus concentrations is reduced (Ohtomo *et al.*, 2004; Viereck *et al.*, 2004);

(ii) long-distance transport from the extraradical mycelium to the intraradical mycelium. Phosphorus is principally transported as polyphosphate from the extraradical mycelium to the root (Callow *et al.*, 1978; Cox *et al.*, 1980) dependent on the high flux rate of phosphorus through the hyphae of AMF (Cooper and Tinker, 1978; Cooper and Tinker, 1981). The lengths of the chain of polyphosphates in the intraradical mycelium are shorter than in the extraradical mycelium, this suggests that polyphosphates initially form in extraradical mycelium and are mobilized again in intraradical mycelium (Solaiman *et al.*, 1999). In ectomycorrhizal and arbuscular mycorrhizal fungi, a tubular vacuole system endowed with motility has been discovered (Ashford and Allaway, 2002; Olsson *et al.*, 2002; Uetake *et al.*, 2002), that enables the polyphosphate transfer via the hyphae separately from the cytoplasmic compartment and permits the fungus to adjust its local cytoplasmic orthophosphate concentration;

(iii) regulation of phosphorus transport. Mycorrhizal fungi regulate the intracellular orthophosphate concentration in the intraradical mycelium and the phosphorus flux to the host by controlling the turnover or/and formation rates of polyphosphate inside the intraradical mycelium or in the Hartig net zone (Bücking and Heyser, 2003; Takanishi *et al.*, 2009; Kiers *et al.*, 2011). During the symbiosis, short-chained polyphosphates

are correlated to the phosphorus transportation, whereas long-chained polyphosphates principally take part in long term storage of phosphorus (Takanishi *et al.*, 2009);

(iv) cation homeostasis. Polyphosphates are polyanions and cations such as Mg^{2+} and K^{+} play the main role in balancing their negative charge (Bücking and Heyser, 1999; Ryan *et al.*, 2003; Bücking and Shachar-Hill, 2005), but polyphosphates can as well trap toxic cations such as heavy metals (Bücking and Heyser, 1999). In ectomycorrhizal fungi, large amounts of nitrogen can also be stored by polyphosphates and the basic amino acid arginine (Arg^{+}) can also play an important role in the charge balance of polyphosphates (Bücking *et al.*, 1998; Bücking and Heyser, 1999).

2.4.7 Mechanisms regulating the arbuscular mycorrhizal symbiosis

Among the elements affecting the production of strigolactones, the availability of nutrients, especially phosphate, has been particularly studied. Indeed, it has long been known that the addition of phosphate fertilizers significantly reduces the invasion of cultivated fields by parasitic plants, and this effect has been linked to lower production of germination inducers (Balzergue, 2012). It has been shown that phosphate deficiency induces the production of strigolactones in clover (Yoneyama, *et al.*, 2007a), tomato (López-Ráez *et al.*, 2008) and rice (Umehara *et al.*, 2008). In sorghum, phosphate and nitrogen deficiency favours the production and exudation of strigolactones (Yoneyama, *et al.*, 2007b).

The fact that plants produce more strigolactones under a deficiency condition is coherent from several angles. Not only does the plant promote the potential establishment of an AMF symbiosis, it also inhibits the branching of the aerial parts to promote root development (more lateral roots and root hairs), allowing a better access to the potential nutrient resources of the soil (Balzergue, 2012). Another mechanism

regulating the production of strigolactones is mycorrhization itself. A study in tomato shows that mycorrhizal plants produce less strigolactones than non-inoculated plants (López-Ráez *et al.*, 2011)

2.4.8 Phosphate as regulating element of arbuscular mycorrhizal symbiosis

The disturbance of the symbiotic interaction of AMF by environmental factors such as P availability has already been demonstrated. In fact, in several experiments, it has been reported that high inorganic phosphate levels suppress AMF colonization (Breuillin *et al.*, 2010; Balzergue *et al.*, 2013; Bonneau *et al.*, 2013). This inhibition can be explained by the carbon cost of the interaction for the plant. In a simplified and finalist way, one can imagine that if the phosphate of the medium is directly available to the plant, then this one does not need the fungus to collect it and therefore limits the interaction with the latter in order to conserve its photosynthates. Today, the mechanisms by which plants control the colonization of their roots according to their nutritional requirements in phosphates are not elucidated (Balzergue, 2012).

In order to assess which nutrients influence AMF development, together with phosphorus, Nouri *et al.* (2014) used *Petunia hybrida* and *Rhizophagus irregularis* as AMF systems to test several elements and check their inhibitory effects on AMF colonization. The results indicated that sulfate, Mg^{2+}, Ca^{2+}, and Fe^{3+} had no effect on root colonization, while nitrate and inorganic phosphate potentially exert negative regulation on AMF. In addition, it has been demonstrated that the starvation of various nutrients, especially nitrate, reverses the inhibitory effect of inorganic phosphate on AMF; this suggests that a dominant AMF-promoting signal is triggered by nutrient starvation (Breuillin *et al.*, 2010; Nouri *et al.*, 2014).

2.4.9 Sources of arbuscular mycorrhizal inoculum

(i) Because it normally contains infected root fragments, mycorrhizal spores and hyphae, soil from the root zone of a mycorrhizal plant can serve as inoculum. Nevertheless, accurate information about the diversity, infectivity and propagule abundance should be available to avoid probable risk of transferring pathogens and weed seeds.

(ii) Crude inoculum can also be used and spores isolated from soils can serve as starters for its production. It can be acquired after growing together a host trap plant (that is, a plant that can be heavily colonized by several arbuscular mycorrhizal fungi species) and a known isolate of arbuscular mycorrhizal fungus in a sterilised medium optimized for arbuscular mycorrhizal propagation. Among the different types of inoculum, the crude inoculum is the most frequently used for large-scale crop inoculation because of its higher concentration in AMF propagules (spores and hyphae),

(iii) And finally, colonized root fragments alone of a specific arbuscular mycorrhizal fungus collected from a trap plant culture can also be used as inoculum.

2.5 Factors determining the success of mycorrhizal inoculation

Arbuscular mycorrhizal fungal colonization gain in plants inoculated under greenhouse conditions are significantly more frequent than in the open-field conditions compared to non-inoculated controls. This could be explained by the AMF propagules often contained in uninoculated control portion of a field, while sterilized substrates used to fill control pots in greenhouses are usually free of AMF propagules (Berruti *et al.*, 2016). Two main approaches for agronomic application of AMF exist: the holistic approach which aims at maximizing fungal diversity to ensure AMF-dependent ecosystem services; the reductionist approach which relies on the inoculation of

optimized AM fungal species adapted to the target crop under cultivation and to specific conditions (Fester and Sawers, 2011). In all cases, consistency of response to AMF inoculation is a requirement for their efficient use in sustainable plant production (Herrera-peraza *et al.*, 2011).

The introduction of new microbes in a soil might have a definitive impact on crop environment and the microbial balance in the soil since every crop plant evolved with its compatible microbiome (Sruthilaxmi and Babu, 2017). The factors limiting the success of biofertilizer microbes are unpredictability of results, gaps in understanding of the interactions between plants and microbes and the challenges related to tracing the inoculated species in the field (Malusà *et al.*, 2016). Intensive agricultural practices such as crop sequence with non-host crops, intensive tillage, fallow and high fertilization reduce mycorrhizal abundance and species diversity (Jansa *et al.*, 2006; Koide and Peoples, 2012; Säle *et al.*, 2015). In fact, soil structure and soil microorganism community assemblages are drastically affected by inappropriate tillage techniques such as high intensity and frequency ploughing (Berruti *et al.*, 2014).

The potential of AMF to promote growth of their hostplants is controlled by the genotype of the fungus, the genotype of the host and their interactions (Baum *et al.*, 2015). Low-quality species were found 'hidden in a crowd' of highly beneficial arbuscular mycorrhizal fungi (Hart *et al.*, 2012). Therefore, high diversity of fungal partners on a root system may not be always beneficial to the hostplants (Werner and Kiers, 2015). To optimize the use of biofertilizers, soil microbial testing is therefore recommended before a cropping season to enhance the field performance of microbial inoculants and hence obtain better crop yields (Sruthilaxmi and Babu, 2017). For successful commercial application, the addition of large amounts of inoculum is

prohibitive and possibly unrealistic (Köhl *et al.*, 2016). Inoculation techniques using smaller amounts of inoculum seem to be more beneficial (Vosátka *et al.*, 2012).

Three key factors have been identified for the success of inoculation and persistence of introduced AMF in soils: species compatibility, priority effects and field carrying capacity (Verbruggen *et al.*, 2013). Evidence has adduced that there were no significant differences in inoculating at sowing or at the seedling stage wile transplanting; to save time and inoculum, the inoculation at sowing is therefore recommended as a better strategy in agricultural practice (Ortas *et al.*, 2011).

2.6 Approaches to using arbuscular mycorrhizal inoculation to enhance P uptake, growth and yield of legumes and residual benefits on subsequent crop

Legume roots are colonized by rhizobia as well as AMF (Harrison, 1999). It has been assumed that all legumes can form symbiosis with arbuscular mycorrhizal fungi with the exception of Lupinus, as the those fungi evolved long before legumes (Sprent and James, 2007). Inoculation with four individual species of AMF increased to the same extent dry matter of three varieties of common bean. There were small but significant differences in N and P uptake, fixed N and AMF frequency for the individual inoculants (Ibijbijen *et al.*, 1996). Mycorrhizal inoculation doubled the concentration of phosphorus in roots and shoots of infected white clover (*Trifolium repens* L.) plants and increased their dry weights (Li *et al.*, 1991).

The response of legumes to AMF inoculation may be affected by the rate of phosphate fertilizer applied. For instance, N and P uptake, fixed N, dry matter and of alfalfa increased with increasing amounts of superphosphate applied, with the inoculated treatments generally superior for most variables in relation to uninoculated treatments at each rate of P applied (Barea and Azcon-Aguilar, 1983).

Plant species pertaining to the Cucurbitaceae, Leguminosae, and Solanaceae exhibited significant responses to the inoculation with arbuscular mycorrhizae under both nonfumigated and fumigated soil conditions (Ortas, 2012). Studies have shown that while enhancing the nitrogen-fixing ability of rhizobium, AMF can improve the capability of soybean to absorb other nutrients and therefore increase yields of soybeans (Tian *et al.*, 2013). Inoculation in pot culture stimulated phosphorus uptake efficiency and increased total biomass of mycorrhizal soybean plants 3.5 and 2.0 times in relation to non-mycorrhizal plants under low and medium-P conditions in Mollisol, respectively (Fernández *et al.*, 2011).

Cozzolino *et al.* (2013) inoculated with *Glomus intraradices* (*R. irregularis*) and applied phosphorus fertilizers in some treatments and not in others on maize (*Zea mays* L.) under field conditions. The results showed that the treatments inoculated and fertilized with N and K without P resulted in phosphorus uptake plant growth and grain yield similar to those obtained with treatments that received phosphate fertilizer (NPK). In several cropping systems, mycorrhizal spores of soils increased following cultivation of mycorrhizal crops (potato, sunflower, soybean, maize, kidney bean, wheat). It resulted in an increase in growth of consecutive crops following mycorrhizal crops (Mohammadi *et al.*, 2011). This effect on P uptake and growth of maize was obvious in dry soil conditions and less pronounced with increasing soil moisture (Osunde *et al.*, 2003; Dahlgren *et al.*, 2004).

2.7 Summary of literature review

Soybean is one of the most important crops worldwide and its grains are important as vegetable oil and protein meal. It is used in Ghana to prepare weaning foods for babies, to fortify various traditional foods and in the animal feed industry. Although soybean, like all cultivated legumes, possess the ability to symbiotically reduce atmospheric dinitrogen and make it available to plants, its growth is generally restricted by scarcity of other resources, such as P, which is strongly bound in soil, resulting in a low rate of diffusion towards the root surface. In fact, phosphorus plays a crucial role in the symbiotic nitrogen fixation process of legumes by improving shoot and root growth, shortening the time necessary for developing nodules to become active and benefit the host legume, increasing the size and number of nodules and the amount of nitrogen assimilated per unit weight of nodules, increasing the total amount and percentage of nitrogen in the harvested portion of the host legume, increasing the density of rhizobia bacteria in the rhizosphere. To overcome that limitation, application of P fertilizers is seemingly inescapable. In Ghana, small-scale soybean producers depend little on fertilizers for increasing production due to the high cost. However, it is estimated that only 15 to 30% of the intake is absorbed by plants and thereby the large addition of phosphates is relatively not effective, increases the cost of production, disturbs microbial diversity, leading to the loss of soil fertility and as a consequence reduces yield of crops.

Phosphate solubilizing microbes have the potential of making these phosphates available to the plants. Microbial inoculants are being used since last century to

improve the efficiency of fertilizer application, but insufficient work has been reported on phosphate solubilizing microorganisms as compared to biological nitrogen fixation. Arbuscular mycorrhizal fungi are the most widely used in agriculture with the aim of enhancing phosphorus availability for crop plants. In several cropping systems, mycorrhizal spores of soils increased following cultivation of mycorrhizal crops including soybean, potato, sunflower, kidney bean, maize and wheat. Identification of the most suitable strains, preparation of effective formulations, and introduction of efficient agronomic managements will be determinant in increasing availability of soil phosphorus and improving phosphorus efficiency through microbial inoculation, while allowing farmers to meet production costs and profit margins.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The study consisted of both greenhouse experiment and field experiments.

3.1 Greenhouse experiment

3.1.1 Symbiotic effectiveness test of four arbuscular mycorrhizal fungi isolates on two soybean lines

3.1.2 Greenhouse conditions

Two pot experiments were conducted for six weeks in the greenhouse of the Department of Horticulture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The greenhouse conditions during the growth period were 57% humidity, 24 hours of 35°C temperature and 486 mmol/m^2/s^2 light intensity, for the river sand from May to June 2016; and 49% humidity and 24 hours of 35°C and 386 mmol/m^2/s^2 light intensity for the soil from December 2016 to January 2017.

3.1.3 Soils used

The first greenhouse experiment was carried out using river sand. For the second greenhouse experiment, soil samples of a Haplic Acrisol were randomly taken from the field and thoroughly mixed into a 100 kg capacity plastic container. Pots were filled with 3 kg of sterilized soil for both experiments. Perforated plastic pots of two litres volume were used. The pots were thoroughly washed with detergent and left to dry for a day before use.

3.1.4 Planting materials

The plant materials used for this study were two soybean (*Glycine max* (L.) Merril) lines: TGx 1989-48 FN and TGx 1989-75 FN. The seeds were obtained from the

Legumes and Oilseeds Division of the Crops Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR) of Fumesua, Kumasi, Ghana.

3.1.5 Arbuscular mycorrhizal inoculum

The mycorrhizal inoculum evaluated consisted of four arbuscular mycorrhizal fungi isolates: *Glomus etunicatum*, *G. fasciculatum*, *G. mosseae* and *Rhizophagus irregularis*. The inoculum was obtained from the Microbial Resource Centre (MIRCEN), the common laboratory for the French National Research Institute for Sustainable Development, Senegalese Institute of Agricultural Research and Cheikh Anta Diop University of Dakar, Senegal. Trapping studies were undertaken for four months by separately inoculating maize crop on sterilized river sand with each of the inocula. The soil mixed with mycorrhizal roots, hyphae and spores was used as crude inoculum (Chalk *et al.*, 2006).

3.1.6 Preparation of soybean seeds and mycorrhizal inocula

The seeds of the two selected lines were sorted and cleaned. They were thoroughly and successively washed with diluted ethanol (89%) and hydrogen peroxide (30%). Afterwards, they were soaked for 3 hours in distilled water and floating seeds were discarded before sowing the same day.

The inocula of the different isolates of arbuscular mycorrhizal fungi were weighed with an electronic precision scale (0.1 mg). They were prepared for four months by inoculating maize in a sterilized soil.

3.1.7 Treatments and experimental design

The treatments consisted of a factorial combination of four mycorrhizal fungi isolates, namely; *Glomus etunicatum*, *G. fasciculatum*, *G. mosseae* and *Rhizophagus irregularis* and two soybean lines. A control was also included and the treatments

arranged in a factorial Completely Randomised Design replicated four times. Each experimental unit was represented by a pot.

3.1.8 Soil sterilization, mycorrhizal inoculation and sowing

The river sand used for the first experiment was washed to remove debris. Afterwards, it was spread on polythene sheet, air-dried for 3 days under shade and sieved through a 2-mm mesh sieve before autoclaving for one hour. After sterilization, it was kept in a plastic container of 100 kg capacity. The topsoil used for the second experiment was however steam sterilized at 80°C after which it was allowed to cool, dry and was sieved through a 2-mm mesh and stored for 5 days before sowing. Both soil types were analysed for selected chemical, physical and biological properties before setting up the experiments.

Two days before sowing, approximately 300 spores (15 g of inoculum) of arbuscular mycorrhizal fungi were carefully mixed with the top 5 cm of the soil following a circular pattern and watered immediately. Two days after inoculation, the seeds were sown in the pots using forceps and immediately watered.

3.1.9 Watering, thinning out and fertilization

Hundred millilitres of distilled water were applied to the pots, daily. Thinning out was undertaken 7 days after sowing to 1 plant per pot. Starter nitrogen (urea) was applied one week after emergence at the recommended dose for legumes in Ghana (20 kg N ha^{-1} = 65 mg urea/pot) to help the young seedlings to acquire nitrogen during the early growth stage (OFRA, 2013).

3.1.10 Cultural practices

All other cultural practices were carried out in accordance with the requirements of the experimental protocol of the trial.

3.1.11 Measured plant parameters

The parameters measured on soybean were plant height, stem girth, shoot dry weight and total plant phosphorus.

3.1.11.1 Plant height and stem girth

Plant height and the stem girth were measured at 20 and 40 DAS using a metallic measurement ruler and a digital caliper, respectively.

3.1.11.2 Shoot biomass yield

Plant shoots were cut at 6 weeks after sowing from each pot at the base and oven-dried at 60°C for 72 hours. The dry weights were taken using an electronic precision scale (0.01 g). The shoot yield per hectare was estimated using the dry weights as described by Okogun *et al.* (2005).

3.1.11.3 Total phosphorus

The plants harvested from each pot and dried in section 3.1.11.2 were cut with scissors, milled using a Perten's laboratory mill 3310® and ashed using a muffle furnace (500 °C for 4 hours). After oven drying, the ash digestion and analysis of plant tissues was used to determine the plant total phosphorus using a spectrophotometer (Milton Roy Company®) at wavelength of 430 nm.

3.1.12 Arbuscular mycorrhizal colonization

At six weeks after sowing, the plants in each pot were harvested. The roots were sampled and thoroughly washed with flowing tap water to remove the adhering sand particles. The root samples were kept in zip lock bags and stored at 4 °C. The roots were then stained using Phillips and Hayman (1970) method and the arbuscular mycorrhizal colonization was estimated using the magnified intersections method as

described by McGonigle *et al.* (1990). The data recorded included arbuscules colonization, vesicles colonization and root length colonization.

3.2 Field assessment of the response of two soybean lines to arbuscular mycorrhizal inoculation and phosphorus fertilizer

3.2.1 Location of the experiment

Two field experiments were conducted in the minor (from September to January, 2016) and major (from April to August, 2017) cropping seasons, respectively on a Haplic Acrisol (IUSS Working Group WRB, 2014), at the Department of Horticulture, KNUST, Kumasi (Latitude 6° 41′ 5.67″ N, Longitude 1° 34′ 13.87″ W) at an altitude of 250 m above mean sea level. The area falls within the moist semi-deciduous forest zone of Ghana with typical bimodal rainfall pattern. The major cropping season is from March to July and the minor cropping season from September to November. This area also experiences a long dry season between December and March and a short one in August. The annual rainfall of the area ranges between 1250 – 1500 mm. The area is characterized by a mean annual temperature of 26.6 °C and a mean annual humidity of 67.6% (Partey, 2010).

3.2.2 Planting materials

The plant materials used for this study were two soybean (*Glycine max* (L.) Merril) lines: TGx 1989-48 FN and TGx 1989-75 FN as used in the greenhouse experiment.

3.2.3 Mass multiplication of arbuscular mycorrhizal microsymbionts The mycorrhizal inoculum consisted of two AMF isolates: *Glomus mosseae* (Nicolson and Gerd.) Gerd. and Trappe and *Rhizophagus irregularis* (Blaszk., Wubet, Renker and Buscot) Walker and Schüßler. The multiplication of mycorrhizal spores was carried out twice: from June to September 2016 and from January to April 2017.

After 24h of incubation in water, a local early maturing maize variety (Omankwa) from Crops Research Institute, Fumesua, Kumasi, was sown as a host plant in a sterile river sand (Miyasaka *et al.*, 2003) containing very low levels of available plant nutrients including phosphorus (1.96 mg P kg^{-1} soil), at a density of 4 plants/pot. Pots of 5 litres in volume were used for each mycorrhizal isolate and randomly placed in a greenhouse with 53% relative humidity, 24 hours of 31°C temperature and 444 mmol/m^2/s^2 light intensity from June to September 2016; and 47% relative humidity and 24 hours of 36°C temperature and 416 mmol/m^2/s^2 light intensity from January to April 2017. A piece of tissue paper was folded and spread at the bottom of the perforated pots to prevent soil and arbuscular mycorrhiza spores from leaching out.

The pots were watered uniformly at 100 ml every day. The plants were supplied with diluted nutrient solution which is poor in phosphate every fortnight (Friberg, 2001). To maintain the inoculum potential, the phosphorus level was kept low (< 20 mg kg^{-1} soil). The total quantity of nutrients supplied in successive doses were: 74.0 N, 14.5 P, 58.8 K, 6.2 Ca, 4.8 Mg, 32.9 S, 10.9 Cl, 3.7 Mn, 1.3 Zn, 0.53 Cu, 0.08 Mo, 0.13 Na (in kg ha^{-1}). The duration of the pot culture was three months. The moisture regime was successively lowered during the last week to stop the growth of plants and allow the AMF sporulation to enhance.

3.2.4 Preparation of soybean seeds and mycorrhizal inocula

The seeds of the two selected lines were sorted and cleaned before sowing. The inoculum of the different isolates of arbuscular mycorrhizal fungi was weighed with an electronic precision scale (0.1 mg). They were prepared for 90 days by inoculating with maize in a sterilized soil with the selected isolates (*Glomus mosseae* and *Rhizophagus irregularis*).

3.2.5 Treatments and experimental design

Three factors were investigated during the field experiments. Two mycorrhizal fungi namely; *Glomus mosseae* (Nicolson and Gerd.) Gerd. and Trappe and *Rhizophagus irregularis* (Blaszk., Wubet, Renker and Buscot) Walker and Schüßler, were used including a control.

The second factor consisted of 3 phosphate rates as follows;

P_0: 0 g of triple superphosphate (TSP)/plot (0 kg P ha^{-1}),

P_1: 9.8 g of TSP/plot (15 kg P ha^{-1}), and P_2:

19.6 g of TSP/plot (30 kg P ha^{-1}).

Soybean lines which were the third factor consisted of two lines; TGx 1989-48 FN and TGx 1989-75 FN.

The treatments were arranged in a split-split plot design and replicated three times. The soybean lines were the main-plot factors while the TSP rates constituted the subplot factors and the mycorrhizal inoculation the sub-sub plot factors. Each plot was represented by 4 rows. The two outside rows were considered as borders. Thinning out was done 15 days after sowing, when the soil was moist and seedlings were well established. The total plot size of the trial was 8 m × 28 m. Plant spacing was kept at 50 cm × 10 cm. The distance between the blocks and sub-blocks was 1 m. Overall, the study consisted of 54 experimental units allotted in 3 blocks of 18 elementary plots each.

3.2.6 Field preparation and mycorrhizal inoculation

The field was slashed and ploughed using a disc plough; this was followed by harrowing to a depth of 15 cm to give a fine tilth. The experimental units were marked out using

ribbon and stakes, and then labelled. Picketing consisted of planting labels at the beginning of each elementary plot with plot numbers and their treatments indicated. For the mycorrhizal inoculation, two ditches of 10 cm depth and 10 cm wide were dug out along the 2 central rows of each plot. Inoculum consisting of soil mixed with mycorrhizal roots, hyphae and spores, was spread out in the ditches and carefully levelled with feet (400 g of inoculum/plot).

3.2.7 Sowing, thinning out and fertilization

A day after inoculation, the soybean seeds were sown in rows at a spacing of 50 × 10 cm. Thinning out was carried out at 15 days after sowing (DAS) to obtain 2 plants per hill. Starter nitrogen (urea) and muriate of potash were supplied as basal application two weeks after sowing at the recommended dose for legumes in Ghana (20 kg N ha^{-1} and 30 kg K ha^{-1}) (OFRA, 2013). Triple superphosphate was also applied concurrently at 0, 15 and 30 kg P ha^{-1} depending on the treatment. Fertilizer was applied along grooves made on one side of the rows at about 5 cm from the seedlings.

3.2.8 Cultural practices

The insecticide Lambda super 25% EC (25 g of Lambda-cyhalothrin per litre in the form of Emulsifiable Concentrate) was sprayed at 50% flowering. First hand weeding with hoe was done 30 DAS, a second weeding at 52 DAS and a third weeding was carried out at 94 DAS. After emergence, missing stands were re-sown.

3.2.9 Soil sampling and sample preparation

A total of eight soil samples were randomly taken from the general plot with steel cores with dimensions of 7 cm diameter and 20 cm height. Samples were taken before inoculation, at 50% flowering and a day after harvest. Soil samples were kept in ziplock plastic bags and were sent to the laboratory where they were thoroughly mixed, and sub-samples were taken and air-dried. Afterwards, they were crushed and sieved

through a 2 mm mesh, thoroughly mixed and kept in zip-lock plastic bags for analysis. Some samples of the fresh soil were stored in a refrigerator and later used to determine soil arbuscular mycorrhizal spore density and the microbial biomass phosphorus.

3.2.10 Soil phosphorus dynamics

During the major cropping season of 2017, soil available phosphorus was assessed at planting, 50% flowering and at harvest. Likewise, microbial biomass phosphorus was determined at 50% flowering and at harvest.

3.2.11 Measured plant parameters

The parameters measured on soybean were plant height, stem girth, grains yield, 100 seed weight, shoot dry weight and plant total phosphorus.

3.2.11.1 Plant height and stem girth

The plant height and the stem girth of 5 tagged plants from the central rows were randomly measured at 40, 60 and 80 DAS using a metallic measurement ruler and a digital caliper, respectively. Unrepresentative plants and/or sick plants were avoided.

3.2.11.2 Shoot dry weight and yield

The shoots of all soybean plants were cut at the base from each plot, oven-dried at 60 °C for 72 h and their dry weights (g) taken using an electronic precision scale (0.01 g). The shoot yield per hectare was estimated using the dry weights as described by Okogun *et al.* (2005).

3.2.11.3 Hundred grain weight and seed yield

Grains were removed from the dried pods, oven-dried at 60 °C for 72 h, and their dry weights (g) were taken using an electronic precision scale (0.01 g). The grain yield per hectare was then estimated using the dry weights as described by Okogun *et al.* (2005).

$$\text{kg} \quad \text{Dry weight} \times 10000$$

$$\text{Grain yield } \left(\frac{}{\text{ha}}\right) = \frac{}{\text{Harvest area}}$$

3.2.11.4 Total phosphorus

After harvest, the shoots were oven dried at 60 °C for 72 h and cut with scissors, milled using a Perten's laboratory mill 3310® and ashed using a muffle furnace (500 °C for 4 hours). The grains were also milled. Subsequently, the dry ash digestion and analysis of plant tissues were used to determine the plant total phosphorus using a spectrophotometer (Milton Roy Company®) at 430 nm wavelength. See detailed description in section 3.10.

3.2.12 Arbuscular mycorrhizal colonization

At 50% flowering, the roots of three plants were randomly sampled from each plot and thoroughly washed under flowing tap water to remove the adhered soil particles and stored in zip lock bags at 4 °C. They were subsequently stained using Phillips and Hayman (1970) method and the arbuscular mycorrhizal colonization was estimated using the magnified intersections method as suggested by McGonigle *et al.* (1990). The data recorded included arbuscules colonization, vesicles colonization and root length colonization (see Appendix 1). Hyphae which were not seen to be connected to arbuscules or vesicles were not counted. The following formula were used to calculate the percentage of root colonization:

$$\%\ \text{RLC} = \frac{G - N}{G} \times 100$$

$$\%\ \text{AC} = \frac{A}{} \times 100\ G$$

$$\%\ \text{VC} = \frac{V}{} \times 100\ G$$

where:

G = Total intersections = N+A+V+H

N = Negative (crosshair did not cut through any arbuscule, vesicle nor hyphae)

A = Arbuscules (crosshair cut through at least one arbuscule)

V = Vesicles (crosshair cut through at least one vesicle)

H = Hyphae only (crosshair cut through at least one hyphae, no arbuscule nor vesicle)

% RLC = Percent root length colonization

% AC = Percent arbuscules colonization

% VC = Percent vesicles colonization

3.3 Residual effects study following two seasons of inoculation with arbuscular mycorrhizal fungi on maize crop

3.3.1 Location of the experiment

The residual effect field experiment was carried out during the minor cropping season of 2017 (from September to December) at the Department of Horticulture of KNUST in Kumasi on a Haplic Acrisol according to IUSS Working Group WRB (2014), following two consecutive cropping of soybean inoculated with AMF inoculation. The study area is as described in section 3.2.1.

3.3.2 Planting material

The test crop used for the residual study was maize (*Zea mays* L.) variety Omankwa which is a quality protein maize and early maturing variety (95 days) released in 2010 with an average yield of 4.5 metric tons per hectare (Adu *et al.*, 2014). The seeds were obtained from the Crops Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR), Fumesua, Kumasi, Ghana.

3.3.3 Field layout and experimental design

The same field layout, agronomic practices and experimental design used for the soybean were maintained during the residual studies. However, sowing was done at intervals of 80 cm × 40 cm.

3.3.4 Measured plant parameters

The data collected on maize included total shoot dry weight, grains weight, root dry weight, plant total phosphorus and total nitrogen.

3.3.4.1 Shoot dry weight

The shoots of all maize plants were cut at the base from each plot, oven-dried at 60 °C for 72 h at 50% tasselling and at harvest and their dry weights (g) taken using an electronic precision scale (0.01 g). The shoot yield per hectare was estimated using the dry weights as described by Okogun *et al.* (2005).

3.3.4.2 Grain weight and yield

Maize cobs were manually collected from each plot at harvest, dried under sun for 7 days, shelled, oven-dried at 60 °C for 72 h and then the grain weight (g) was recorded using an electronic precision scale (0.01 g). The grain yield per hectare was estimated using the dry weights as described by Okogun *et al.* (2005).

3.3.4.3 Root dry weight

At 30, 50 and 70 DAS, two maize plants were randomly uprooted from each plot and the roots thoroughly washed under flowing tap water to remove adhered soil particles, oven-dried at 60 °C for 72 h after sampling for mycorrhizal colonization and weighed using an electronic precision scale (0.01 g).

3.3.4.4 Total phosphorus

The plant total phosphorus was assessed at 50% tasseling and at harvest. The shoots were oven dried at 60 °C for 72 h and cut with scissors, milled using a Perten's laboratory mill 3310® and ashed using a muffle furnace. After that, the dry ash digestion and analysis of plant (Motsara *et al.*, 2008; Westerman *et al.*, 1990; Munter *et al.*, 1984) were performed to determine the plant total phosphorus using a spectrophotometer (Milton Roy Company®) at 430 nm wavelength. See detailed description in section 3.10.

3.3.4.5 Plant total nitrogen

The plant total nitrogen was assessed at 50% tasseling and at harvest. The macro Kjeldahl method involving digestion followed by a distillation was used to determine the plant total nitrogen as described by Bremner and Mulvaney (1982). Two grams of dried and milled maize samples from seed and shoot were separately weighed into 500 ml long-necked Kjeldahl flask and the process completed as described in the Kjeldahl method in section 3.6.3 were followed.

3.3.5 Arbuscular mycorrhizal colonization

At 30, 50 and 70 DAS, the roots of two maize plants were randomly sampled in each plot and thoroughly washed under flowing tap water to remove the sand particles and stored in zip lock bags at 4 °C. The same staining process used for the soybean in section 3.8 was subsequently applied to them and the percent root colonization was determined as detailed in section 3.2.12.

3.4 Laboratory protocols used for soil analyses

Initial soil analyses were conducted on the sampled soil at the Soil Chemistry laboratory of the Department of Crop and Soil Sciences at KNUST. The parameters determined

were soil pH in 1: 1 soil: water ratio using the Electrometric method (Page *et al.*, 1982); organic carbon using Walkley – Black wet oxidation method (Nelson and Sommers, 1982); available P using the Bray No. 1 method (Bray and Kurtz, 1945); microbial biomass P using chloroform fumigation followed by extraction of available P using the Bray No. 1 method (Oberson *et al.*, 1997); total nitrogen using Kjeldahl distillation method (Bremner and Mulvaney, 1982); exchangeable acidity using titration method (Mclean, 1965); determination of exchangeable basic cations using ammonium acetate (Moss, 1961; Cottenie, 1980); texture (Clay, Silt, Sand) using hydrometer method; and the initial arbuscular mycorrhizal fungi spore density in the soil using wet sieving and sucrose techniques (Dalpé and Hamel, 2006) before setting up the experiment. Detailed descriptions of these analyses are given in sections 3.6 and 3.7.

3.5 Statistical analyses

The data from the greenhouse and field experiments were subjected to analysis of variance using the GenStat® 12th edition statistical software. When the analysis of variance indicated significant treatment effects, Fisher's Protected LSD (least significant differences in average) test at 5% was used to detect differences among treatment means. The data on root length colonization were analysed statistically to obtain the correlation matrices for the various parameters such as shoot phosphorus uptake, plant height, stem girth and shoot biomass yield for the greenhouse experiments. For the field experiments, data on mycorrhizal inoculation effect concerning soybean were also analysed using Pearson's correlation for shoot phosphorus uptake, grain phosphorus uptake, phosphorus use efficiency, microbial biomass phosphorus, available phosphorus, shoot biomass yield and grain yield.

3.6 Determination of soil chemical properties

3.6.1 Soil pH

The electrometric method (Page *et al.*, 1982) was used to determine the soil pH in a 1:1 soil: solution ratio. Ten grams of air-dried soil was weighed and put into a 50 ml plastic beaker. Using a pipette, 10 ml of distilled water was added to the soil and the suspension was vigorously stirred using stirring rods for the following 20 minutes and allowed to stand for 30 minutes to settle out the suspended clay from the suspension. The glass electrode pH meter was first calibrated with blank at pH of 7 and 4 respectively, after which the electrode was inserted into the partly settled suspension and the pH value read on the pH meter was recorded.

3.6.2 Soil organic carbon

The modified Walkley and Black method was used to determine the soil organic carbon (Nelson and Sommers, 1982). It consists of a modification of the wet oxidation method based on the reduction of the dichromate ($Cr_2O_7^{2-}$) by organic matter. The excess dichromate was subsequently determined by titrating it with standard ferrous sulphate solution. The amount of substance oxidized was then calculated from the quantity of dichromate reduced. One gram of that soil sample was weighed into a 500 ml Erlenmeyer flask and thoroughly mixed. A reference sample was also added as a blank. Exactly 10 ml of 1 equivalent (0.1667 M) potassium dichromate ($K_2Cr_2O_7$) solution was added using a pipette, followed by 20 ml of concentrate sulfuric acid from a burette to facilitate the reaction by the heat generated. The mixture was swirled and left for 30 mins on an asbestos sheet to cool down. Afterwards, 200 ml of distilled water was added, and then 10 ml of orthophosphoric acid. One equivalent (1.0 *N*) of ferrous sulphate solution was used to titrate the solution till a colour change of dark blue was obtained, and then to a green end-point after adding 2 ml of diphenylamine used as

indicator. The titre value was recorded and corrected based on the blank solution (≥ 10.5).

Calculation:

$$\% \text{ C} = \frac{M \times (\text{Vbl} - \text{Vs}) \times 0.003 \times 1.33 \times 100}{\text{g}}$$

where:

M = Molarity of $FeSO_4$

Vbl = ml $FeSO_4$ of blank titration Vs

= ml $FeSO_4$ of soil sample titration g

= mass of soil taken in grams

0.003 = milli-equivalent weight of C in grams (12/4000)

1.33 = correction factor used to convert the wet combustion C value to the true C value since the Wet combustion method is about 75% efficient in estimating C value, (i.e. 100/75 = 1.33)

The following formula was used to determine organic matter content:

% organic C × 1.724 (1.724 is the Van Bemellean factor)

3.6.3 Total nitrogen

The macro Kjeldahl method involving digestion followed by distillation was used to determine the soil total nitrogen as described by Bremner and Mulvaney (1982). After being uniformly mixed, 10 g of the air-dried soil sample was weighed into a 500 ml long-necked Kjeldahl flask, 10 ml distilled water was added and the mixture was allowed to stand for 10 minutes to moisten. One spatula full of Kjeldahl catalyst

(mixture of l part Selenium + 10 parts copper sulphate + 100 parts sodium sulphate) and 30 ml concentrate sulphuric acid were added. The mixture was then digested until clear and colourless or light greenish (30 minutes to one hour) solution was obtained and the flask allowed to cool. The digest was decanted into a 100 ml volumetric flask and made up to the mark with distilled water with the rinsing from the digestion flask. An aliquot of 10 ml of digest was afterwards transferred into the Kjeldahl distillation apparatus using a pipette followed by the addition of 20 ml of sodium hydroxide solution concentrated at 40%. Over 10 ml of boric acid (4%) and three drops of mixed indicator and about 100 ml of distillate were collected into a 500 ml conical flask for 5 minutes. A blank determination was also performed in the absence of the soil sample to trace the nitrogen in the water used and the reagents. The presence of nitrogen turned to a light blue colouration. The collected distillate (about 100 ml) was titrated with 0.1 *N* hydrochloric acid (HCl), using methyl red and Bromocresol green as an indicator, until the blue colour developed turned grey and then to pink.

Calculation:

14 g of N contained in one equivalent weight of NH_3:

$$\text{Weight of N in the soil} = \frac{14 \times (V - B) \times N}{1000}$$

Thus, the percentage of nitrogen in the soil sample is:

$$\% \text{ N} = \frac{14.01 \times (V - B) \times N \times R \times 100}{1000 \times \text{Weight of sample}}$$

where:

R= ratio between total volume of digest and digest volume (aliquot) for distillation

N = normality of HCl = 0.1 *N*

V = volume of HCl titrated for the sample

B = digested blank titration volume

3.6.4 Available phosphorus

The Bray No. 1 method consisting of a dilute acid fluoride (HCl: NH_4F mixture) extraction procedure was used to extract the more available forms of P in the soil as described by Bray and Kurtz (1945). Three grams of air-dried and sieved soil using a 2 mm mesh sieve were weighed into centrifuge tubes and 15 ml of Bray 1 solution (0.025 N HCl + 0.03 N NH_4F) were added. The tubes were placed on a mechanical shaker, shaken for 5 minutes, and centrifuged for 5 mins at 3000 rpm after allowing them to stand for 2 mins. After that, 2 ml of the supernatant solution was pipetted into different clean centrifuge tubes. Ten millilitres of distilled water was added and the solution well mixed. Afterwards, 2 ml of colour reagent was added by mixing the solution well again, and 2 ml of ascorbic acid solution was finally added and the mixture thoroughly mixed. The solution was allowed to stand 15 mins to develop a blue colour and the colour intensity was measured on a Milton Roy Company® Spectrophotometer at the wavelength of 650 nm. The unknown samples were read and the ppm P obtained by interpolation on a standard curve C plotted using absorbance verse. Calculation:

ppm P (mg P/kg soil) = C × 15/3

= C × 5

3.6.5 Soil exchangeable acidity

The exchangeable acidity was determined using the titration method by Mclean (1965).

3.6.5.1 Exchangeable hydrogen

Three grams of air-dried soil ground to pass a 2 mm sieve was weighed into folded Whatman No. 42 filter paper, then placed on a funnel and on an Erlenmeyer flask. Fifty millilitres of 1.0 *N* potassium chloride solution were carefully poured in the filter paper, through the soil. The leachate was collected into the Erlenmeyer flask and five drops of phenolphthalein indicator were added to it and titrated with 0.05 *N* sodium hydroxide to pink end-point and then the volume (ml) of sodium hydroxide used (V) was recorded.

3.6.5.2 Exchangeable aluminium

To the titrated extract, 4 ml of 3.0 *N* sodium fluoride (NaF) was added and the mixture was titrated with hydrochloric acid (0.05 *N*) to colourless end-point and the volume (ml) of hydrochloric acid used (V) was recorded.

3.6.5.3 Calculations:

$$\text{Exchangeable hydrogen (meq/100g)} = \frac{\text{V} \times 0.05 \times 100}{W} = \text{V} \times 1.67$$

where:

V = Titre volume of NaOH used (ml)

W = weight of soil sample used (3 g)

Normality of NaOH = 0.05 *N*

$$\text{Exchangeable aluminium (meq/100g)} = \frac{\text{V} \times 0.05 \times 100}{W} = \text{V} \times 1.67$$

where:

V = Titre volume of hydrochloric acid (HCl) used (ml)

Normality of HCl = 0.05 *N*

W = weight of soil sample used (3 g)

3.6.6 Exchangeable cations

Potassium, sodium, calcium and magnesium in the soil were determined by ammonium acetate (NH_4OAc) extraction method. A volume of 57 ml of glacial acetic acid was diluted to 800 ml with distilled water. The solution was neutralized with concentrated NH_4OH to pH 7.0. The solution was then diluted to one litre in a volumetric flask.

3.6.6.1 Exchangeable potassium and sodium

The concentration of potassium (K) and sodium (Na) in the soil extract was determined using flame photometry (Cottenie, 1980). A standard solution of K + Na concentrations were prepared by dissolving 2.542 g sodium chloride in about 200 ml deionised water; dissolving 1.907 g potassium chloride in about 200 ml deionized water; mixing the two solutions and made up to 1000 ml with deionised water. Afterwards, 50 ml of the 1000 mg L^{-1} (stock solution) was diluted into 1000 ml to give 50 mg L^{-1} for K + Na. Portions of 0, 4, 8, 12, 16 and 20 ml of the 50 mg L^{-1} standard solution was placed into separate 200 ml volumetric flasks. To each of the flasks, 100 ml of one equivalent of ammonium acetate solution was added and using distilled water, made to volume (200 ml). The standard series obtained was 0, 2, 4, 6, 8 and 10 mg L^{-1} for potassium plus sodium (K + Na).

A 100 ml of one equivalent of ammonium acetate solution was added onto 10 g of soil initially weighed into extraction bottle which was placed with content on a mechanical shaker and the mixture shaken for 2 hours. Through Whatman filter paper No. 42, the supernatant solution was filtered and a 10 ml aliquot was pipetted and read for potassium and sodium on a flame photometer at a wavelength of 766.5 nm after the

photometer was calibrated with standard solutions previously prepared. The meter reading standard curve was used to determine the concentration of potassium or sodium in the soil extract.

Calculations:

$$\text{Exchangeable K (cmol/kg soil)} = \frac{1000 \times (a - b) \times \text{mcf}}{10 \times 39.1 \times s}$$

$$\text{Exchangeable Na (cmol/kg soil)} = \frac{1000 \times (c - d) \times \text{mcf}}{10 \times 22.9 \times s}$$

where:

a = mg K L^{-1} in the diluted sample b =

mg K L^{-1} in the diluted blank sample c =

mg Na L^{-1} in the diluted sample d = mg

Na L^{-1} in the diluted blank sample mcf =

moisture correcting factor s = air-

dried soil sample weight in grams

3.6.6.2 Determination of exchangeable calcium and magnesium

The concentrations of calcium and magnesium were determined using titration method with ethylene diamine tetraacetic acid (EDTA) solution (Moss, 1961).

3.6.6.2.1 Calcium

A 10 ml aliquot of the sample solution extracted with 1.0 *N* ammonium acetate and filtrate was transferred into a 100 ml conical flask. Ten millilitres of 10% potassium hydroxide solution was added, and then 1 ml of 30% Triethanolamine. Three drops of

10% potassium cyanide solution and a few crystals of cal-red indicator were added and the mixture was vigorously shaken to get it uniform. The mixture was titrated with 0.02 *N* EDTA solution from a red to blue end-point and the titre value of calcium was recorded.

3.6.6.2.2 Calcium and magnesium

A 10 ml aliquot of the same sample solution extracted with 1.0 *N* ammonium acetate and filtered as described in section 3.6.6 were transferred into a 100 ml conical flask. Five millilitres of ammonium chloride-ammonium hydroxide buffer solution were added, and then 1 ml of triethanolamine. Three drops of 10% potassium cyanide solution were added and a few drops of Eriochrome Black T (EBT) indicator solution and the mixture was vigorously shaken to get it uniform. The mixture was titrated with 0.02 *N* EDTA solution from a red to blue end-point. The titre value was recorded again and to get the titre value for Mg, the titre value of Ca was subtracted from this value.

Calculation:

$$\text{Concentration of Ca+Mg (cmol(+)/kg)} = \frac{0.02 \times (V_a - V_b) \times 1000}{0.1 \times W}$$ where:

W = weight (g) of air-dry soil

V_a = volume (ml) of 0.02 *N* EDTA used in the sample titration

V_b = volume (ml) of 0.02 *N* EDTA used in the blank titration

0.02 = concentration of EDTA used

NB: Ca = Titre value of Ca × 2 in cmol/kg or Meq/100 g soil

3.7 Determination of soil physical and biological properties

The particle size distribution and the soil microbial biomass phosphorus were assessed at the Soil Chemistry Laboratory, KNUST while the soil arbuscular mycorrhizal spore density was done at Soil Microbiology Laboratory of Crop and Soil Science Department, KNUST.

3.7.1 Particle size distribution

Bouyoucos hydrometer method (Bouyoucos, 1936) was used to determine particle size distribution. In a 1 litre flask, 35.7 g of sodium hexametaphosphate was dissolved in 750 ml of deionized water. Then 7.49 g of sodium carbonate were added and made up to 1 litre with deionized water. The solution (Calgon) was vigorously shaken to dissolve the hexametaphosphate. After that, 51 g of air-dried soil (< 2mm) was weighed and transferred into a 250 ml beaker. Fifty millilitres of the Calgon solution prepared and 100 ml of deionized water was dispensed onto the soil and the suspension was vigorously stirred for one minute using a glass rod and left to stand for 30 mins. The suspension was then transferred to the mixer and mixed for 15 minutes at a medium speed. After mixing, it was transferred to the sedimentation cylinder and made up to 1 litre with deionized water.

The measurements were taken as follow: in the cylinder placed on a flat surface, the suspension was mixed again by several vertical movements of the plunger (2 minutes). The time was recorded and the soil hydrometer was forthwith placed into the suspension and slowly slid into it until it was floating. The first reading was taken on the hydrometer at 40 seconds after the cylinder was set down (H_1). The hydrometer was then removed and the temperature of the suspension was measured with a thermometer (T_1 in ^oF). The steps of measurements were repeated to take a duplicate reading. The suspension was left to stand for 3 h after the first two hydrometer readings

(H_1), and then a second reading (H_2) was taken. The temperature of the suspension (T_2 in ^oF) was also taken.

The suspension was mixed in the cylinder by several vertical movements of the plunger (1 – 2 minutes), the cylinder was placed on a flat surface and the time recorded. The soil hydrometer was immediately placed into the suspension and the hydrometer slowly slid into the suspension until it was floating. The first hydrometer reading was taken on the at 40 seconds (H_1). The hydrometer was removed and the temperature of the suspension was measured using a thermometer (T_1 in ^{o} F). Step 1 to 4 were repeated to take a duplicate reading. After the first two hydrometer readings (H_1), the suspension was left to stand for 3 hours and a second reading (H_2) was taken. Also, take the temperature of the suspension (T_2 in ^{o} F).

Calculations of the percentage of sand, clay and silt were done as follows:

$$\% \text{ Sand} = 100 - 2 \times (H_1 + T_1 - 68 \times 0.2)$$

$$\% \text{ Clay} = H_2 - 2 + T_2 - 68 \times 0.2 \times 2$$

$$\% \text{ Silt} = 100 - \% \text{ Sand} + \% \text{ Clay}$$

The textural triangle was used to determine the soil textural class and the various portions were expressed in percentage.

3.7.2 Extraction of arbuscular mycorrhizal spores from soil

Wet sieving and sucrose techniques was used as described by Dalpé and Hamel (2006). Fifty grams of soil was weighed and poured into a 1 L flask containing 300 mL of water. The suspension was vigorously shaken for 1 minute and allowed to soak for 30 min. The water and soil mix were poured through the sieves piled in a decreasing order of mesh size (212, 90, 63 and 45 µm with the largest mesh on the top to retain debris),

recovery of the entire soil mix was ensured by carefully rinsing the flask. This allowed the recovery of all soil material for spore extraction. The soil was then washed with running water, manually and carefully breaking soil aggregates when required. The entire soil was recovered from each sieve, the soil material distributed in centrifuge tubes (Corex® tubes), the tubes were filled with a 50% (w = v) sucrose solution, the tubes content was thoroughly mixed using magnetic stirring and centrifuged at 1800 rpm for 4 min. The supernatants were recovered on a 45 µm sieve and carefully washed to dilute sucrose concentration. The spores were transferred into plastic petri dishes containing water for examination under an optical microscope. The extracted spores were then counted. For each centrifuge tube, the spore suspension was placed in a haemocytometer, the surface of which is gridded to facilitate the counting of the spores. The total number of spores per 100 grams of dry soil was then calculated.

3.7.3 Soil microbial biomass phosphorus

After defrosting the fresh soil samples kept frozen, 5 g of it was weighed, put into a crucible and fumigated in a desiccator for five days with 30 ml of chloroform. Both unfumigated and fumigated samples were shaken for 10 minutes with 35 ml Bray No. 1 extracting solution (0.03 *M* NH_4F + 0.025 *M* HCl) and filtered. Adsorption of phosphorus during fumigation was corrected by simultaneously counterbalancing unfumigated soil with a series of phosphorus containing standard solutions before extracting with the Bray-1 solution. The quantity of chloroform released phosphorus was determined using the relationship between phosphorus extracted by the Bray-1 solution and phosphorus added (from microbial lysis or standard solutions) (Oberson *et al.*, 1997). Phosphorus adsorption during balance was described according to Barrow and Shaw (1975) by the following equation adapted by Morel *et al.* (1997):

$$Ext_p = Ext_0 + b_1 Pad^{b2}$$

where:

Ext_p = Pi concentration (mg/l) extracted after equilibration with different amounts of P added,

Ext_0 = Pi concentration extracted without P addition, b_1, b_2 = coefficients estimated by non-linear regression of mean values of Ext_p against Pad,

Pad = amount of P added (0 – 20 mg kg^{-1}).

Chloroform released P corresponds to a P addition and was calculated from the equation:

$$P_{chl.} = \left[\frac{(Ext_{chl} - Ext_0)}{b_1}\right]^{1/b2}$$

where:

$P_{chl.}$ = chloroform released P (mg kg^{-1}),

$Ext_{chl.}$ = Pi concentration in extracts of fumigated samples.

The amount of microbial P was estimated by assuming a kp factor of 0.4 (Brookes *et al.*, 1982; McLaughlin and Alston, 1986).

3.8 Roots staining and observation

Root cleaning and staining were done as described by Dalpé and Hamel (2006) in order to evaluate mycorrhizal infection rates. The roots previously sampled were carefully rinsed with tap water to remove soil particles, ensuring that no soil adhered to the root pieces to be stained. The root samples were placed in the McCartney bottles labelled with a lead pencil (ink vanishes with potassium hydroxide), covered with 10% potassium hydroxide (KOH) solution, placed on a water bath and boiled (90 °C) for 1 hour to clear the roots. The potassium hydroxide allows the roots to be discoloured and

to empty the cytoplasmic contents of root cells. The KOH solution was then discarded and the McCartney bottles rinsed several times with tap water and in 30% hydrogen peroxide. The roots were then placed in a 0.05% Trypan blue solution for staining. The McCartney bottles containing the roots soaked in the Trypan blue solution were placed in a water bath at 90 °C for 30 minutes. The stained roots were placed in a destaining solution: 500 mL glycerol, 450 mL distilled water, and 50 mL 1% hydrochloric acid over-night. The stained roots were stored in destaining solutions at 4 °C.

These roots were mounted between slide and coverslip in glycerol. For each experimental plot, 50 fragments spread over 5 slides were observed per treatment after each harvest. Those fragments have previously been stained with Trypan blue (Phillips and Hayman, 1970) to make more visible fungi structures (vesicles, hyphae and arbuscules).

3.9 Mycorrhizal dependency and mycorrhizal inoculation effect

Mycorrhizal dependency (MD) was defined by Gerdemann (1964) as: " at a given level of soil fertility, the degree to which a plant species depends on the mycorrhizal infection to show maximum growth or yield ".

Calculation:

When using a AMF inoculant in sterile soil, the formula below was proposed by Menge *et al.* (1978) to calculate the relative mycorrhizal dependency (RMD) of crop plants:

$$\text{RMD}\ (\%) = \frac{\text{Dry weight of mycorrhizal plants}}{\text{Dry weight of nonmycorrhizal plants}} \times 100\%$$

In the presence of indigenous AMF, to evaluate the growth enhancement brought about by a mycorrhizal fungus inoculated to non-sterile soil, Bagyaraj (1992) proposed a formula to calculate mycorrhizal inoculation effect (MIE) as:

$$\text{MIE (\%)} = \frac{\text{DW of inoculated plants} - \text{DW of uninoculated plants}}{\text{Dry weight of inoculated plants}} \times 100\%$$

where DW = Dry weight

MIE is useful for assessing the extent to which introduced fungi compete with native endophytes to bring about plant growth responses. This information is of great value in practical agriculture, especially in developing countries, where farmers do not fumigate or sterilize either nursery or field sites (Bagyaraj, 1992).

3.10 Determination of plant total phosphorus

The dry ash digestion and analysis of plant tissues (Munter *et al.*, 1984; Westerman *et al.*, 1990; Motsara *et al.*, 2008) were performed to determine the plant total phosphorus. The plant samples were oven-dried at 60 °C for 72 hours, cut using scissors and milled (Perten's laboratory mill 3310®). One gram sample was weighed into a clean ceramic crucible whose weight had been recorded to the nearest 0.001 g. An empty crucible was included as a blank and the whole was placed in a muffle furnace at a temperature of 500 °C over a period of four hours. The ash samples were left in the oven to cool, removed from it and poured into 50 ml centrifuge tubes that are labelled. Ten millilitres of distilled water was used to rinse the crucibles into the centrifuge tubes followed by 10 ml of Aqua Regia. The samples were then shaken (Vortex) for 5 mins to ensure proper mixing and centrifuged for 10 mins at 3000 rpm. After decanting the supernatants into clean 100 ml volumetric flasks, they were made up to volume using distilled water.

A standard series was initially prepared by pipetting respectively 0, 2, 4, 6, 8, 10 and 20 ml of a standard solution of 50 ppm (µg P/ml) into 50 ml volumetric flasks. Vanadomolybdate reagent was added to each flask at a volume of 10 ml and the volume was made up to the 50 ml mark. These concentrations were measured on a spectrophotometer (Milton Roy Company®) at 430 nm wavelength to prepare the standard curve. The corresponding absorbance was recorded and the standard curve was plotted. Ten millilitres of supernatant were measured into a volumetric flask of 50 ml capacity, 10 ml of vanadomolybdate reagent was added and made to volume with distilled water. The solution was vigorously shaken and allowed to stand for 30 minutes. A yellow colour developed and a spectrophotometer was used to measure the transmission at 430 nm wavelength. The absorbance was determined from the percent transmittance recorded, and hence the phosphorus content was determined from the standard curve.

Calculation:

$$\text{P content (g) in 100 g plant sample (\%P)} = \frac{C \times df \times 100}{1{,}000{,}000} = \frac{C \times 100 \times 100}{1{,}000{,}000}$$

$$\text{P content (g) in 100 g plant sample (\%P)} = \frac{C}{10}$$

Where:

C = concentration of P (µg/ml) as read from the Standard Curve df = dilution factor, which is 100 × 10 = 1000 and is calculated as 1.0 g of sample made to 100 (100 times)

10 ml of sample solution made up to 100 ml (10 times)

3.11 Phosphorus uptake and use efficiency

The phosphorus uptake by plants was calculated by using the formula described by Sharma *et al.* (2012).

$$\text{P uptake (kg/ha)} = \frac{\text{P(\%)} \times \text{dry matter (kg/ha)}}{100}$$

Phosphorus use efficiency as described by Mosier *et al.* (2004) were used to evaluate the beneficial effects of arbuscular mycorrhizal inoculation. The following formula was used:

$$\text{Phosphorus use efficiency (PUE) (kg/kg)} = \frac{\text{Yield increase (kg)}}{\text{Phosphorus applied (kg)}}$$

3.12 Economic analysis

The formulas used to calculate overall and adjusted yields, gross revenues, net profits, marginal costs, net marginal benefit and the marginal rate of return were derived from an economics training manual (CIMMYT, 1988). The percentage marginal rate of return was calculated as:

$$\text{Marginal rate of return (\%)} = \frac{\text{Marginal net benefits}}{\text{Marginal costs}} \times 100$$

CHAPTER FOUR

4.0 RESULTS

4.1 Symbiotic effectiveness test of four arbuscular mycorrhizal fungi isolates on two soybean lines: a greenhouse study

4.1.1 Physical and chemical properties of soils used

Results of the selected chemical, physical and biological properties of the sterile river sand and non-sterile soil (Haplic Acrisol) are presented in Table 4.1. From the soil testing interpretation manual by Landon (2014), the results indicate that the sterile river sand was acidic with only traces of available P, total nitrogen, organic carbon, exchangeable potassium and exchangeable calcium, except for exchangeable magnesium. The non-sterile soil was medium in available phosphorus, very low in total nitrogen and organic carbon, low in exchangeable potassium and medium in exchangeable calcium and magnesium. It was slightly acidic and sandy clay loam in texture, and was suitable for soybean cultivation according to Belfield *et al.* (2011). It was found to contain low densities of AMF spores (< 3-4 spores in some fields examined on the petri dish, and no spores in others) based on the standard set by INVAM (2017).

Table 4.1. Selected properties of soils

Soil property	Soils	
	Sterile river sand	Non-sterile soil
pH (1:1 H_2O)	5.95	6.27
Organic carbon (%)	0.09	1.37
Total nitrogen (%)	0.04	0.06
Available P (mg kg^{-1})	1.96	39.43
Exchangeable K ($cmol_c$ kg^{-1}soil)	0.32	0.03
Exchangeable Ca ($cmol_c$ kg^{-1} soil)	0.82	6.62
Exchangeable Mg ($cmol_c$ kg^{-1} soil)	0.86	0.60
Exchangeable Na ($cmol_c$ kg^{-1} soil)	0.34	0.22
Textural class	Sand	Sandy Clay Loam
AMF spore density (spores/100 g of soil)	-	284
Soil classification (IUSS Working Group WRB, 2014)	-	Haplic Acrisol

4.1.2 Arbuscular mycorrhizal inoculation on root length colonization Results of ANOVA for arbuscular mycorrhizal fungi inoculation on the root length colonization (RLC) of the two soybean lines under greenhouse conditions are presented in Table 4.2. At six weeks after sowing, the ANOVA showed significant differences in mycorrhizal RLC of soybean that varied with AMF isolates, the soybean lines and the soil types. In both soils used, uninoculated control remained nonmycorrhizal. Mycorrhizal RLC was higher in the sterile river sand than in the nonsterile soil. All the other isolates inoculated to the sterile river sand significantly ($P < 0.001$) increased the mycorrhizal RLC of plants over *Glomus etunicatum*. However, in the non-sterile soil all the inoculants significantly ($P < 0.001$) increased the mycorrhizal RLC of plants over *G. fasciculatum*.

Among the four AMF species used, *G. mosseae* showed the highest percent RLC (83.84% in sterile river sand and 33.07% in non-sterile soil), followed by *G. fasciculatum* in sterile river sand (75.69%) and *G. etunicatum* in non-sterile soil (21.49%), while *Rhizophagus irregularis* maintained the third position for both soil types (73.85% in sterile river sand and 20.08% in non-sterile soil).

In the sterile river sand, mycorrhizal RLC was significantly ($P < 0.001$) higher in plants inoculated with *G. mosseae* and *G. fasciculatum*, with no significant difference in root colonization between treatments with *G. fasciculatum* and *R. irregularis* (Table 4.2). Inoculation with *G. mosseae* in sterile river sand significantly ($P < 0.001$) increased soybean RLC by 25% over *G. etunicatum*, 13% over *R. irregularis* and 11% over *G. fasciculatum*.

In the non-sterile soil, mycorrhizal RLC was significantly higher ($P < 0.001$) in plants inoculated with *G. mosseae* and *G. etunicatum*, with no significant differences in RLC between *G. etunicatum* and *R. irregularis* (Table 4.2). The RLC by *G. mosseae* increased by 54% over *G. etunicatum*, 65% over *R. irregularis* and 92% over *G. fasciculatum*.

The RLC was not consistent for the two soybean lines from one medium to the other, even though there was a significant difference in both cases ($P = 0.003$ for sterile river sand and $P < 0.001$ for non-sterile soil) (Table 4.2). The line TGx 1989-48 FN showed the highest RLC, an increase of 3% over TGx 1989-75 FN in sterile river sand. On the contrary, RLC was higher for the line TGx 1989-75 FN in non-sterile soil and increased of 37% over TGx 1989-48 FN.

There was a significant ($P < 0.001$) interaction between the AMF isolates and soybean lines in both sterile river sand and non-sterile soil with regard to RLC (Appendix 2). The RLC was highest (89.90%) when the isolate *G. mosseae* was inoculated to the line TGx 1989-48 FN and lowest (64.30%) when *G. etunicatum* was inoculated to the line TGx 1989-75 FN in sterile river sand. In the non-sterile soil, the RLC was highest (35.90%) when *G. mosseae* was inoculated to the line TGx 1989-48 FN and lowest (12.10%) when *G. fasciculatum* was inoculated to the same line.

Table 4.2. Root length colonization of two soybean lines by the four AMF isolates in sterile river sand and non-sterile soil Root length colonization Treatments (%)

	Sterile river sand	Non-sterile soil
AMF isolates		
Glomus etunicatum	67.01	21.49
Glomus fasciculatum	75.69	17.22
Glomus mosseae	83.84	33.07
Rhizophagus irregularis	73.85	20.08
Control	0.00	0.00
F pr.	< 0.001	< 0.001
LSD (5%)	2.35	2.22
CV (%)	3.8	11.7
Soybean lines (S)		
TGx 1989-48 FN	60.86	15.52
TGx 1989-75 FN	59.29	21.23
F pr.	0.003	< 0.001
LSD (5%)	0.58	1.20
CV (%)	0.4	2.9
F pr. **S × AMF isolates**	< 0.001	< 0.001

4.1.3 Mycorrhizal inoculation on relative mycorrhizal dependency of two soybean lines

Table 4.3 shows the results of mycorrhizal inoculation on the relative mycorrhizal dependency (RMD) of the two soybean lines under greenhouse conditions. In the sterile river sand, inoculation with *R. irregularis* resulted in the highest value of RMD (114 %) and *G. etunicatum*, the least (93%). The values were significantly ($P < 0.001$)

different among the different AMF isolates except between *G. fasciculatum* and *G. etunicatum*. The effect of the inoculation on RMD in the sterile river sand ranked as follows: *R. irregularis* > *G. mosseae* > *G. fasciculatum* > *G. etunicatum*. There was a significant ($P < 0.001$) interaction between the AMF isolates and soybean lines for RMD in both sterile river sand and non-sterile soil (Appendix 3). The RMD was highest (119%) when *R. irregularis* was inoculated to TGx 1989-48 FN and least (92%) with the isolate *G. etunicatum* inoculated to the same line in sterile river sand. There was a significant ($P = 0.021$) difference in RMD between the two soybean lines in sterile river sand only. The line TGx 1989-48 FN showed more dependency (105%) on the AMF isolates than TGx 1989-75 FN (101%) (Table 4.3).

In the non-sterile soil, RMD significantly ($P < 0.001$) increased with the mycorrhizal isolates tested and ranged from 93% (*G. fasciculatum*) to 118% (*R. irregularis*). The effect of the inoculation on RMD was in the order: *R. irregularis* > *G. mosseae* > *G. etunicatum* > *G. fasciculatum*. There was a significant ($P < 0.001$) interaction between the AMF isolates and soybean lines for RMD which was highest when *R. irregularis* was inoculated to TGx 1989-75 FN (128%) and least with *G. fasciculatum* isolate (88%) inoculated to the same line (Appendix 3).

Table 4.3. Relative mycorrhizal dependency of two soybean lines inoculated with four AMF isolates in sterile river sand and non-sterile soil Relative Mycorrhizal Dependency Treatments (%)

	Sterile river sand	**Non-sterile soil**
AMF isolates		
Glomus etunicatum	93	103
Glomus fasciculatum Glomus	95	92
mosseae	110	112
Rhizophagus irregularis	114	118
F pr.	< 0.001	< 0.001
LSD (5%)	2.60	5.00
CV (%)	2.4	4.3

Soybean lines (S)		
TGx 1989-48 FN	105	105
TGx 1989-75 FN	101	108
F pr.	0.021	0.445
LSD (5%)	2.70	NS
CV (%)	1.2	4.5
F pr.		
S × AMF isolates	< 0.001	< 0.001

NS = Not Significant at $P \leq 0.05$.

4.1.4 Mycorrhizal inoculation on shoot phosphorus uptake by the two soybean lines

There were significant differences in shoot phosphorus (P) uptake of the soybean lines in both soils (Table 4.4). In the sterile river sand, inoculation with *R. irregularis* and *G. mosseae* significantly ($P < 0.001$) increased soybean shoot P uptake over the control, *G. etunicatum* and *G. fasciculatum*. Soybean shoot P uptake was highest (2.43 kg ha^{-1}) when inoculated with the isolate *R. irregularis*, followed by *G. mosseae* (2.09 kg ha^{-1}) and the difference was significant between these two, with the former being 16% higher than the latter. This represented an increase in shoot P uptake of 62% and 39% over the control for *R. irregularis* and *G. mosseae*, respectively. However, the treatments inoculated with the isolates *G. etunicatum* and *G. fasciculatum* did not show any significant difference in relation to the control.

In the non-sterile soil, inoculation with *R. irregularis* and *G. mosseae* significantly ($P < 0.001$) increased soybean shoot P uptake over the control, *G. etunicatum* and *G. fasciculatum* (Table 4.4). Shoot P uptake was highest (18.59 kg ha^{-1}) with inoculation with *R. irregularis* which represented significant increases over uninoculated control, *G. fasciculatum*, *G. etunicatum* and *G. mosseae*. The difference in shoot P uptake was significant between *R. irregularis* and *G. mosseae*, but not between *G. fasciculatum* and the control. The lowest soybean shoot P uptake was recorded in the control

treatment for the sterile river sand, and on treatments inoculated with *G. fasciculatum* in the non-sterile soil.

For both soils, the difference in shoot P uptake between the two soybean lines was not significant. However, in the sterile river sand there was a significant (P = 0.043) interaction between AMF isolates and soybean lines for shoot P uptake which was highest (2.74 kg ha^{-1}) when *R. irregularis* was inoculated to the line TGx 1989-48 FN, and lowest (1.45 kg ha^{-1}) with the control of the same line (Appendix 4). The interaction was not significant in the non-sterile soil.

Table 4.4. Shoot phosphorus uptake of two soybean lines inoculated with four AMF isolates under greenhouse conditions in sterile river sand and non-sterile soil Shoot phosphorus uptake Treatments (kg ha^{-1})

	Sterile river sand	**Non-sterile soil**
AMF isolates		
Glomus etunicatum	1.74	15.06
Glomus fasciculatum	1.62	12.60
Glomus mosseae	2.09	17.06
Rhizophagus irregularis	2.43	18.59
Control	1.50	12.70
F pr.	< 0.001	< 0.001
LSD (5%)	0.30	0.58
CV (%)	16.2	7.4
Soybean lines (S)		
TGx 1989-48 FN	1.99	15.22
TGx 1989-75 FN	1.77	15.18
F pr.	0.086	0.960
LSD (5%)	NS	NS
CV (%)	6.6	6.1
F pr.		
S × AMF isolates	0.043	0.107

NS = Not Significant at $P \leq 0.05$.

4.1.5 Mycorrhizal inoculation on growth parameters of the two soybean lines

Results for mycorrhizal inoculation on some growth parameters of the two soybean lines under greenhouse conditions are presented in Tables 4.5, 4.6 and 4.7. The

performance in growth resulting from inoculation with mycorrhizal isolates was different in both soils. Stem girth was not significantly affected by inoculation in the sterile river sand, and plant height and shoot biomass yield were not affected in the non-sterile soil.

In the sterile river sand, inoculation with all the AMF isolates, except *G. fasciculatum* at 20 DAS, significantly ($P < 0.001$) improved plant height (Tables 4.5), stem girth (Tables 4.6) and shoot biomass yield (Tables 4.7) except *G. etunicatum* relative to the uninoculated control. Inoculation with *R. irregularis* showed the highest increase in plant height (24%) followed by *G. mosseae* (20%) at 20 DAS, and 19 and 15% at 40 DAS over the control treatment, respectively (Table 4.5). Similarly, these two isolates led to an improvement in shoot biomass yield of 42 and 22% increases over the control for *R. irregularis* and *G. mosseae*, respectively (Table 4.7). The inoculation significantly increased the stem girth over the control, irrespective of the AMF isolate. However, they were highest with *G. mosseae* and *R. irregularis* (Table 4.6).

In the non-sterile soil, all the mycorrhizal isolates significantly ($P < 0.001$) stimulated plant height and stem girth at 20 and 40 DAS and shoot biomass yield (except *G. fasciculatum* in terms of shoot biomass yield) relative to the uninoculated control. Inoculation with *R. irregularis* showed the highest improvement in plant height (47%) followed by *G. mosseae* (35%) at 20 DAS, and 26 and 22% at 40 DAS over the control treatment, respectively (Table 4.5). Likewise, these two isolates increased shoot biomass yield by 24 and 15% over the control for *R. irregularis* and *G. mosseae*, respectively (Table 4.7). The major significant growth improvements were mainly brought about by *R. irregularis* and *G. mosseae*, except plant height at 40 DAS, where the inoculation significantly increased the stem girth over the control, irrespective of the AMF isolate (Table 4.6).

Table 4.5. Plant height of two soybean lines following inoculation with four AMF isolates in sterile river sand and non-sterile soil

Treatments	Plant height (cm)			
	Sterile river sand		Non-sterile soil	
	20 DAS	40 DAS	20 DAS	40 DAS
AMF isolates				
Glomus etunicatum	33.90	52.20	36.70	142.30
Glomus fasciculatum	32.50	50.50	38.10	140.80
Glomus mosseae	36.20	53.80	39.50	143.80
Rhizophagus irregularis	37.20	55.90	43.00	148.30
Control	30.10	46.80	29.30	118.10
F pr.	< 0.001	< 0.001	< 0.001	< 0.001
LSD (5%)	2.90	3.60	2.40	8.50
CV (%)	5.8	6.7	6.1	5.9
Soybean lines (S)				
TGx 1989-48 FN	33.00	51.00	38.00	140.00
TGx 1989-75 FN	35.00	53.00	36.00	138.00
F pr.	0.022	0.206	0.283	0.496
LSD (5%)	1.10	NS	NS	NS
CV (%)	2.3	3.6	5.5	2.5
F pr.				
S × AMF isolates	0.255	0.017	< 0.001	0.800

NS = Not Significant at $P \leq 0.05$.

Table 4.6. Stem girth of two soybean lines following inoculation with four AMF isolates in sterile river sand and non-sterile soil

Treatments	Stem girth (mm)			
	Sterile river sand		Non-sterile soil	
	20 DAS	40 DAS	20 DAS	40 DAS

AMF isolates				
Glomus etunicatum	1.53	2.08	1.96	3.72
Glomus fasciculatum	1.54	2.07	2.05	3.68
Glomus mosseae	1.58	2.10	2.59	4.20
Rhizophagus irregularis	1.59	2.14	2.23	3.96
F pr.	0.008	0.036		< 0.001
LSD (5%)	0.08	0.15	0.27	0.30
CV (%)	5.3	7.0	12.6	7.7
Soybean lines (S)				
TGx 1989-48 FN	1.56	2.10	2.16	3.92
TGx 1989-75 FN	1.51	2.02	2.04	3.60
F pr.	0.094	0.190	0.207	0.001
LSD (5%)	NS	NS	NS	0.08
CV (%)	1.8	3.0	5.0	1.0
F pr.				
S × AMF isolates	0.367	0.285	0.174	0.037
NS = Not Significant at $P \leq 0.05$.		Control	1.44	1.91
	1.68			
	< 0.001	3.22		

Table 4.7. Shoot biomass yield of two soybean lines following inoculation with four AMF isolates in sterile river sand and non-sterile soil Shoot biomass yield Treatments (kg ha^{-1})

	Sterile river sand	**Non-sterile soil**
AMF isolates		
Glomus etunicatum	881	1980
Glomus fasciculatum	911	1860
Glomus mosseae	1029	2047
Rhizophagus irregularis	1200	2178
Control	842	1751
F pr.	< 0.001	< 0.001
LSD (5%)	63.21	185.36
CV (%)	6.3	9.1
Soybean lines (S)		
TGx 1989-48 FN	1046	1991
TGx 1989-75 FN	899	1935
F pr.	0.005	0.306
LSD (5%)	62.17	NS
CV (%)	2.8	3.3
F pr.		
S × AMF isolates	< 0.001	0.232

NS = Not Significant at $P \leq 0.05$.

4.2 Field assessment of the response of two soybean lines to arbuscular mycorrhizal inoculation and different phosphorus fertilizer rates

4.2.1 Properties of soil at the experimental site

Results on some selected chemical, physical and biological properties of the Haplic Acrisol are presented in Table 4.8. With reference to the soil test interpretation manual by Landon (2014), the results indicated that the soil of the experimental site was medium in available phosphorus, very low in total nitrogen and organic carbon, low in exchangeable potassium and sodium, medium in exchangeable calcium and magnesium. The soil was slightly acidic sandy clay loam in texture and suitable for soybean cultivation according to Belfield *et al.* (2011). It was found to contain low densities of AMF spores (< 3-4 spores in some fields examined on the petri dish and no spores in others) based on the standard set by INVAM (2017).

Table 4.8. Selected properties of soil at the experimental site

Soil parameters	Value
pH (1:1 H_2O)	6.27
Organic carbon (%)	1.37
Total nitrogen (%)	0.06
Available P (mg kg^{-1})	39.43
Exchangeable K ($cmol_c$ kg^{-1} soil)	0.03
Exchangeable Ca ($cmol_c$ kg^{-1} soil)	6.62
Exchangeable Mg ($cmol_c$ kg^{-1} soil)	0.60
Exchangeable Na ($cmol_c$ kg^{-1} soil)	0.22
Sand (%)	71.12
Clay (%)	21.32
Silt (%)	7.56
Textural class	Sandy Clay Loam
AMF spore density (spores/100 g of soil)	284
Soil classification (IUSS Working Group WRB, 2014)	Haplic Acrisol

4.2.2 Arbuscular mycorrhizal inoculation and phosphorus fertilizer application on soybean root length colonization

Table 4.9 show mycorrhizal inoculation and phosphorus fertilizer application on the root length colonization (RLC) by the arbuscular mycorrhizal fungi of the two soybean lines under field conditions. Plants inoculated with AMF showed higher RLC than uninoculated control plants during the minor cropping season of 2016 and the differences were significant ($P < 0.001$). There was a significant difference between the two AMF isolates, and the increase in soybean RLC over the control treatment by the inoculation with *G. mosseae* was greater (169.04%) than the one observed with *R. irregularis* (106.78%).

Root length colonization significantly ($P < 0.001$) decreased by 41.16% when TSP was applied at 30 kg P ha^{-1} compared to the control (0 kg P ha^{-1}) (Table 4.9). However,

there was no significant decrease with the application of 15 kg P ha^{-1} (Table 4.9). Line TGx 1989-48 FN significantly (P = 0.003) increased in RLC by 192.70% over TGx 1989-75 FN. There was a significant (P < 0.001) interaction between the soybean lines, phosphorus rates and AMF isolates for RLC (Appendix 5). The highest soybean RLC (38.94%) was observed with TGx 1989-48 FN inoculated with *G. mosseae* with no TSP applied (0 kg P ha^{-1}) followed by the application of 15 kg P ha^{-1} on the same soybean line with the same AMF (23.02%). The lowest soybean RLC (1.66%) was observed with TGx 1989-75 FN uninoculated and with no TSP applied.

During the major cropping season of 2017, mycorrhizal inoculation significantly (P < 0.001) increased the RLC over the uninoculated control and there was no significant difference between the two AMF isolates (Table 4.9). The RLC was increased by 250.17 and 245.86% by *G. mosseae* and *R. irregularis*, respectively. Addition of 15 kg P ha^{-1} significantly (P < 0.001) increased soybean RLC by 30.51% over 0 kg P ha^{-1} while 30 kg P ha^{-1} significantly (P < 0.001) decreased it by 29.20% relative to 0 kg P ha^{-1}. On the other hand, TGx 1989-48 FN increased in RLC by 64.19% over TGx 1989-75 FN, which was consistent with the result obtained in the minor cropping season of 2016. The interaction between the soybean lines, phosphorus rates and AMF isolates for RLC was also significant (P < 0.001) during the major cropping season of 2017 (Appendix 6). The highest RLC (50.67%) was obtained on TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 15 kg P ha^{-1}, and the lowest (0%) with the two lines uninoculated and fertilized with 30 kg P ha^{-1}.

Table 4.9. Mycorrhizal inoculation and phosphorus fertilizer application on soybean root length colonization Root length colonization Treatments (%)

	Minor cropping season 2016	Major cropping season 2017

AMF isolates		
Glomus mosseae	15.47	31.69
Rhizophagus irregularis	11.89	31.30
Control	5.75	9.05
F pr.	< 0.001	< 0.001
LSD (5%)	1.56	2.26
CV (%)	20.6	13.7
P rates (P)		
15 kg P ha^{-1}	12.54	32.26
30 kg P ha^{-1}	7.62	16.49
0 kg P ha^{-1}	12.95	23.29
F pr.	< 0.001	< 0.001
LSD (5%)	2.08	2.40
CV (%)	14.1	7.5
Soybean lines (S)		
TGx 1989-48 FN	16.45	29.85
TGx 1989-75 FN	5.62	18.18
F pr.	0.003	< 0.001
LSD (5%) 2.75 0.60 CV (%) 7.1 0.7		
F pr.		
S × P	0.002	< 0.001
S × AMF isolates	< 0.001	< 0.001
P × AMF isolates	< 0.001	0.355
S × P × AMF isolates	< 0.001	< 0.001

4.2.3 Mycorrhizal inoculation and phosphorus fertilizer on mycorrhizal inoculation effect of soybean under field conditions

Inoculation with *G. mosseae* had a significantly (P < 0.001) higher Mycorrhizal inoculation effect (MIE) than inoculation with *R. irregularis* during the minor cropping season of 2016 (Table 4.10). A similar trend was observed during the major cropping season of 2017. The MIE of *G. mosseae* was 62.50 and 56.50% higher than that of *R. irregularis* during the minor cropping season of 2016 and the major cropping season of 2017, respectively.

Mycorrhizal inoculation effect significantly (P < 0.001) decreased by 104.5% of the control (0 kg P ha^{-1}) when 30 kg P ha^{-1} was applied, but there was no significant

decrease with the addition of 15 kg P ha^{-1} in the minor cropping season of 2016 (Table 4.10). During the major cropping season of 2017, the addition of 15 kg P ha^{-1} significantly (P = 0.035) increased the MIE by 23.08 and 33.33% of the control and application of 30 kg P ha^{-1}, respectively.

During the minor cropping season of 2016, the MIE was significantly (P = 0.011) greater for TGx 1989-48 FN (19%) relative to TGx 1989-75 FN (10%) (Table 4.10). Conversely, it was significantly (P = 0.006) higher for TGx 1989-75 FN (16%) relative to TGx 1989-48 FN (11%) in the major cropping season of 2017.

There was a significant (P < 0.001) interaction between the soybean lines, phosphorus rates and AMF isolates for MIE during the two cropping seasons. During the minor cropping season of 2016, the highest MIE (57%) was observed with TGx 1989-48 FN inoculated with *G. mosseae* with no TSP (0 kg P ha^{-1}), followed by the inoculation with *R. irregularis* to same line (TGx 1989-48 FN) with no TSP. The lowest and negative MIE (-18%) was observed with the same line inoculated with *R. irregularis* under 30 kg P ha^{-1} (Appendix 7).

During the major cropping season of 2017, the highest MIE (38%) was observed with TGx 1989-75 FN inoculated with *G. mosseae* with 15 kg P ha^{-1}. The lowest MIE (7%) was observed with TGx 1989-48 FN inoculated with *R. irregularis* with no TSP (Appendix 8). There was a significant positive correlation (P < 0.001, R^2 = 0.7287) between root length colonization (RLC) and mycorrhizal inoculation effect (MIE) in the major cropping season of 2017 (Figure 4.1).

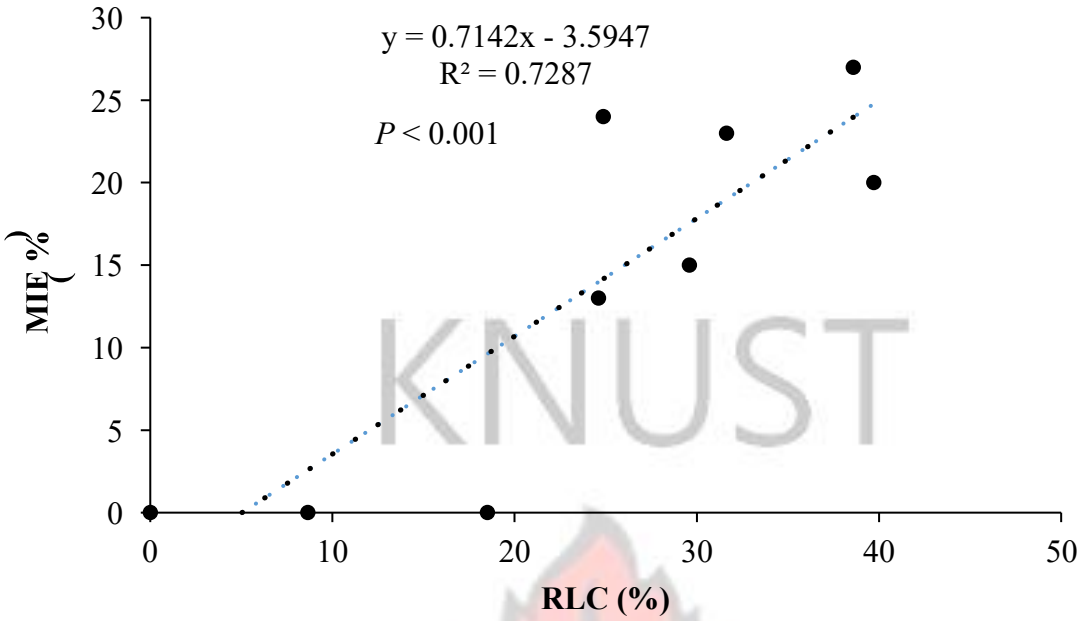


Figure 4.1. Relationship between mycorrhizal inoculation effect and the root length colonization of soybean during the major cropping season of 2017

Table 4.10. Mycorrhizal inoculation and phosphorus fertilizer on mycorrhizal inoculation effect of soybean **Mycorrhizal inoculation effect Treatments (%)**

	Minor cropping season 2016	Major cropping season 2017
AMF isolates		
Glomus mosseae	26	25
Rhizophagus irregularis	16	16
F pr.	< 0.001	< 0.001
LSD (5%)	2.00	1.70
CV (%)	20.1	17.9
P rates (P)		
15 kg P ha^{-1}	23	16
30 kg P ha^{-1}	-1	12
0 kg P ha^{-1}	22	13

F pr.	< 0.001	0.035
LSD (5%)	3.80	2.50
CV (%)	19.4	13.8
Soybean lines (S)		
TGx 1989-48 FN	19	11
TGx 1989-75 FN	10	16
F pr.	0.011	0.006
LSD (5%)	4.00	1.50
CV (%)	7.8	3.2
F pr.		
S × P	< 0.001	< 0.001
S × AMF isolates	< 0.001	< 0.001
P × AMF isolates	< 0.001	0.021
S × P × AMF isolates	< 0.001	< 0.001

4.2.4 Mycorrhizal inoculation and phosphorus fertilizer application on phosphorus uptake

Significant differences were observed in grain and shoot P uptake during the minor cropping season of 2016 as indicated in Table 4.11. The AMF inoculation significantly ($P < 0.001$) increased the grain P uptake over the uninoculated control. The isolate *G. mosseae* produced a significantly greater increase in grain P uptake (37.09%) over the control treatment than *R. irregularis* (15.29%). Mycorrhizal inoculation did not significantly ($P = 0.273$) improve the shoot P uptake.

The results indicated significant differences in grain and shoot P uptake with the addition of the TSP fertilizer. The addition of TSP fertilizer increased grain P uptake by 54.65 and 42.54% and shoot P uptake by 36.18 and 22.48% of the non-fertilized control following the applications of 15 kg P ha^{-1} and 30 kg P ha^{-1}, respectively. There was no significant ($P = 0.213$) difference in grain P uptake between the two soybean lines, but the shoot P uptake was significantly ($P = 0.018$) greater in TGx 1989-75 FN (16.57 kg ha^{-1}) than TGx 1989-48 FN (15.01 kg ha^{-1}).

There was a significant ($P < 0.001$, $P = 0.029$) interaction between the soybean lines, phosphorus rates and AMF isolates for grain and shoot P uptake, respectively (Appendix 9). The highest grain P uptake (18.56 kg ha^{-1}) was obtained in TGx 198948 FN inoculated with *G. mosseae* and fertilized with 15 kg P ha^{-1}, and the lowest grain P uptake (3.79 kg ha^{-1}) in the uninoculated treatment with no TSP application (0 kg P ha^{-1}). There were no significant differences in shoot P uptake in TGx 1989-48 FN fertilized with 15 kg P ha^{-1} and inoculated with either *G. mosseae* (18.87 kg ha^{-1}) or *R. irregularis* (19.87 kg ha^{-1}), and TGx 1989-75 FN uninoculated and fertilized with 30 kg P ha^{-1}, which produced the highest shoot P uptake (20.08 kg ha^{-1}). The lowest shoot P uptake (6.72 kg ha^{-1}) was obtained in uninoculated TGx 1989-48 FN and with no application of TSP.

During the major cropping season of 2017, mycorrhizal inoculation significantly ($P < 0.001$) increased the grain P uptake over the uninoculated control. Inoculation with *G. mosseae* induced a significantly greater increase in grain P uptake (50.32%) over the control treatment than *R. irregularis* (37.84%). Inoculation with *G. mosseae* significantly improved shoot P uptake by 44.74 and 21.02% over *R. irregularis* and uninoculated control, respectively.

The results indicated significant ($P < 0.001$) differences in grain and shoot P uptake with the addition of the TSP fertilizer, which significantly increased grain P uptake by 40.80 and 35.34%, and 24.97 and 19.82% for shoot P uptake over non-fertilized treatments upon the application of, with 30 and 15 kg P ha^{-1}, respectively.

There was no significant ($P = 0.846$) difference in grain P uptake between the two soybean lines, but the shoot P uptake was significantly ($P = 0.043$) greater for TGx 1989-48 FN (29.28 kg ha^{-1}) than TGx 1989-75 FN (23.38 kg ha^{-1}).

There was a significant ($P = 0.040$) interaction between the soybean lines, phosphorus rates and AMF isolates for grain P uptake (Appendix 10). The highest grain P uptake (22.06 kg ha^{-1}) was obtained from TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 15 kg P ha^{-1} while the lowest grain P uptake (8.10 kg ha^{-1}) was produced by the same line with no inoculation and TSP application (0 kg P ha^{-1}). However, there was no significant difference in grain P uptake between TGx 1989-48 FN fertilized with 15 kg P ha^{-1} and inoculated with *G. mosseae* (22.06 kg ha^{-1}), and TGx 1989-75 FN fertilized with 30 kg P ha^{-1} and inoculated with *R. irregularis* (19.80 kg ha^{-1}). Similar observation was made in the uninoculated treatment supplied with 30 kg P ha^{1} for TGx 1989-75 FN (19.45 kg ha^{-1}).

There was a significant positive correlation ($P < 0.001$, $R^2 = 0.6389$) between the mycorrhizal inoculation effect (MIE) and the P uptake (grain P uptake + shoot P uptake) during the major cropping season of 2017 (Figure 4.2).

Table 4.11. Mycorrhizal inoculation and phosphorus fertilizer application on P uptake in soybean grain and shoot

Treatments	Phosphorus uptake (kg ha^{-1})					
	Minor cropping season 2016			Major cropping season 2017		
AMF isolates	Grain	Shoot	Total	Grain	Shoot	Total
G. mosseae	12.64	15.95	28.59	18.67	31.38	50.05
R. irregularis	10.63	16.01	26.64	17.12	25.93	43.05
Control	9.22	15.42	24.64	12.42	21.68	34.11
F pr.	< 0.001	0.273	< 0.001	< 0.001	< 0.001	< 0.001
LSD (5%)	0.52	NS	1.05	0.65	2.15	2.73

CV (%)	6.9	7.5	5.8	12.2	11.9	9.4
P rates (P)						
15 kg P ha^{-1}	12.65	17.99	30.64	17.35	27.45	44.80
30 kg P ha^{-1}	11.65	16.18	27.83	18.05	28.63	46.68
0 kg P ha^{-1}	8.18	13.21	21.39	12.81	22.91	35.72
	< 0.001		< 0.001	< 0.001	< 0.001	
F pr.		< 0.001				< 0.001
LSD (5%) CV	1.12	0.80	1.66	0.50	1.64	2.17
(%)	7.7	3.8	4.7	5.4	4.7	3.8
Soybean lines (S)						
TGx 1989-48 FN	10.58	15.01	25.59	16.04	29.28	45.32
TGx 1989-75 FN	11.08	16.57	27.65	16.11	23.38	39.49
F pr.	0.213	0.018	0.050	0.846	0.043	0.066
LSD (5%)	NS	0.92	NS	NS	5.43	NS
CV (%)	3.1	1.7	2.2	2.5	5.9	4.6
F pr.						
S × P	< 0.001	0.002	< 0.001	0.003	0.001	0.002
S × AMF isolates	< 0.001	< 0.001	< 0.001	0.250	< 0.001	< 0.001
P × AMF isolates	< 0.001	< 0.001	< 0.001	< 0.001	0.034	0.004
S × P × AMF isolates	< 0.001	0.029	< 0.001	0.040	0.181	0.064

NS = Not Significant at $P \leq 0.05$.

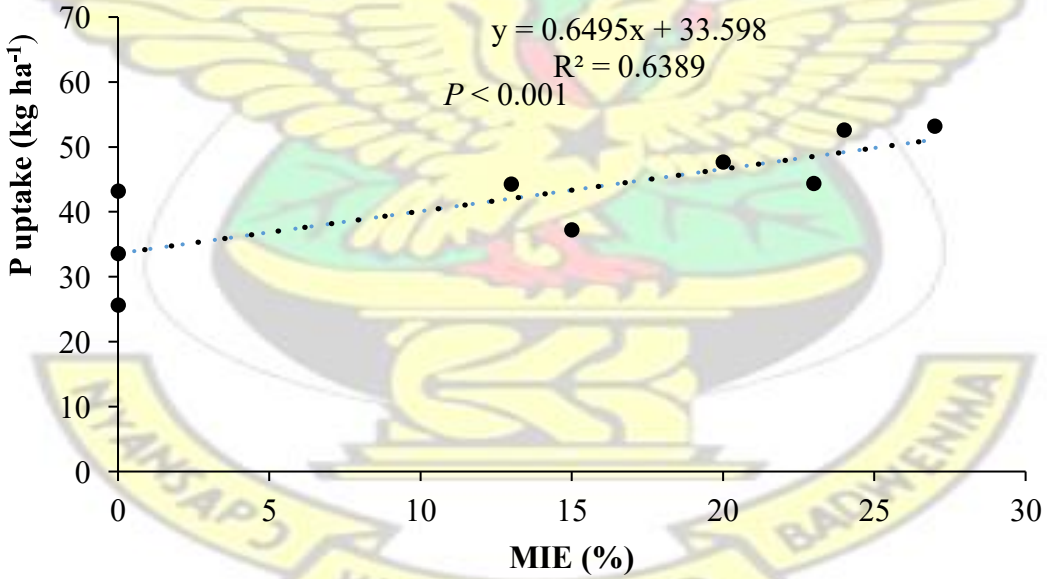


Figure 4.2. Relationship between mycorrhizal inoculation effect and total phosphorus uptake of soybean during the major cropping season of 2017

4.2.5 Mycorrhizal inoculation and phosphorus fertilizer application on phosphorus use efficiency of soybean

Effects of mycorrhizal inoculation and P fertilizer on phosphorus use efficiency (PUE) of the two soybean lines under field conditions are presented in Table 4.12. Mycorrhizal inoculation resulted in a significantly ($P < 0.001$) higher PUE as compared to the control treatment. During both cropping seasons, the difference in PUE was not significant between the two mycorrhizal isolates. Inoculation with *G. mosseae* significantly increased PUE by 36.84 and 50.00% over the control treatment during the minor and major cropping seasons, respectively. Inoculation with *R. irregularis* significantly ($P < 0.001$) increased PUE by 31.58 and 37.50% over the uninoculated control during the minor and major cropping seasons, respectively. Fertilization with 15 kg P ha^{-1} resulted in a significantly ($P < 0.001$) higher PUE over 30 kg P representing 59.26 and 48.00% increase during the minor and major cropping seasons, respectively (Table 4.12).

There were significant ($P < 0.001$) interaction between the soybean lines, phosphorus rates and AMF isolates in terms of their PUE during the minor and major cropping seasons (Appendix 11). The inoculated line TGx 1989-48 FN with *G. mosseae* and fertilized with 15 kg P ha^{-1} showed the highest PUE of 63 kg kg^{-1} and 61 kg kg^{-1} during the minor and major cropping seasons, respectively. The lowest PUE was observed in uninoculated TGx 1989-75 FN fertilized with 30 kg P ha^{-1} in the minor (20 kg kg^{-1}) and major (17 kg kg^{-1}) cropping seasons.

Table 4.12. Phosphorus use efficiency of soybean in response to arbuscular mycorrhizal inoculation and phosphorus fertilizer application Phosphorus use efficiency (kg kg^{-1}) Treatments

	Minor cropping season 2016	Major cropping season 2017
AMF isolates		
Glomus mosseae	26	24
Rhizophagus irregularis	25	22
Control	19	16
F pr.	< 0.001	< 0.001
LSD (5%)	2.20	2.50
(%)	13.9	17.7
P rates (P)		
kg P ha^{-1}	43	37
kg P ha^{-1}	27	25
F pr.	< 0.001	< 0.001
LSD (5%) CV	4.70	1.10
(%)	15.1	4.0
Soybean lines (S)		
TGx 1989-48 FN	28	26
TGx 1989-75 FN	18	15
F pr.	0.006	0.002
LSD (5%)	3.40	2.20
(%)	4.1	3.1
F pr.		
S × P	0.004	< 0.001
S × AMF isolates	0.002	0.004
P × AMF isolates	< 0.001	< 0.001
S × P × AMF isolates	0.014	0.013

4.2.6 Mycorrhizal inoculation and phosphorus fertilizer application on microbial biomass phosphorus

Effects of mycorrhizal inoculation and phosphorus fertilizer on microbial biomass phosphorus under field conditions during the major cropping season of 2017 are presented in Table 4.13. Mycorrhizal inoculation had no significant ($P = 0.307$) effect on microbial biomass phosphorus at 50% flowering. However, inoculation with *G. mosseae* resulted in a significantly ($P < 0.001$) reduced microbial biomass phosphorus by 16.02% at harvest as compared to the uninoculated treatment. On the other hand,

inoculation with *R. irregularis* had no significant effect on microbial biomass phosphorus.

Addition of 30 kg P ha^{-1} resulted in a significant ($P < 0.001$) increase in microbial biomass phosphorus at 50% flowering as compared to 15 kg P ha^{-1} and control treatment, representing 34.14 and 87.24% increase, respectively. The trend was the same at harvest ($P < 0.001$), representing 14.93 and 28.15% increase over 15 kg P ha^{1} and the control treatment, respectively (Table 4.13). The application of 15 kg P ha^{-1} significantly ($P = 0.010$) increased the microbial biomass phosphorus at 50% flowering by 39.58% over the control, notwithstanding, the increase was not significant at harvest.

There were significant ($P < 0.001$) interactions between the soybean lines, phosphorus rates and AMF isolates for microbial biomass phosphorus at 50% flowering and at harvest (Appendix 12). The uninoculated line TGx 1989-48 FN fertilized with 30 kg P ha^{-1} showed the highest microbial biomass phosphorus of 51.01 mg kg^{-1} and 53.35 mg kg^{-1} at 50% flowering and at harvest, respectively. The lowest microbial biomass phosphorus of 14.06 mg kg^{-1} and 14.86 mg kg^{-1} at 50% flowering was obtained under uninoculated TGx 1989-75 FN and TGx 1989-48 FN and with no P fertilization, respectively.

Table 4.13. Mycorrhizal inoculation and phosphorus fertilizer application on microbial biomass phosphorus during the major cropping season of 2017

Treatments	Microbial biomass phosphorus (mg kg^{-1})	
	50% Flowering	Harvest
AMF isolates		
Glomus mosseae	23.87	27.88
Rhizophagus irregularis	26.21	35.21
Control	25.50	33.20

F pr.	0.307	< 0.001
LSD (5%)	NS	3.33
CV (%)	18.2	15.1
P rates (P)		
15 kg P ha^{-1}	24.72	31.61
30 kg P ha^{-1}	33.16	36.33
0 kg P ha^{-1}	17.71	28.35
F pr.	< 0.001	0.010
LSD (5%)	4.15	4.45
CV (%)	12.4	10.4
Soybean lines (S)		
TGx 1989-48 FN	26.48	32.21
TGx 1989-75 FN	23.91	31.98
F pr.	0.097	0.792
LSD (5%)	NS	NS
CV (%)	4.2	2.9
F pr.		
S × P	0.148	0.010
S × AMF isolates	0.039	< 0.001
P × AMF isolates	< 0.001	< 0.001
S × P × AMF isolates	0.001	< 0.001

NS = Not Significant at $P \leq 0.05$.

4.2.7 Effect of mycorrhizal inoculation on soil available phosphorus

Effects of mycorrhizal inoculation on soil available phosphorus under field conditions during the major cropping season of 2017 are presented in Table 4.14. Mycorrhizal inoculation significantly ($P < 0.001$) increased the soil available P pre-planting, at 50% flowering and at harvest over the uninoculated control, and the difference between the two AMF isolates was significant only at pre-planting (Table 4.14). Inoculation with *G. mosseae* and *R. irregularis* increased soil available phosphorus by 28.61 and 16.59% at pre-planting, by 7.68 and 7.50% at 50% flowering and by 7.80 and 4.50% at harvest compared to the uninoculated control. However, there was no significant increase in soil available P induced by inoculation with *R. irregularis* over the control treatment at harvest.

The interaction between the soybean lines, P rates and AMF isolates on soil available

P at pre-planting was significant ($P = 0.011$) (Appendix 13). The highest soil available P (103.76 mg kg^{-1}) was observed with TGx 1989-75 FN inoculated with *G. mosseae* and fertilized with 30 kg P ha^{-1}, followed by the applications on the same line with the same AMF isolate, but fertilized with 15 kg P ha^{-1} (90.73 mg kg^{-1}). The lowest soil available P (56.82 mg kg^{-1}) was obtained from uninoculated TGx 1989-48 FN with no TSP application. There was a significant positive correlation ($P < 0.001$, $R^2 = 0.6890$) between the MIE and soil available phosphorus at pre-planting during the major cropping season of 2017 (Figure 4.3, Table 4.22).

Table 4.14. Soil available phosphorus as affected by mycorrhizal inoculation in the major cropping season of 2017

Treatments	Available phosphorus (mg kg^{-1})		
	Pre-planting	**50% flowering**	**Harvest**
AMF isolates			
Glomus mosseae	85.95	148.53	120.21
Rhizophagus irregularis	77.92	148.29	116.53
Control	66.83	137.94	111.51
F pr.	< 0.001	< 0.001	0.007
LSD (5%) CV	4.17	3.60	5.16
(%)	7.9	3.6	6.5
F pr.			
S × AMF isolates	0.038	0.067	0.136
P × AMF isolates	0.005	< 0.001	0.242
S × P × AMF isolates	0.011	0.509	0.066

NS = Not Significant at $P \leq 0.05$. S = soybean lines, P = P rates

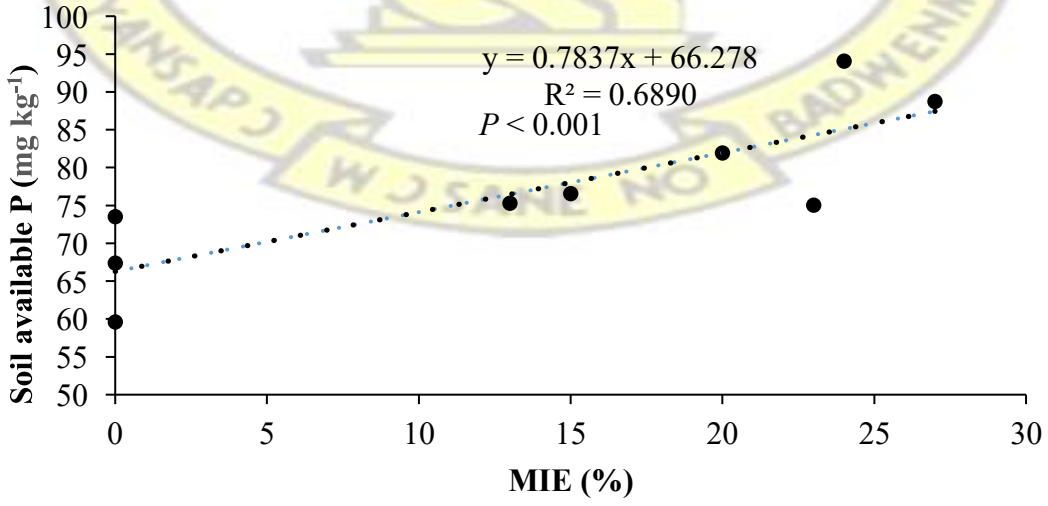


Figure 4.3. Relationship between soil available P pre-planting and mycorrhizal inoculation effect major during the cropping season of 2017

4.2.8 Mycorrhizal inoculation and phosphorus fertilizer application on plant height of soybean

Effects of mycorrhizal inoculation and phosphorus fertilizer on plant height of the two soybean lines under field conditions are as presented in Tables 4.15 and 4.16. During the minor cropping season of 2016, plants inoculated with AMF were significantly ($P < 0.001$) taller than the uninoculated treatment at 40 and 60 DAS (Table 4.15). At 40 DAS, there was a significant difference between the two mycorrhizal isolates and the increase in plant height induced by *G. mosseae* over uninoculated treatment was greater (16.01%) than that obtained with *R. irregularis* (12.99%). Inoculation with *G. mosseae* and *R. irregularis* increased plant height by 8.18 and 8.02% of the uninoculated treatment at 60 DAS, respectively, and there was no significant difference between the two mycorrhizal isolates. There was no significant ($P = 0.734$) difference in plant height at 80 DAS between the inoculated and uninoculated control plants.

Plant height at 60 and 80 DAS significantly ($P = 0.025$ and $P = 0.006$, respectively) increased with P fertilization (Table 4.15). The line TGx 1989-75 FN significantly ($P = 0.045$ and $P = 0.049$) increased in height relative to TGx 1989-48 FN at 60 and 80 DAS, respectively. However, there was significant ($P = 0.002$) interactive effects between the soybean lines and AMF isolates on plant height at 40 DAS (Appendix 14). The tallest soybean plants (52.60 cm) were observed from the line TGx 1989-48 FN inoculated with *G. mosseae*, and the shortest plants (42.00 cm) from the uninoculated treatment of the same line. There was also a significant ($P < 0.001$) interaction between phosphorus rate and AMF isolate for plant height at 40 DAS

(Appendix 15). Inoculated soybean plants with or without TSP applied were significantly ($P < 0.001$) taller than the uninoculated control plants, except when 30 kg P ha^{-1} was applied, where there was no significant difference in plant height between inoculated and uninoculated plants.

During the major cropping season of 2017, mycorrhizal inoculation significantly ($P < 0.001$) increased plant height at 40, 60 and 80 DAS over the uninoculated treatment, however, there was no significant difference between the two AMF isolates (Table 4.16). The plant height increased by 12.68 and 11.97% at 40 DAS, 14.51 and 11.32% at 60 DAS, and 9.97 and 9.04% at 80 DAS over the uninoculated treatment and when inoculated with *G. mosseae* and *R. irregularis*, respectively.

The only significant ($P = 0.002$) increase in soybean plant height induced by P fertilization was obtained at 80 DAS (Table 4.16). The addition of 15 kg P ha^{-1} and 30 kg P ha^{-1} increased plant height by 7.50 and 8.03% over 0 kg P ha^{-1}, respectively. On the other hand, TGx 1989-48 FN significantly ($P = 0.014$) increased plant height by 17.55% of TGx 1989-75 FN at 80 DAS, which was inconsistent with the result obtained in the minor cropping season of 2016.

There was a significant ($P < 0.001$) interaction between the phosphorus rates and AMF isolates for plant height at 40 and 80 DAS. Inoculated soybean plants with or without TSP applied were significantly ($P < 0.001$) taller than uninoculated control plants, except for 30 kg P ha^{-1}, where there was no significant difference in plant height between inoculated and uninoculated control. The interaction between soybean lines, P rates and AMF isolates on plant height was also significant ($P < 0.001$) at 40 DAS. The tallest soybean plants (35.00 cm) were obtained with TGx 1989-48 FN inoculated

with *G. mosseae* with 15 kg P ha^{-1} applied, and the shortest plants (20.20 cm) with TGx 1989-75 FN uninoculated and with no TSP application (Appendix 16).

Table 4.15. Mycorrhizal inoculation and phosphorus fertilizer application on soybean plant height during the minor cropping season of 2016

Treatments	Plant height (cm)		
	40 DAS	**60 DAS**	**80 DAS**
AMF isolates			
Glomus mosseae	50.00	68.80	69.70
Rhizophagus irregularis	48.70	68.70	70.50
Control	43.10	63.60	69.50
F pr.	< 0.001	< 0.001	0.734
LSD (5%)	1.15	2.18	NS
CV (%)	7.3	4.7	5.3
P rates (P)			
15 kg P ha^{-1} 30	49.00	69.00	71.60
kg P ha^{-1}	46.60	68.10	70.90
0 kg P ha^{-1}	46.20	64.00	67.20
F pr.	0.251	0.025	0.006
LSD (5%)	NS	3.50	2.42
CV (%)	6.2	3.9	2.6
Soybean lines (S)			
TGx 1989-48 FN	47.20	64.40	66.90
TGx 1989-75 FN	47.30	69.70	72.90
F pr.	0.924	0.045	0.049
LSD (5%)	NS	4.99	5.97
CV (%)	2.9	2.1	2.4
F pr.			
S × P	0.688	0.866	0.171
S × AMF isolates	0.002	0.071	0.379
P × AMF isolates	< 0.001	0.083	0.751
S × P × AMF isolates	0.208	0.434	0.999

NS = Not Significant at $P \leq 0.05$.

Table 4.16. Mycorrhizal inoculation and phosphorus fertilizer application on soybean plant height during the major cropping season of 2017

Treatments	Plant height (cm)		
	40 DAS	**60 DAS**	**80 DAS**
AMF isolates			
Glomus mosseae	32.00	71.80	82.70
Rhizophagus irregularis	31.80	69.80	82.00
Control	28.40	62.70	75.20

F pr.	< 0.001	< 0.001	< 0.001
LSD (5%)	1.50	3.83	2.12
CV (%)	7.1	8.2	3.8
P rates (P)			
15 kg P ha^{-1}	31.70	68.80	81.70
30 kg P ha^{-1}	31.20	69.70	82.10
0 kg P ha^{-1}	29.30	65.70	76.00
F pr.	0.211	0.177	0.002
LSD (5%)	NS	NS	2.78
CV (%)	7.4	5.1	2.6
Soybean lines (S)			
TGx 1989-48 FN	30.80	70.80	86.40
TGx 1989-75 FN	30.70	65.40	73.50
F pr.	0.961	0.071	0.014
LSD (5%)	NS	NS	6.49
CV (%)	9.0	2.7	2.3
F pr.			
S × P	0.435	0.349	0.764
S × AMF isolates	0.001	0.041	0.009
P × AMF isolates	< 0.001	0.172	< 0.001
S × P × AMF isolates	< 0.001	0.218	0.173

NS = Not Significant at $P \leq 0.05$.

4.2.9 Mycorrhizal inoculation and phosphorus fertilizer application on stem girth of soybean

Tables 4.17 and 4.18 show mycorrhizal inoculation and phosphorus fertilizer effects on stem girth of two soybean lines under field conditions. During the minor cropping season of 2016, plants inoculated with AMF had greater stem girth than uninoculated control plants at 40 and 60 DAS, and the differences were significant (P = 0.002 and P = 0.005, respectively). Inoculation with *G. mosseae* and *R. irregularis* increased stem girth by 8.16% and 13.59% at 40 DAS and 11% and 14.16% at 60 DAS over uninoculated control, respectively. There was a significant difference in stem girth between the treatments inoculated with *G. mosseae* or *R. irregularis* at 60 DAS but not at 40 DAS. The difference in stem girth between the inoculated and uninoculated control plants was not significant at 80 DAS (Table 4.17).

There were no significant differences in stem girth induced by phosphorus application at 40, 60 and 80 DAS during the minor cropping season of 2016 (Table 4.17). Similarly, the difference in stem girth between the two soybean lines was not significant. However, there was a significant (P = 0.004) interaction between soybean lines and phosphorus rates for stem girth at 40 DAS (Appendix 17). The greatest stem girth (5.83 mm) was obtained when TGx 1989-48 FN was fertilized with 30 kg P ha^{-1} and the smallest (5.09 mm) with the same line fertilized with 15 kg P ha^{-1}.

The inoculation during the major cropping season of 2017 significantly (P < 0.001) increased the stem girth at 40, 60 and 80 DAS over the uninoculated control. The difference between the two AMF isolates was significant at 40 DAS, where *G. mosseae* performed better, but not at 60 and 80 DAS (Table 4.18). The stem girth increased by 14.70 and 11.50% at 40 DAS, by 15.50 and 14.20% at 60 DAS and by 7.03 and 7.49% at 80 DAS over the uninoculated control and when inoculated with *G. mosseae* and *R. irregularis*, respectively.

The was a significant (P = 0.043 and P = 0.026) increase in stem girth of soybean induced by P fertilization at 60 and 80 DAS, respectively, but not significant (P = 0.525) at 40 DAS (Table 4.18). The application of 15 kg P ha^{-1} and 30 kg P ha^{-1} increased the stem girth of soybean over 0 kg P ha^{-1} by 2.06 and 6.74% at 60 DAS, and by 2.08 and 6.38% at 80 DAS, respectively. The TGx 1989-48 FN significantly (P = 0.003) increased in stem girth by 13.99% over TGx 1989-75 FN at 80 DAS.

There was a significant (P = 0.043 and P = 0.014) interaction between the soybean lines, phosphorus rates and AMF isolates for stem girth at 40 and 80 DAS, respectively (Appendix 18). At 40 DAS, the greatest stem girth (8.01 mm) was obtained with TGx 1989-48 FN inoculated with *R. irregularis* and with no TSP applied (0 kg P ha^{-1}) while the smallest stem girth (5.63 mm) with the same line uninoculated and with no TSP

applied. At 80 DAS, the greatest stem girth (15.43 mm) was obtained with TGx 198975 FN inoculated with *R. irregularis* and with no 30 kg P ha^{-1} applied while the smallest stem girth (11.68 mm) with TGx 1989-48 FN uninoculated and with no TSP applied.

Table 4.17. Mycorrhizal inoculation and phosphorus fertilizer application on stem girth of soybean during the minor cropping season of 2016

Treatments	Stem girth (mm)		
	40 DAS	60 DAS	80 DAS
AMF isolates			
Glomus mosseae	5.57	8.78	10.12
Rhizophagus irregularis	5.85	9.03	10.23
Control	5.15	7.91	10.22
F pr.	0.002	0.005	0.943 NS
LSD (5%)	0.36	0.66	11.1
CV (%)	9.6	11.3	
P rates (P)			
15 kg P ha^{-1}	5.41	8.75	10.56
30 kg P ha^{-1}	5.62	8.59	10.08
0 kg P ha^{-1}	5.54	8.38	9.93
F pr.	0.281	0.588	0.126
LSD (5%)	NS	NS	NS
CV (%)	5.8	7.1	4.8
Soybean lines (S)			
TGx 1989-48 FN	5.55	8.65	10.49
TGx 1989-75 FN	5.49	8.49	9.89
F pr.	0.830	0.151	0.108
LSD (5%)	NS	NS	NS
CV (%)	5.3	1.0	2.6
F pr.			
S × P	0.004	0.746	0.956
S × AMF isolates	0.933	0.918	0.676
P × AMF isolates	0.656	0.716	0.419
S × P × AMF isolates	0.410	0.613	0.664

NS = Not Significant at $P \leq 0.05$.

Table 4.18. Mycorrhizal inoculation and phosphorus fertilizer application on stem girth of soybean during the major cropping season of 2017

Treatments	Stem girth (mm)		
	40 DAS	60 DAS	80 DAS

AMF isolates			
Glomus mosseae	7.18	11.55	14.15
Rhizophagus irregularis	6.98	11.42	14.21
Control	6.26	10.00	13.22
F pr.	< 0.001	< 0.001	< 0.001
LSD (5%)	0.38	0.75	0.40
CV (%)	8.2	9.9	4.2
P rates (P)			
15 kg P ha^{-1}	6.91	10.90	13.76
30 kg P ha^{-1}	6.85	11.40	14.34
0 kg P ha^{-1}	6.66	10.68	13.48
F pr.	0.525	0.043	0.026
LSD (5%)	NS	0.55	0.58
CV (%)	5.7	3.8	3.2
Soybean lines (S)			
TGx 1989-48 FN	7.25	10.87	13.45
TGx 1989-75 FN	6.36	11.11	14.27
F pr.	0.003	0.639	0.179
LSD (5%)	0.21	NS	NS
CV (%)	0.9	4.9	3.6
F pr.			
S × P	0.900	0.387	0.164
S × AMF isolates	0.108	0.846	0.052
P × AMF isolates	0.032	0.664	0.055
S × P × AMF isolates	0.043	0.387	0.014

NS = Not Significant at $P \leq 0.05$.

4.2.10 Mycorrhizal inoculation and phosphorus fertilizer application on soybean shoot biomass yield

Results for mycorrhizal inoculation and phosphorus fertilizer effects on shoot biomass yield of two soybean lines under field conditions are presented in Table 4.19. Inoculation with *G. mosseae* resulted in a significantly ($P < 0.001$) higher soybean shoot biomass yield relative to the control during the minor cropping season of 2016. It also resulted in a significantly ($P < 0.001$) higher soybean shoot biomass yield relative to treatments inoculated with *R. irregularis* and control treatment during the major cropping season of 2017.

The increase in shoot biomass yield inoculation following with *G. mosseae* represented 4.86% (over *R. irregularis*) and 20.15% (over the control) during the minor cropping season of 2016, and 20.10 (over *R. irregularis*) and 96.92% (over the control) during the major cropping season of 2017. The treatments with *R. irregularis* significantly ($P < 0.001$) increased shoot biomass yield by 14.57 (minor cropping season) and 63.96% (major cropping season) over the control.

During the minor cropping season of 2016, 30 kg P ha^{-1} supported a significantly ($P < 0.001$) greater shoot biomass yield relative to 15 kg P ha^{-1} and control treatment, representing 26.00 and 40.62% increase, respectively. The 15 kg P ha^{-1} treatment did not significantly increase the shoot biomass yield over the controls. The applications of 15 kg P ha^{-1} and 30 kg P ha^{-1} during the major cropping season of 2017 supported a significantly ($P < 0.001$) greater shoot biomass yield over the control treatment, representing 67.62 and 74.36% increase, respectively (Table 4.19).

The TGx 1989-75 FN significantly ($P = 0.008$) increased shoot biomass yield by 15.17% over TGx 1989-48 FN during the minor cropping season of 2016. Inversely, TGx 1989-48 FN significantly ($P = 0.002$) increased shoot biomass yield by 24.60% over TGx 1989-75 FN during the major cropping season of 2017 (Table 4.19).

There was a significant ($P < 0.001$) interaction between the soybean lines, phosphorus rates and AMF isolates for shoot biomass yield during the major cropping season of 2017 (Appendix 19). The line TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 30 kg P gave the highest soybean shoot biomass yield (4486 kg ha^{-1}), followed by the inoculation with the same isolate on the same line which received 15 kg P (4392 kg ha^{-1}) with no significant difference in shoot biomass between these two

treatments. The lowest shoot biomass yield (877 kg ha^{-1}) was obtained with TGx 198948 FN uninoculated AMF and with no TSP applied.

Table 4.19. Mycorrhizal inoculation and phosphorus fertilizer application on soybean shoot biomass yield Shoot biomass yield (kg ha^{-1}) Treatments

	Minor cropping season 2016	Major cropping season 2017
AMF isolates		
Glomus mosseae	2910	3639
Rhizophagus irregularis	2775	3030
Control	2422	1848
F pr.	< 0.001	< 0.001
LSD (5%)	226.70	293.80
(%)	12.2	15.0
P rates (P)		
kg P ha^{-1}	2569	3230
kg P ha^{-1}	3237	3360
kg P ha^{-1}	2302	1927
F pr.	< 0.001	< 0.001
LSD (5%)	287.30	364.60
(%)	8.0	9.6
Soybean lines (S)		
TGx 1989-48 FN	2512	3150
TGx 1989-75 FN	2893	2528
F pr.	0.008	0.002
LSD (5%)	144.60	104.00
(%)	1.5	1.0
F pr.		
S × P	0.096	0.985
S × AMF isolates	0.090	< 0.001
P × AMF isolates	< 0.001	0.474
S × P × AMF isolates	0.805	< 0.001

NS = Not Significant at $P \leq 0.05$.

4.2.11 Mycorrhizal inoculation and phosphorus fertilization on soybean grain yield

Table 4.20 shows results for mycorrhizal inoculation and phosphorus fertilizer effect on grain yield of two soybean lines under field conditions. Inoculation with *G. mosseae* resulted in a significantly ($P < 0.001$) higher soybean grain yield relative to *R. irregularis* and the control during both cropping seasons. Inoculation with *G. mosseae*

increased grain yield by 12.86 (over *R. irregularis*) and 39.11% (over the control) during the minor cropping season, and 7.19 (over *R. irregularis*) and 56.03% (over the control) during the major cropping season. The treatments with *R. irregularis* significantly ($P < 0.001$) increased the grain yield by 23.26 (minor cropping season) and 45.56% (major cropping season) over the control.

During the minor cropping season of 2016, fertilization with 30 kg P ha^{-1} supported a significantly ($P < 0.001$) greater soybean grain yield over 15 kg P ha^{-1} and the control, representing 11.92 and 49.68% increase, respectively. A similar trend was observed during the major cropping season of 2017, 30 kg P ha^{-1} increased by 4.05 and 33.47% grain yield over 15 kg P ha^{-1} and control treatment, respectively (Table 4.20). The fertilization with 15 kg P ha^{-1} significantly ($P < 0.001$) increased the grain yield by 33.73 and 28.28% over the control during the minor and major cropping seasons, respectively.

The TGx 1989-75 FN significantly ($P = 0.045$) increased grain yield by 15.17% over TGx 1989-48 FN during the minor cropping season. However, the difference in grain yield between the soybean lines was not significant during the major cropping season (Table 4.20).

There was significant ($P < 0.001$) interactions between the soybean lines, phosphorus rates and AMF isolates for grain yield during the minor and major cropping seasons (Appendix 20). The TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 15 kg P ha^{-1} exhibited the highest soybean grain yield of 2369 kg ha^{-1} and 2679 kg ha^{1} respectively during the minor and major cropping seasons. The lowest grain yields

(499 kg ha^{-1} and 954 kg ha^{-1}) were obtained with uninoculated treatments with no TSP applied on the line TGx 1989-48 FN during the minor cropping season of 2016, and the line TGx 1989-75 FN during the major cropping season of 2017.

There were significant positive correlations ($P < 0.001$, $R^2 = 0.6504$; $P < 0.001$, $R^2 = 0.8031$) between the shoot phosphorus uptake and grain yield during the minor cropping season of 2016 and during the major cropping season of 2017, respectively (Figures 4.4 and 4.5, Tables 4.21, 4.22). During the major cropping season of 2017, there was a significant positive correlation ($P < 0.001$, $R^2 = 0.6140$) between the mycorrhizal inoculation effect (MIE) and grain yield (Figure 4.6, Table 4.22).

Table 4.20. Grain yield response of soybean to mycorrhizal inoculation and phosphorus fertilizer application

Treatments	**Grain yield (kg ha^{-1})**	
	Minor cropping season 2016	**Major cropping season 2017**
AMF isolates		
Glomus mosseae	1597	2356
Rhizophagus irregularis	1415	2198
Control	1148	1510
F pr.	< 0.001	< 0.001
LSD (5%)	42.70	111.70
CV (%)	4.5	8.0
P rates (P)		
15 kg P ha^{-1}	1451	2150
30 kg P ha^{-1}	1624	2237
0 kg P ha^{-1}	1085	1676
F pr.	< 0.001	< 0.001
LSD (5%)	67.20	81.80
CV (%)	3.6	3.0
Soybean lines (S)		
TGx 1989-48 FN	1421	2057
TGx 1989-75 FN	1353	1986
F pr.	0.045	0.215
LSD (5%)	64.10	NS
CV (%)	1.3	2.4
F pr.		
S × P	< 0.001	< 0.001

S × AMF isolates	< 0.001	0.006
P × AMF isolates	< 0.001	< 0.001
S × P × AMF isolates	< 0.001	< 0.001

NS = Not Significant at $P \leq 0.05$.

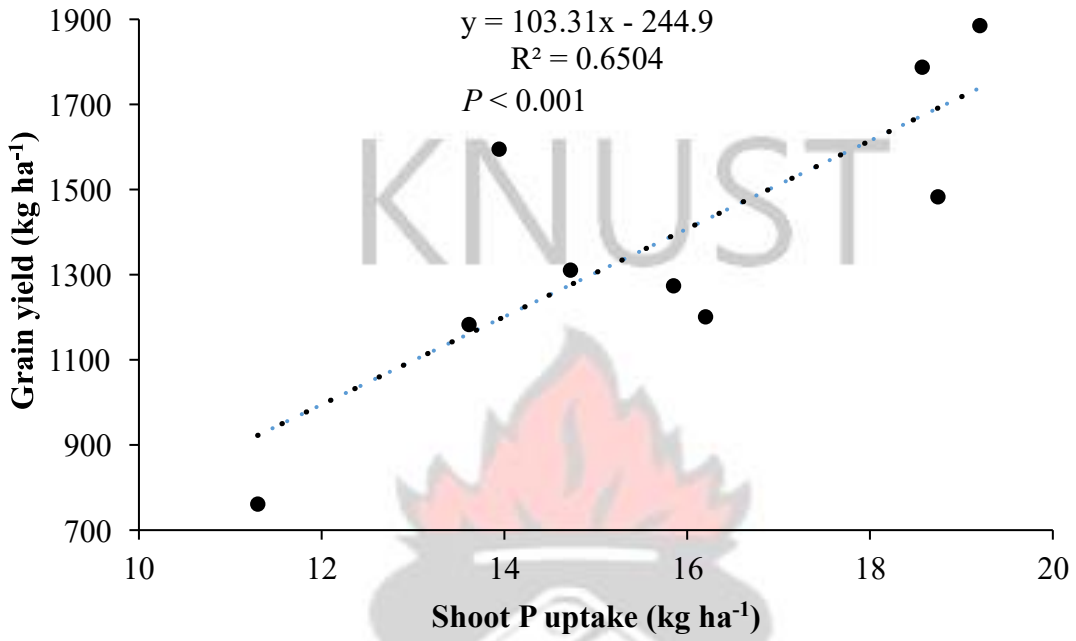


Figure 4.4. Relationship between soybean grain yield and shoot P uptake during the minor cropping season of 2016

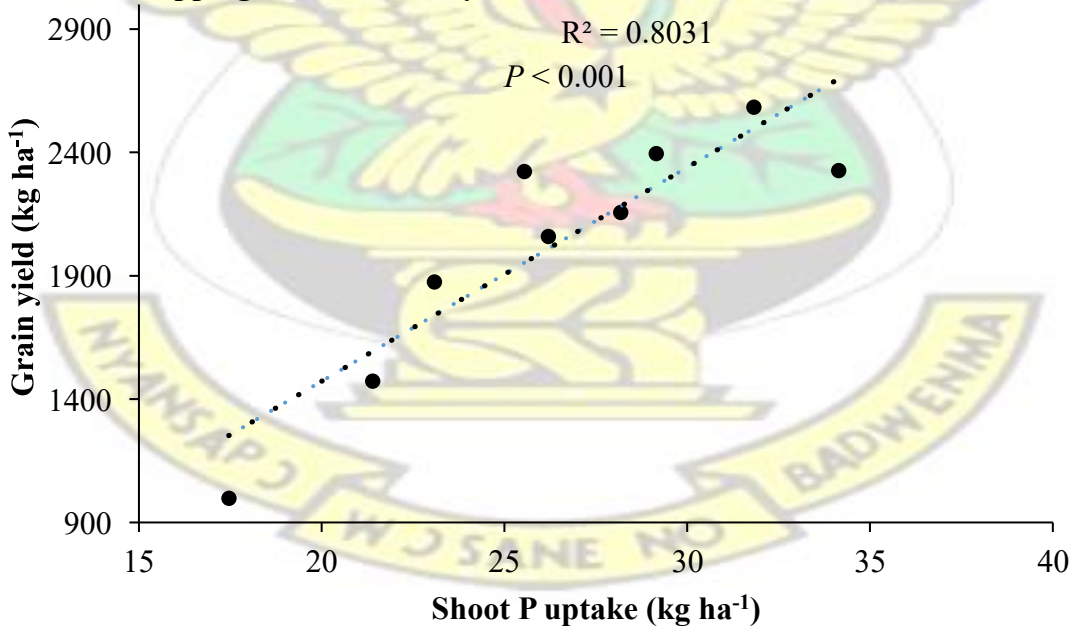


Figure 4.5. Relationship between soybean grain yield and shoot P uptake during the major cropping season of 2017

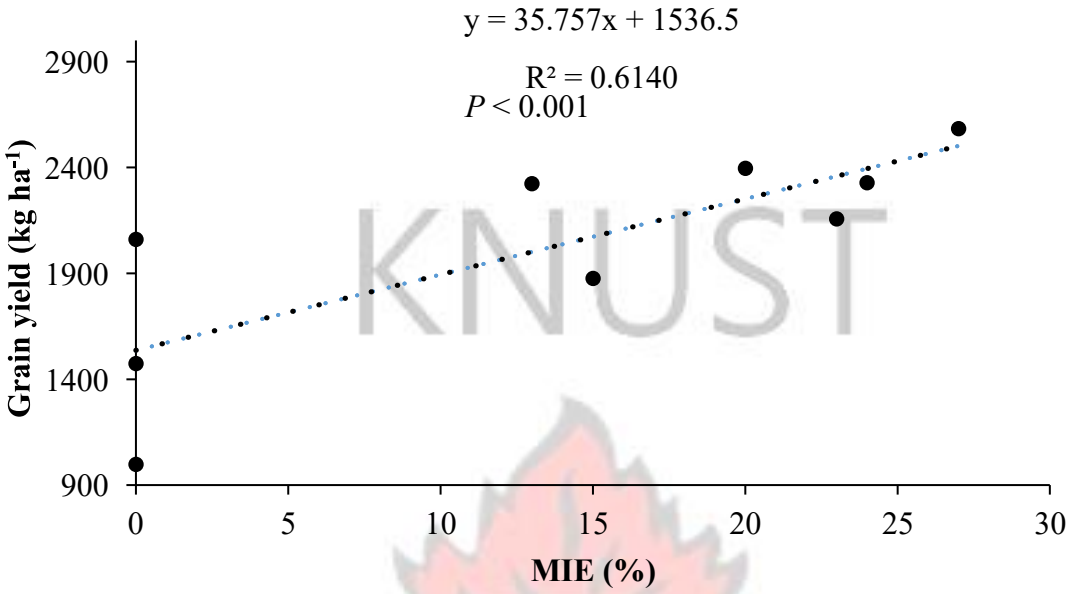


Figure 4.6. Relationship between soybean grain yield and mycorrhizal inoculation effect during the major cropping season of 2017

Tables 4.21 and 4.22 represent the Pearson's correlation matrices between mycorrhizal inoculation effect, shoot phosphorus uptake, grain phosphorus uptake, shoot yield, grain yield, phosphorus use efficiency, microbial biomass phosphorus and available phosphorus during the minor cropping season of 2016 and during the major cropping season of 2017, respectively.

Table 4.21. Pearson's correlation matrix for plant, microbial and soil parameters during the minor cropping season of 2016

	RLC (%)	SPU (kg ha^{-1})	PUE (kg kg^{-1})	MIE (%)	GY (kg ha^{-1})	Av P harv (mg kg^{-1})
RLC (%)	-					
SPU (kg ha^{-1})	0.0238	-				
PUE (kg kg^{-1})	0.0694	0.6438***	-			
MIE (%)	0.0633	0.1459	0.2214	-		
GY (kg ha^{-1})	0.0805	0.6608***	0.7660***	0.4950***	-	
Av P harv (mg kg^{-1})	0.0473	0.3023*	0.3311*	0.0947	0.4409***	-

RLC = root length colonization, SPU = shoot phosphorus uptake, PUE = phosphorus use efficiency, MIE = mycorrhizal inoculation effect, GY = grain yield, Av P = available phosphorus at harvest (harv). Levels of significance: * = $P < 0.05$, *** = $P < 0.001$.

Table 4.22. Pearson's correlation matrix for plant, microbial and soil parameters during the major cropping season of 2017

	RLC (%)	SPU (kg ha^{-1})	PUE (kg kg^{-1})	MBP harv (mg kg^{-1})	MBP flow (mg kg^{-1})	MIE (%)	GY (kg ha^{-1})	Av P plan (mg kg^{-1})	Av P harv (mg kg^{-1})	Av P flow (mg kg^{-1})
RLC (%)	-									
SPU (kg ha^{-1})	0.4402***	-								
PUE (kg kg^{-1})	0.3681**	0.4146**	-							
MBP harv (mg kg^{-1})	-0.0604	0.0161	0.1027	-						
MBP flow (mg kg^{-1})	-0.1188	0.2202	0.3349*	0.6290***	-					
MIE (%)	0.5386***	0.4757***	0.2394	-0.1849	-0.1738					
GY (kg ha^{-1})	0.4696***	0.6736***	0.5938***	0.1210	0.3943**	0.5819***	-			
Av P plan (mg kg^{-1})	0.2126	0.3352*	0.3593**	-0.1703	0.2136	0.5151***	0.5437***	-		
Av P harv (mg kg^{-1})	0.1767	0.3641**	0.2400	-0.0294	0.1854	0.2241	0.4371***	0.3120*	-	
Av P flow (mg kg^{-1})	0.14	0.2436	0.5959***	0.1327	0.3360*	0.4180**	0.4792***	0.6219***	0.1023	-

RLC = root length colonization, SPU = shoot phosphorus uptake, PUE = phosphorus use efficiency, MBP = microbial biomass phosphorus, MIE = mycorrhizal inoculation effect, GY = grain yield, Av P = available phosphorus pre-planting (plan), 50% flowering (flow) and harvest (harv). Levels of significance: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

4.2.12 Economic profitability of arbuscular mycorrhizal inoculation on soybean

4.2.12.1 Net benefits

Tables 4.23 and 4.24 represent the partial cost benefit analysis on the basis of soybean grain yield of the trials during the minor cropping season of 2016 and the major cropping season of 2017, respectively. Table 4.23 shows that for the two AMF species and the control, grain yield considerably increased following application of 15 kg P ha^{-1} in relation to 0 kg P ha^{-1}; an increase of 440 kg ha^{-1} for uninoculated control, 574 kg ha^{-1} for treatments inoculated with *G. mosseae* and 604 kg ha^{-1} for *R. irregularis*. The increase in grain yield remained important for the passage of 0 kg P ha^{-1} to 30 kg P ha^{-1} for all treatments. However, increasing TSP applied from 15 kg P ha^{-1} to 30 kg P ha^{-1}, the grain yield of treatments inoculated decreased by 290 kg ha^{-1} (*G. mosseae*) and 243 kg ha^{-1} (*R. irregularis*) in relation to the increase from 0 kg P ha^{-1} to 15 kg P ha^{-1}. The costs of the fertilizer and its application remained the same for all inoculation treatments, but they varied according to the rate of TSP applied. They respectively amounted to GH ¢91 and GH ¢30 for 15 kg P ha^{-1}, GH ¢183 and GH ¢40 for 30 kg P ha^{-1}.

The net benefits increased considerably following application of 15 kg P ha^{-1} over the control (0 kg P ha^{-1}) (Figure 4.7), a difference of GH ¢605 ha^{-1} for uninoculated control treatment and GH ¢827 ha^{-1} for soybean plants inoculated with *G. mosseae* and GH ¢876 ha^{-1} for soybean plants inoculated with *R. irregularis*. With the exception of the uninoculated control, the net benefits obtained in the 15 kg P ha^{-1} were relatively higher than those obtained in 30 kg P ha^{-1}. The change from 0 to 30 kg P ha^{-1} gave only smaller increases in net benefits than those obtained by changing from 0 to 15 kg P ha^{-1} for soybean inoculated with *G. mosseae* (GH ¢246ha^{-1}) and for *R. irregularis*

(GH ¢373 ha^{-1}), except for the uninoculated control which was highest (GH ¢969 ha^{-1}).

For the major cropping season of 2017, Table 4.24 shows that for the two AMF species and the control, grain yield considerably increased from 0 kg P ha^{-1} to 15 kg P ha^{-1}; an increase of 475 kg ha^{-1} for the uninoculated control, 426 kg ha^{-1} for soybean plants inoculated with *G. mosseae* and 520 kg ha^{-1} for soybean plants inoculated with *R. irregularis*. On the other hand, increasing 15 kg P ha^{-1} to 30 kg P ha^{-1}, the grain yields

of soybean plants inoculated decreased by 256 kg ha^{-1} (*G. mosseae*) and 72 kg ha^{-1} (*R. irregularis*).

The net benefits increased considerably from 0 kg P ha^{-1} to 15 kg P ha^{-1} (Figure 4.7), an increase of GH ¢663 ha^{-1} for uninoculated control treatment, GH ¢583 ha^{-1} for soybean plants inoculated with *G. mosseae* and GH ¢738 ha^{-1} for soybean plants inoculated with *R. irregularis*. With the exception of uninoculated control, the net benefits obtained in 15 kg P ha^{-1} were higher than 30 kg P ha^{-1}. The change from 0 to 30 kg P ha^{-1} gave smaller increases in net benefits than those obtained by changing from 0 to 15 kg P ha^{-1} for soybean plants inoculated with *G. mosseae* (GH ¢58 ha^{-1}) and *R. irregularis* (GH ¢517 ha^{-1}), except for the uninoculated control which was highest (GH ¢1531 ha^{-1}).

Table 4.23. Partial cost benefit analysis on the basis of soybean grain yield during the 2016 minor cropping season

Adjusted yield (kg ha^{-1})	685	1081	1335	1180	1697	1436	1065	1608	1390
Grain yield (kg ha^{-1})	761	1201		1311	1885		1183		1544
10% less than actual yield at farmer's level (kg ha^{-1})	76	120	148	131	189	160	118	179	154
AMF isolates		**C**			**Gm**			**Ri**	
P (kg ha^{-1})	**0**	**15**	**30**	**0**	**15**	**30**	**0**	**15**	**30**
Gross income (soybean price: GH ¢200/109kg bag) *	1257	1983	1483 2449	2165	3113	1595 2634	1954	1787 2951	2550
Fertiliser amount used (kg TSP ha^{-1})	0	32.6	65.2	0	32.6	65.2	0	32.6	65.2
Cost of fertilizer (TSP price: GH ¢140/50kg bag) **	0	91	183	0	91	183	0	91	183
Fertilizer application cost (GH ¢10/man/day)	0	30	40	0	30	40	0	30	40
Net benefit (GH ¢ ha^{-1})	1257	1862		2165	2992		1954		2327
Variable cost (GH ¢ ha^{-1})	0	121	223 2226	0	121	223 2411	0	121 2830	223

* Price from insyt (2017), ** price from central market of Kumasi, C = control, Gm = *Glomus mosseae*, Ri = *Rhizophagus irregularis.*

Table 4.24. Partial cost benefit analysis based on the soybean grain yield of during the 2017 minor cropping season

Adjusted yield (kg ha^{-1})	898	1326	1854	1941	2325	2094	1688	2156	2091
Grain yield (kg ha^{-1})	998	1473		2157	2583		1875		2323
10% less than actual yield at farmer's level (kg ha^{-1})	100	147	206	216	258	233	188	240	232
AMF isolates		**C**			**Gm**			**Ri**	
P (kg ha^{-1})	**0**	**15**	**30**	**0**	**15**	**30**	**0**	**15**	**30**
Gross income (soybean price: GH ¢200/109kg bag) *	1648	2432	2060 3402	3562	4266	2327 3843	3096	2395 3955	3836
Fertiliser amount used (kg TSP ha^{-1})	0	32.6	65.2	0	32.6	65.2	0	32.6	65.2
Cost of fertilizer (TSP price: GH ¢140/50kg bag) **	0	91	183	0	91	183	0	91	183
Fertilizer application cost (GH ¢10/man/day)	0	30	40	0	30	40	0	30	40

Net benefit (GH ¢ ha^{-1})	1648	2311	3562	4145		3096		3613	
Variable cost (GH ¢ ha^{-1})	0	121	223 3179	0	121	223 3620	0	121 3834	223

*Price from insyt (2017), ** price from central market of Kumasi, C = control, Gm = *Glomus mosseae*, Ri = *Rhizophagus irregularis*.

4.2.12.2 Marginal rate of return

Tables 4.25 and 4.26 represent the marginal rate of return for the different rates of TSP fertilizer and AMF inoculation treatments based on the field trials during the minor (2016) and the major (2017) cropping seasons. During the minor cropping season of 2016 (Table 4.25), soybean plants with 30 kg P ha^{-1} had the highest variable costs. For all treatments, the marginal net benefit obtained for the change from 0 to 15 kg P ha^{-1} was higher than that obtained for the change from 15 to 30 kg P ha^{-1}. Similarly, the marginal rate of return was lower for the change from 15 to 30 kg P ha^{-1} and higher for the change from 0 to 15 kg P ha^{-1} (Figure 4.8). On average, the marginal rate of return of treatments with 15 and 30 kg P ha^{-1} were 634% and -712%, respectively.

The trend remained the same during the major cropping season of 2017 with exception of the uninoculated control (Table 4.26) where treatments with 30 kg P ha^{-1} had the highest variable costs. For soybean plants inoculated with *G. mosseae* and *R. irregularis*, the marginal net benefit obtained for the change from 0 to 15 kg P ha^{-1} was higher than that obtained for the change from 15 to 30 kg P ha^{-1}, but not for the uninoculated control. Similarly, the marginal rate of return was lower for the change from 15 to 30 kg P ha^{-1} and higher for the change from 0 to 15 kg P ha^{-1} except for the

control treatment (Figure 4.8). On average, the marginal rate of return of 15 kg and 30 kg P ha^{-1} were 546% and 121%, respectively.

Table 4.25. Calculation of the marginal rate of return for the different doses of TSP fertilizer combined with AMF inoculation - 2016 trial

AMF isolates	Phosphorus applied (kg ha^{-1})	TSP rates (kg ha^{-1})	Cost (GH ¢ ha^{-1})	Net Benefit (GH ¢ ha^{-1})	Marginal Cost (GH ¢ ha^{-1})	Marginal Net Benefit (GH ¢ ha^{-1})	Marginal Rate of Return (%)
C	0	0	0 121	1257	0	0 605	0%
	15	32.6		1862	121		499%
	30	65.2	223	2226	101	364	359%
Gm	0	0	0 121	2165	0	0 827	0%
	15	32.6		2992	121		682%
	30	65.2	223	2411	101	-581	-574%
Ri	0	0	0 121	1954	0	0 876	0%
	15	32.6		2830	121		722%
	30	65.2	223	2327	101	-503	-497%

Cost = Cost for particular treatment (GH ¢)
Marginal cost = The increase in variable cost which occurs in changing from one production alternative to another
Marginal net benefit = The increase in net benefit which can be obtained by changing from one production alternative to another
In this study, marginal net benefit is calculated for changing from 0 to 32.6 kg TSP ha^{-1} and for changing from 32.6 to 65.2 kg TSP ha^{-1}, respectively.

Table 4.26. Calculation of the marginal rate of return for the different doses of TSP fertilizer combined with AMF inoculation - 2017 trial

AMF isolates	Phosphorus applied (kg ha^{-1})	TSP rates (kg ha^{-1})	Cost (GH ¢ ha^{-1})	Net Benefit (GH ¢ ha^{-1})	Marginal Cost (GH ¢ ha^{-1})	Marginal Net Benefit (GH ¢ ha^{-1})	Marginal Rate of Return (%)
C	0	0	0 121	1648	0	0 663	0%
	15	32.6		2311	121		547%
	30	65.2	223	3179	101	868	857%
Gm	0	0	0 121	3562	0	0 583	0%
	15	32.6		4145	121		481%
	30	65.2	223	3620	101	-525	-518%
Ri	0	0	0 121	3096	0	0 738	0%
	15	32.6		3834	121		609%
	30	65.2	223	3613	101	-221	-218%

Cost = Cost for particular treatment (GH ¢)
Marginal cost = The increase in variable cost which occurs in changing from one production alternative to another
Marginal net benefit = The increase in net benefit which can be obtained by changing from one production alternative to another
In this study, marginal net benefit is calculated for changing from 0 to 32.6 kg TSP ha^{-1} and for changing from 32.6 to 65.2 kg TSP ha^{-1}, respectively.

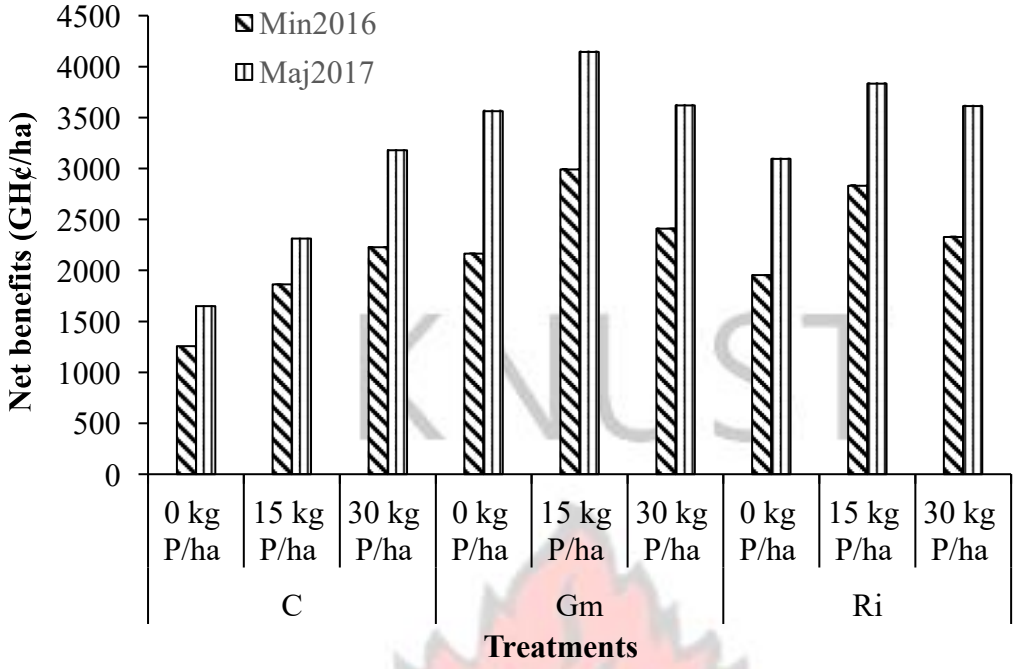


Figure 4.7. Comparison of net benefits for the different TSP fertilizer rates combined with AMF inoculation treatments - 2016 and 2017 trials

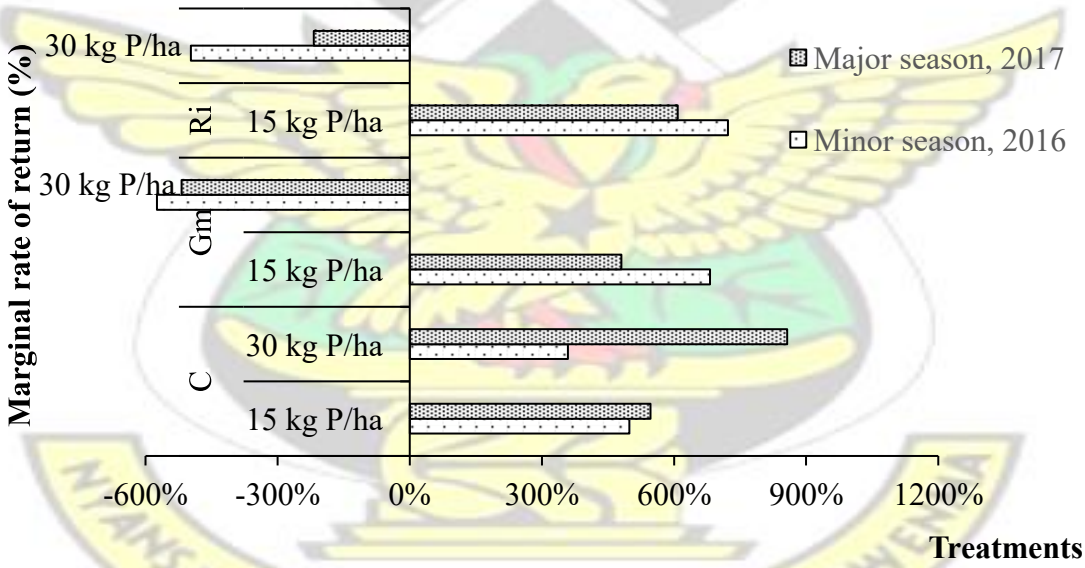


Figure 4.8. Marginal rates of return for the use of AMF and different levels of TSP fertilizer on soybean - 2016 and 2017 trials

4.3 Residual effects following arbuscular mycorrhizal fungi inoculation on maize

4.3.1 Maize root length colonization

Maize plants on plots previously inoculated with AMF showed significantly ($P <$ 0.001) higher RLC than uninoculated control at 30, 50 and 70 DAS with a significant

difference between the two mycorrhizal isolates (Table 4.27). The increase in RLC induced by *R. irregularis* was greater than that obtained with *G. mosseae* only at 30 DAS. The RLC increased with plots previously inoculated with *G. mosseae* and *R. irregularis* by 77.61 and 63.73% at 50 DAS and by 197.82 and 88.86% at 70 DAS over uninoculated control, respectively.

The RLC at 30, 50 and 70 DAS significantly ($P < 0.001$) increased in plots previously fertilized with 15 kg P ha^{-1} and significantly ($P < 0.001$) decreased with 30 kg P ha^{-1} relative to 0 kg P ha^{-1} (Table 4.27).

There was a significant ($P < 0.001$) interaction between phosphorus rate and AMF isolate for RLC at 30, 50 and 70 DAS (Figure 4.9, Appendix 21). Plots previously inoculated with *G. mosseae* where 15 kg P ha^{-1} was added showed the highest RLC at 50 DAS (55.11%) and 70 DAS (53.59%). The lowest RLC was obtained in uninoculated plots with 30 kg P ha^{-1} applied.

Table 4.27. Residual effects of mycorrhizal inoculation and P fertilizer on maize root length colonization by AMF Root length colonization Treatments (%)

	30 DAS	50 DAS	70 DAS
AMF isolates			
G. mosseae	21.05	34.03	38.24
R. irregularis	23.79	31.37	24.25
Control	4.65	19.16	12.84

F pr.	< 0.001	< 0.001	< 0.001
LSD (5%)	0.992	0.545	0.649
CV (%)	5.9	1.9	2.5
P rates (P)			
15 kg P ha^{-1}	21.39 9.83	40.94	32.15
30 kg P ha^{-1}	18.27	21.67	19.15
0 kg P ha^{-1}		21.95	24.03
F pr.	< 0.001	< 0.001	< 0.001
LSD (5%)	0.617	0.237	0.950
CV (%)	1.7	3.9	1.7
F pr.			
P × AMF isolates	< 0.001	< 0.001	< 0.001

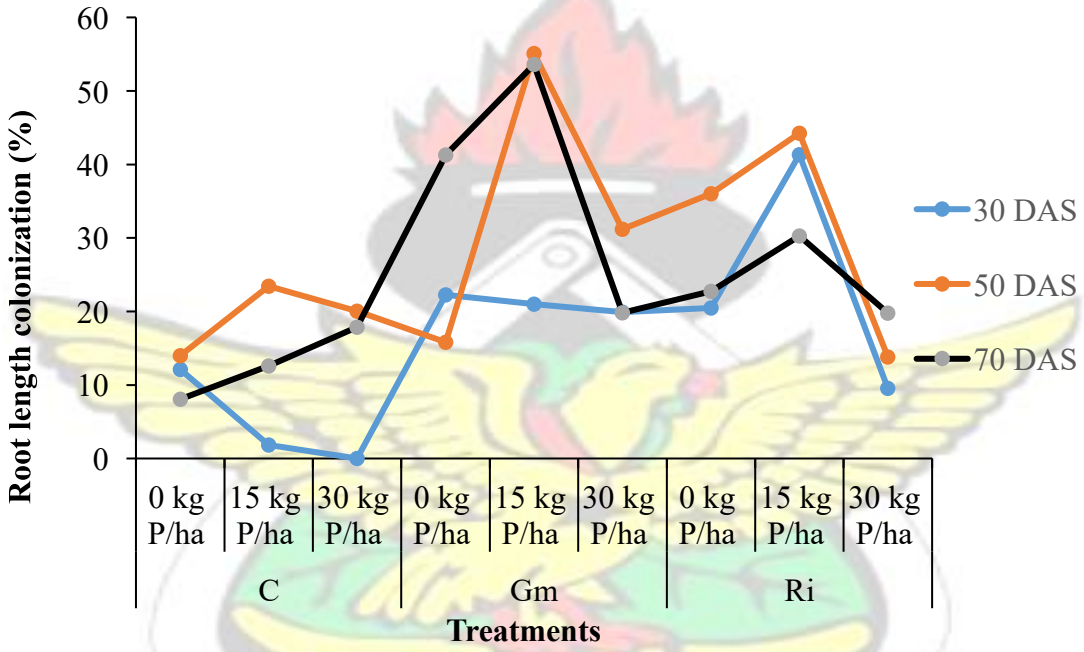


Figure 4.9. Residual effects of mycorrhizal inoculation and P fertilizer application on maize root length colonization at 30, 50 and 70 DAS

4.3.2 Mycorrhizal inoculation and P fertilizer application on nitrogen and phosphorus uptake in maize shoot and grain

Maize shoot N uptake was significantly (< 0.001) highest in plots previously inoculated with *G. mosseae* followed by that of uninoculated control and plots with *R. irregularis* (Table 4.28). Shoot N uptake increased in plots previously inoculated with *G. mosseae* by 28.20% (over *R. irregularis*) and 8.52% (over uninoculated control). The trend was similar with shoot P uptake where plots previously inoculated with *G.*

mosseae showed an increase of 26.64 (over *R. irregularis*) and 14.91% (over uninoculated control). The plots previously inoculated with AMF significantly (< 0.001) increased in maize grain P uptake but not in grain N uptake in relation to uninoculated control plots. The treatments with *G. mosseae* and *R. irregularis* resulted in a significant increase of 29.32 and 15.18% in grain P uptake over that of uninoculated control, respectively (Table 4.28).

The plots previously supplied with 30 kg P ha^{-1} recorded a significantly ($P < 0.001$) higher shoot N and P uptake and grain N uptake than other treatments followed by that of the control treatments. The 15 kg P ha^{-1} recorded the lowest values even though the difference in shoot P uptake and grain N uptake was not significant between them and the control treatment. However, the highest grain P uptake was observed in plots previously fertilized with 15 kg P ha^{-1} (Table 4.28). The treatments with 30 kg P ha^{-1} previously applied resulted in 49.01, 19.21 and 8.50% increase in shoot N uptake, shoot P uptake and grain N uptake over the control treatment, respectively. On the other hand, grain P uptake resulting from 15 kg P ha^{-1} was significantly higher than that of 30 kg P ha^{-1} and increased by 4.11% over the control treatment.

There was a significant interaction between phosphorus rate and AMF isolate for shoot N ($P = 0.025$) and shoot P ($P < 0.001$) uptake (Appendix 22). Plots previously inoculated with *G. mosseae* where 30 kg P ha^{-1} was applied showed the highest shoot N uptake (43.37 kg ha^{-1}) and shoot P uptake (13.19 kg ha^{-1}). The lowest shoot N uptake (20.71 kg ha^{-1}) and shoot P uptake (7.92 kg ha^{-1}) were obtained in plots previously inoculated with *R. irregularis* and supplied with 15 kg P ha^{-1}. There was no significant interaction in grain N ($P = 0.534$) and P ($P = 0.102$) uptake between the different treatments.

Table 4.28. Residuals effects of mycorrhizal inoculation and P fertilizer application on nitrogen and phosphorus uptake into maize shoot and grain

Treatments	Shoot		Grain	
	N uptake	P uptake	N uptake	P uptake
	(kg ha^{-1})			
AMF isolates				
G. mosseae	32.23	11.79	47.22	2.47
R. irregularis	25.14	9.31	42.27	2.20
F pr.		< 0.001	0.083	
LSD (5%)	1.868	0.620	NS	0.197
CV (%)	6.3	5.08	9.8	8.8
P rates (P)				
15 kg P ha^{-1}	24.12	9.68	42.90	2.28
30 kg P ha^{-1}	37.67	11.79	46.98	2.11
0 kg P ha^{-1}	25.28	9.89	43.30	2.19
F pr.	< 0.001	< 0.001	0.004	0.005
LSD (5%)	0.148	0.394	1.619	0.068
CV (%)	0.2	1.17	1.6	1.4
F pr.				
P × AMF isolates	0.025	< 0.001	0.534	0.102
	29.70			1.91
Control	< 0.001	10.26	43.69	< 0.001

NS = Not Significant at P ≤ 0.05.

4.3.3 Residual effects of mycorrhizal inoculation and P fertilizer application on maize shoot biomass and grain yields

Plots previously inoculated with *G. mosseae* significantly ($P < 0.001$) increased in shoot biomass yield (5.63%) while plots with *R. irregularis* significantly ($P < 0.001$) reduced it (13.13%) in relation to uninoculated control treatment (Table 4.29). The 30

kg P ha^{-1} treatments gave the highest shoot biomass yield which was significantly (P < 0.001) greater than 15 kg P ha^{-1} and control treatment, an increase of 31.29 and 31.05%, respectively. The differences in shoot biomass yield between the 15 kg P ha^{1} and control treatment were not significant. Plots previously inoculated with *G. mosseae* resulted in the highest mean grain yield (2847 kg ha^{-1}) and it represented a significant (P = 0.007) increase of 12.53% over uninoculated control treatment compared to 2.92% increase from *R. irregularis*. The difference in grain yield between 15 kg P ha^{-1} and 30 kg P ha^{-1} treatments was not significant.

There was a significant (P = 0.009) interaction between phosphorus rates and the AMF isolates for maize shoot biomass yield but not (P = 0.284) for grain yield as indicated in Appendix 23. Plots previously inoculated with *G. mosseae* where 30 kg P ha^{-1} was applied showed the highest shoot biomass yield (4946 kg ha^{-1}). The lowest shoot biomass yield (2994 kg ha^{-1}) was obtained in plots previously inoculated with *R. irregularis* and fertilized with 15 kg P ha^{-1}.

Table 4.29. Residual effects of mycorrhizal inoculation and P fertilizer application into maize shoot biomass and grain yields

Shoot biomass yield	Grain yield Treatments	(kg ha-1)
AMF isolates		
G. mosseae	4055	2847
R. irregularis	3335	2604
Control	3839	2530
F pr.	< 0.001	0.007
LSD (5%)	228.7	183.4
CV (%)	5.9	6.7
P rates (P)		
15 kg P ha^{-1}	3388	2678
30 kg P ha^{-1}	4448	2684
0 kg P ha^{-1}	3394	2619

F pr.	< 0.001	0.040
LSD (5%)	65.9	49.45
CV (%)	0.8	0.8
F pr.		
P × AMF isolates	0.009	0.284

NS = Not Significant at P ≤ 0.05.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Symbiotic effectiveness test of four arbuscular mycorrhizal fungi isolates on two soybean lines: a greenhouse study

5.1.1 Mycorrhizal inoculation on root length colonization of soybean in sterile river sand and in non-sterile soil

The root length colonization (RLC) of the soybean lines by AMF was increased by inoculation with all the AMF isolates in both soils (Table 4.2). This finding agrees with that of Cely *et al.* (2016) who reported an increase of around 20% of root colonization in both cotton and soybean upon inoculation with *Rhizophagus clarus* under field conditions. This current finding supports previous meta-analyses of several inoculation trials (Mcgonigle, 1988; Lekberg and Koide, 2005) that established that root colonization by AMF is usually enhanced by inoculation.

For the sterile river sand, inoculation with the different isolates showed a high rate of mycorrhizal root length colonization ranging from 67.01 to 83.84% of the control. The degree of root colonization in the non-sterile soil was relatively low and ranged from 17.22% (*G. fasciculatum*) to 33.07 (*G. mosseae*). Higher levels of root length colonization by the mycorrhizal isolates in the sterile river sand compared to the nonsterile soil might be explained by the low available phosphorus in the sterile river sand (Table 4.1), and also low nutrient holding capacity (Blanchart *et al.*, 2005) which has an effect on AMF colonization. In fact, because they are more porous, warmer,

drier and less fertile than soils with finer textures (Sylvia and Williams, 1992), sandy soils give the optimum conditions for AMF development from spore germination to arbuscules formation (Schenck and Schroder, 1974; Saif, 1981; Tommerup, 1983; Bowen, 1987). This study confirmed that AMF root colonization is also influenced by the soil properties as indicated by Carrenho *et al.* (2007).

In the present study, root length colonization of soybean lines exceeded 50%, regardless of the mycorrhizal isolate in sterile river sand, but below 50% in non-sterile soil. Similar trends were observed with the root infection of the tropical leguminous tree, *Acacia holosericea* inoculated with *G. intraradices* (currently *R. irregularis*) under nursery conditions (Duponnois *et al.*, 2005). These results could be explained by the relatively higher soil available P in the non-sterile soil (Table 4.1). Suppression of AMF colonization due to high inorganic phosphate levels has been reported by Balzergue *et al.* (2013).

5.1.2 Mycorrhizal inoculation on relative mycorrhizal dependency of soybean in sterile river sand and in non-sterile soil

The relative mycorrhizal dependency (RMD) was classified into three categories by Cruz *et al.* (1999): not dependent (RMD ≤ 10 %), moderate dependency (10 < RMD < 40%) and high dependency (RMD > 40%).

It was observed in this study that both soybean lines exhibited a high dependency to all AMF isolates in both sterile and non-sterile soils. However, they significantly depended more on *R. irregularis* and *G mosseae* (Table 4.3) to improve their phosphorus uptake, growth and dry matter production (Tables 4.4, 4.5, 4.6 and 4.7). The TGx 1989-48 FN was more dependent on the AMF isolates than TGx 1989-75 FN in sterile river sand but at par in non-sterile soil (Table 4.3). The findings support the

fact that soils with low fertility limit plant development and increase the dependence of plants on mycorrhizal association as reported by Siqueira and Saggin-Júnior (1995). Inversely, plant dependence on the AMF symbiosis generally decreases with increasing soil fertility (Smith and Read, 1997).

The high RMD could also be explained by the shift of the ratio between the plant Direct Pathway (DP) and Mycorrhizal Pathway (MP) for phosphorus uptake towards the MP after a strong suppression of the DP as suggested by Bücking and Kafle (2015). Indeed, the plant phosphorus transporters involved in the uptake via the DP have been reported to be down-regulated in response to the mycorrhizal symbiosis (Chiou *et al.*, 2009), whereas mycorrhiza-specific transporters involved in phosphorus uptake from the MP are induced (Harrison *et al.*, 2007).

5.1.3 Mycorrhizal inoculation on shoot P uptake, plant height, stem girth and shoot biomass yield of soybean in sterile river sand and in non-sterile soil

Inoculation of soybean lines with mycorrhizal isolates had a positive impact on shoot phosphorus uptake, plant height, stem girth and shoot biomass yield in both sterile and non-sterile soil types (Tables 4.4, 4.5, 4.6 and 4.7). Plant growth improvement by the AMF has been attributed to enhanced inorganic nutrients transport, absorption and uptake in plant tissues, especially phosphorus (Smith and Read, 2008). Furthermore, the different AMF isolates are not the only factor inducing variation in performance of plant growth, but also the growth medium because of an eventual influence of soil properties and the varieties (genotype or line) on the establishment of AM fungal symbiosis. This was supported by the significant interaction observed between inoculation and the soybean lines (Appendix 2). These results corroborated that of Baum *et al.* (2015) who suggested that the capability of arbuscular mycorrhizae to

enhance quality and growth of their host plants is governed by the genotype of the fungus, the genotype of the host and their interactions.

Among the four AMF tested, *R. irregularis* and *G. mosseae* had the best effect on plant performance in terms of phosphorus uptake, plant height, stem girth and shoot biomass yield in sterile river sand and in non-sterile soil (Tables 4.4, 4.5, 4.6 and 4.7). However, the higher root colonization of *G. mosseae* did not result in a better performance than *R. irregularis* in terms of growth improvement as suggested by Hetrick *et al.* (1992), that AMF colonization of plant roots is not necessarily related to their growth.

The relative mycorrhizal dependency (Table 4.3) showed that the soybean lines were significantly more dependent on *R. irregularis* to improve their phosphorus uptake, growth and dry matter production (Tables 4.4, 4.5, 4.6 and 4.7) than *G. mosseae* in both soil types under greenhouse conditions. This corroborates the findings of Wang *et al.* (2016) who established that *R. irregularis* was more beneficial in increasing shoot phosphorus uptake of two soybean genotypes under three phosphorus supply conditions compared with *G. custos* and *G. aggregatum* and under greenhouse conditions. Similarly, co-inoculation with some native rhizobial strains and AMF in a calcareous soil was greatly affected by the AMF species. The *G. intraradices* (*R. irregularis*) performed better than *G. mosseae* in increasing nutrient uptake and plant growth of lentil (*Lens culinaris* cv. 'Ziba') (Zarei *et al.*, 2006). The results of this study demonstrated the importance of the soil type and soybean line as modulating factors on AMF root length colonization and plant growth.

Another observation was that available P, N, Ca and organic carbon were relatively higher in non-sterile soil than in the sterile river sand (Table 4.1). Some previous studies have demonstrated the influence of nutrient content and soil texture on

mycorrhizal function (Liu *et al.*, 2000; Carrenho *et al.*, 2007). In addition, the nonsterile soil contained more clay, which probably played a major role in holding nutrients and making them available to plants (Table 4.1). Significant positive correlations were observed between mycorrhizal RLC and the growth parameters (plant height and stem girth at 20 and 40 DAS), shoot P uptake and shoot biomass yield. The Pearson's correlation showed that the RLC accounted for the highest differences between the averages (Appendices 24 and 25).

5.2 Field assessment of the response of two soybean lines to arbuscular mycorrhizal inoculation and different phosphorus fertilizer rates

5.2.1 Mycorrhizal inoculation and phosphorus fertilizer application on root length colonization

Soybean root length colonization (RLC) was significantly increased by arbuscular mycorrhizal inoculation on the Haplic Acrisol during the minor and major cropping seasons of 2016 and 2017 (Table 4.9). This positive response corroborates several other reports of increase in RLC of soybean after inoculation with arbuscular mycorrhizal fungi (Meghvansi *et al.*, 2008; Fernandez *et al.*, 2009; Meghvansi and Mahna, 2009; Cely *et al.*, 2016). In addition, after three successive years of inoculation with some selected AMF, Ortas (2012) observed an increase in root colonization in twelve different crops including soybean as compared with uninoculated treatments.

The positive response of soybean to mycorrhizal inoculation observed could be due to the flat ploughing performed at the field using a disc tractor followed by a harrowing at a depth of 15 cm some days before planting. This management practice had surely influenced the special aggregation and microbial competitive interactions. Verbruggen *et al.* (2013) reported that aggregation of the indigenous fungi is reduced by 'intensive' tillage before inoculation and that will greatly affect the success of the introduced

allochthonous one. In fact, if they are applied patchily after tillage, there is a possibility for the new introduced fungi to be locally far more abundant than the resident fungi because of the 'dilution' of the latter through mixing (Verbruggen *et al.*, 2013). In addition, Thomas and Sekhar (2016) indicated that the presence of beneficial mycorrhizal species in a given soil does not necessarily imply that it is metabolically active in the rhizosphere.

The positive response could also be attributed to the adaptability and higher competitiveness of the two AMF isolates with the indigenous mycorrhizal population with a density of 284 spores per 100 g of soil. Indeed, the two mycorrhizal isolates used for this study (*Rhizophagus irregularis* and *Glomus mosseae*) are very generalist symbionts widely distributed, can survive long-term storage, colonize a wide variety of host plants (Öpik *et al.*, 2010) and can be massively and easily propagated (Berruti *et al.*, 2016). The studies carried out by Köhl *et al.* (2016) on eight different unsterilized field soils with diverse indigenous arbuscular mycorrhizal communities, soil types and chemical properties revealed that the isolate *R. irregularis* has a wide niche, can compete with indigenous arbuscular mycorrhizal fungi and hence is able to establish successfully in a wide range of soils with highly variable chemical properties.

Unlike in the legume-rhizobial symbiosis, the successful establishment of AMF species inoculated to a soil where they did not originate from does not depend on the population size of the indigenous AMF. According to Wagg *et al.* (2015), the composition in AMF species could be more influential than the diversity of species in determining the way species function within a partnership. The present study supports therefore the previous observations made on these two isolates because both *G. mosseae* and *R. irregularis* increased by more than 100% the RLC under field conditions. The *G. mosseae* showed a significantly higher RLC than *R. irregularis*

during the minor cropping season but the increase in RLC was not significant during the major cropping season. This observation was similar to the findings of Sohrabi *et al.* (2015) who observed that inoculation of *G. mosseae* was more effective than *R. irregularis* in increasing root colonization in chickpea. Furthermore, Jie *et al.* (2013) reported *G. mosseae* and *Glomus* spp. to be the dominant AMF in the experimental fields of various soybean cultivars.

Application of 30 kg P ha^{-1} significantly decreased soybean RLC over the control (0 kg P ha^{-1}) during the minor and major cropping seasons of 2016 and 2017. There was no significant decrease when 15 kg P ha^{-1} was applied during the minor cropping season of 2016 (Table 4.9). This supports the argument of Balzergue *et al.* (2013) who asserted that high phosphate levels suppress arbuscular mycorrhizal colonization. Johnson *et al.* (1997) reported that AM fungi may reduce plant growth when phosphorus is abundant in the soil. Furthermore, Berruti *et al.* (2014) reported that high levels of fertilizer present in a particular soil drastically alters the interaction between plants and dwelling microbial communities, and due to their cardinal role in plant nutrition, AMF are very susceptible to variations in soil nutrient availability. In addition, Fernández *et al.* (2011) reported that, mycorrhizal root colonization in soybean was significantly and negatively related to soil available phosphorus in a Mollisol.

During the major cropping season of 2017, addition of 15 kg P ha^{-1} significantly increased soybean RLC by 30.51% over the control (0 kg P ha^{-1}). This is likely to confirm the assertion by Chalk *et al.* (2006) that soils with low available phosphorus would respond better to mycorrhizal inoculation when they are corrected by superphosphate application than unfertilized soils. It is confirmed by the significant interaction obtained during the major cropping season, where the highest RLC was

obtained with TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 15 kg P ha-1.

The TGx 1989-48 FN had consistently higher RLC than TGx 1989-75 FN during the minor and major cropping seasons in 2016 and 2017, respectively. This is consistent with the findings of Jie *et al.* (2013) where the mycorrhizal colonization rates differed according to the soybean cultivar and the year. The root length colonization by AMF in soybean at 50% flowering (Table 4.9) showed that the colonization of AMF in soybean over two years of continuous cropping was higher than that under one year of cropping. This suggests that longer continuous cropping is beneficial for AM fungal colonization in soybean roots. Jie *et al.* (2013) asserted that this practice gradually transforms a soil from high fertility soil which is "bacterial type" to low fertility soil which is "fungi type".

5.2.2 Inoculation and phosphorus application on mycorrhizal inoculation effect on two soybean lines in Haplic Acrisol

The data shown in Table 4.10 indicates that the mycorrhizal inoculation effect (MIE) was significantly higher when the soybean lines were inoculated with *G. mosseae* than with *R. irregularis* during both the minor and major cropping seasons. The MIE was significantly reduced by the addition of 30 kg P ha^{-1} in relation to the control (0 kg P ha^{-1}) with no significant decrease when 15 kg P ha^{-1} was applied during the minor cropping season of 2016. However, the addition of 15 kg P ha^{-1} during the major cropping season of 2017 increased the MIE over the control and 30 kg P ha^{-1}. This follows the same trend as the root length colonization (Table 4.9) and it is illustrated by the significant positive correlation between the RLC and MIE (Figure 4.1).These results are in accordance with the findings of Ortas (2012), where plant species

belonging to Leguminosae showed high responses to MIE under non-fumigated and fumigated soil conditions, and the MIE was higher under low phosphate fertilizer supply than under high phosphate fertilizer supply. In addition, among all the factors influencing MIE, phosphorus is well known to be the most influential element in AMF development and efficiency (Ortas, 2012).

The response of the two soybean lines was not consistent with regard to the MIE during the two cropping seasons. The MIE was significantly higher for TGx 1989-48 FN during the minor cropping season of 2016 and significantly higher for TGx 1989-75 FN during the major cropping season of 2017. According to Sruthilaxmi and Babu (2017), inconsistency in the performance of inoculated microbial isolates under field conditions could be due to negative effects of interaction with existing native microbes and incompatibility in colonizing different varieties.

5.2.3 Mycorrhizal inoculation and phosphorus application on shoot and grain phosphorus uptake of two soybean lines

5.2.3.1 Shoot phosphorus uptake

The increase in shoot P uptake by the mycorrhizal inoculation was not significant during the minor cropping season of 2016 but it was significant during the major cropping season of 2017 as indicated in Table 4.11. That significant increase in shoot P uptake was brought about by the inoculation of *G. mosseae*. This is in agreement with several findings of previous researches on the high performance of *G. mosseae* in improving plant shoot P uptake (Ortas *et al.*, 2011; Pellegrino *et al.*, 2011; Smith and Smith, 2011; Yaseen *et al.*, 2011).

Application of the TSP fertilizer significantly increased the shoot P uptake over nonP-fertilized control (Table 4.11). During the minor cropping season of 2016, fertilization

with 15 kg P ha^{-1} supported greater shoot P uptake than the fertilization with 30 kg P ha^{-1}. This is in agreement with the assertion by Kaschuk (2009) that plants associated with AMF often produce more grains and biomass than fertilized plants.

Nevertheless, shoot P uptake under 15 kg P ha^{-1} was lower compared to fertilization with 30 kg P ha^{-1} during the major cropping season of 2017, but the difference was not statistically significant. It has been demonstrated that phosphate fertilizer supply to legumes increases shoot phosphorus content and plant biomass by increasing the rate of nitrogen fixation (Olivera *et al.*, 2004; Ofori, 2016).

The interaction between the soybean lines, phosphorus rates and AMF isolates showed that there was no significant difference between TGx 1989-48 FN fertilized with 15 kg P ha^{-1} and inoculated with either of the mycorrhizal isolates (*G. mosseae* or *R. irregularis*) and TGx 1989-75 FN uninoculated and fertilized with 30 kg P ha^{-1} which recorded the highest shoot P uptake (Appendix 9). This is in agreement with Wang *et al.* (2016) who reported that inoculation with *R. irregularis* increased P concentration in the tissues of two soybean genotypes in roots and shoots under low and medium P conditions. Nevertheless, no difference in shoot P concentrations was found between uninoculated or mycorrhizal plants when the plants were supplied with high P (Wang *et al.*, 2016).

In this present study, the shoot P uptake was significantly higher for TGx 1989-75 FN (16.57 kg ha^{-1}) than TGx 1989-48 FN (15.01 kg ha^{-1}) during the minor cropping season. Inversely, it was higher for TGx 1989-48 FN (29.28 kg ha^{-1}) than TGx 198975 FN (23.38 kg ha^{-1}) during the major cropping season. Although it was not consistent between the two lines, this could be explained by a difference in their root structure as suggested by Vandamme *et al.* (2013), that differences in phosphorus uptake efficiency

among soybean genotypes is closely related to colonization by AMF and to a greater extent to variations in root hair development.

5.2.3.2 Grain phosphorus uptake

The grain P uptake was significantly increased by mycorrhizal inoculation during the minor and major cropping seasons as shown in Table 4.11. However, inoculation with *G. mosseae* consistently and significantly yielded the highest increase in grain P uptake over *R. irregularis* and the uninoculated control. This confirms several assertions by many authors on the ability of these two AMF species to increase phosphorus uptake in diverse crops including soybean (Barea *et al.*, 1983; Douds *et al.*, 2007; Meghvansi *et al.*, 2008; Antunes *et al.*, 2009; Cozzolino *et al.*, 2013; Williams *et al.*, 2013). Furthermore, it follows the same trend as the RLC where *G. mosseae* was leading *R. irregularis* in terms of root infection.

The effect of the application of TSP fertilizer on grain P uptake followed the same trend than observed on shoot P uptake. The 15 kg P ha^{-1} supported a greater grain P uptake than 30 kg P ha^{-1} during the minor cropping season of 2016. Nevertheless, it was significantly lower in relation to fertilization with 30 kg P ha^{-1} during the major cropping season of 2017. This infers that 30 kg P ha^{-1} is the maximum rate to induce an increase in soybean grain P uptake in response to P fertilizer application. This was the observation made by Ofori (2016) who noted no significant increase in grain P uptake following addition of 34.35 kg P_2O_5 and 68.70 kg P_2O_5 to soybean in a Gleyic Lixisol.

The difference in grain P uptake between the two soybean lines investigated was not significant. However, significant interactions between the soybean lines, phosphorus rates and AMF isolates for grain P uptake indicated that the combination of *G. mosseae*

inoculated to TGx 1989-48 FN and fertilized with 15 kg P ha^{-1} was the best treatment that resulted in highest grain P uptake during both minor and major cropping seasons. This suggest that inoculating *G. mosseae* to TGx 1989-48 FN and adding 15 kg P ha^{-1} is a promising approach in reducing the P fertilizer applied to soybean while maintaining grain P uptake similar to the sole addition of 30 kg P ha^{-1} without inoculation. This is confirmed by the fact that there were no significant differences in grain P uptake between the following treatment combinations: TGx 1989-75 FN × 30 kg P ha^{-1} × *R. irregularis*, TGx 1989-75 FN × 30 kg P ha^{-1} × uninoculated control and TGx 1989-48 FN × 15 kg P ha^{-1} × *G. mosseae*. However, it was noticed that the response to inoculation with *R. irregularis* was not hindered by the addition of 30 kg P ha^{-1}.

Several authors have reported a negative impact of P fertilizer addition on plantmycorrhizal interaction (Khan *et al.*, 2010; Fernández *et al.*, 2011; Sharma *et al.*, 2013; Berruti *et al.*, 2014; Yang *et al.*, 2014). However, studies carried out by Gosling *et al.* (2013) on maize (*Zea mays*), field violet (*Viola arvensis*), and soybean (*Glycine max*) have indicated that high soil phosphorus supply is not always detrimental to arbuscular mycorrhizal fungi diversity under field conditions. Furthermore, Köhl *et al.* (2016) demonstrated that AMF inoculation, particularly *R. irregularis* can be successful, despite the abundance of indigenous AMF communities and the high soil P

availability. The beneficial impact of the inoculation with the two isolates investigated is supported by the significant positive correlation (R^2 = 0.6389) between the total P uptake (grain P uptake + shoot P uptake) and mycorrhizal inoculation effect (MIE) during the major cropping season of 2017 (Figure 4.2).

5.2.4 Mycorrhizal inoculation and phosphorus fertilizer application on phosphorus use efficiency of two soybean lines

Mycorrhizal inoculation significantly increased phosphorus use efficiency of soybean compared to the control treatments (Table 4.12). Inoculation with *G. mosseae* significantly increased PUE over *R. irregularis* during the minor cropping season, but there was no significant difference between the two isolates during the major cropping season. This confirms that, the spatial precision with which plant species can select partners vary with plants as suggested by Werner and Kiers (2015).

The data in Table 4.12 indicates that PUE of soybean significantly increased with low phosphorus rates (15 kg P) applied during the minor and major cropping seasons. This response of soybean to low quantities of P added in terms of increased PUE agrees with the assertion by Veneklaas *et al.* (2012) that crops growing in soils with very low phosphorus content and where no or little phosphorus fertilizer is applied are expected to yield the largest benefits of improved PUE. This could be explained by the relatively ineffective large addition of phosphates since only 15 to 30% of the intake is estimated to be absorbed by plants (Cordell *et al.*, 2009).

The TGx 1989-75 FN showed a significantly greater increase in PUE of 18.75 and 11.59% over TGx 1989-48 FN during the minor and major cropping seasons, respectively (Table 4.12). However, the significant interactions between the soybean lines, phosphorus rates and AMF isolates for PUE during the minor and major cropping seasons indicated that TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 15 kg P ha^{-1} gave the highest PUE (Appendix 11). Following the same trend as the RLC, MIE, shoot biomass and grain yield, *G. mosseae* combined with application of 15 kg P ha^{-1} was the most suitable treatment combination for the soybean lines investigated.

5.2.5 Mycorrhizal inoculation and phosphorus fertilizer application on microbial biomass phosphorus

Mycorrhizal inoculation had no significant effect on microbial biomass P at 50% flowering. However, inoculation with *G. mosseae* significantly reduced microbial biomass phosphorus by 16.02% at harvest relative to uninoculated control treatment. On the other hand, inoculation with *R. irregularis* had no significant effect on microbial biomass P (Table 4.13). The most likely plausible explanation for this observation is the difference in performance between the two AMF isolates. In fact, the findings of Jie *et al.* (2013) established that *G. mosseae* and *Glomus* spp. were the dominant AMF species in the root systems of various soybean cultivars.

Application of 30 kg P ha^{-1} significantly increased microbial biomass P at 50% flowering and at harvest as compared to 15 kg P ha^{-1} and the control treatments (Table 4.13). The addition of 15 kg P ha^{-1} also significantly increased the microbial biomass P at 50% flowering over the control but the increase was not significant at harvest. This could be attributed to the immobilization of phosphorus by rhizospheric microorganisms as it was also reported in several studies (Marschner *et al.*, 2010; Sheng *et al.*, 2010; Jin *et al.*, 2014; Morais and Gatiboni, 2015). In fact, the microbial communities surrounding the rhizosphere of plants play a key role in the nutrient cycling mainly N and P (Barea *et al.*, 2005).

It has been demonstrated that AMF secrete organic acids and acid phosphatases that solubilize organic complexes to release phosphorus (Ezawa *et al.*, 2005; Alvarez *et al.*, 2012). The results on soil microbial biomass P suggest that inoculation with *G. mosseae* had an influence on the activity of other soil microorganisms and stimulated the release rather than immobilization of the low mobility element which is P. In fact, Taktek *et al.* (2015) established that several phosphate solubilizing bacteria (PSB) were

strongly attached to the hyphae of AMF especially *R. irregularis*, and some of them exhibit high potential of mobilizing organic and inorganic P.

The findings of the present research indicate that in the absence of effective and compatible AMF species, a large amount of available P will be immobilized by the microbial communities around the root zone resulting in a higher microbial biomass P. The AM fungus has therefore a central role by collecting via the extraradical hyphae the soluble Pi from the soil and PSB and conveying it through the intraradical hyphae to the nutrient exchange interface inside root cells as reported by Smith and Smith (2011). In return, the PSB will benefit from some extraradical exudates from the hyphae of the AMF for their nutrition (Taktek *et al.*, 2015).

In contrast, low microbial biomass P in the root zone could be related to a high microbial biomass carbon (C) since more P solubilized would result in more organic nutrients available via extraradical exudation to the rhizospheric microbial community. This is corroborated by the findings of Zarea *et al.* (2009) who reported that inoculation with *G. mosseae* to clover increased soil microbial biomass carbon from 256 to 444 mg carbon kg^{-1}. Similarly, Vilela *et al.* (2014) reported that microbial biomass carbon and soil acid phosphatase activity were increased by inoculation with *G. macrocarpum* on a Cerrado Oxisol. In contrast, Stamou *et al.* (2017) reported that the inoculation with *R. irregularis* to tomato in a sandy loam mixture in pot experiment was contrasted with previous reports demonstrating a positive correlation between the soil enzymatic activity and the soil total microbial biomass.

5.2.6 Effect of mycorrhizal inoculation on soil available phosphorus

Mycorrhizal inoculation significantly increased phosphorus availability on the Haplic Acrisol from previous season, at 50% flowering and at harvest over the uninoculated

control during the major cropping season of 2017 (Table 4.14). Treatments inoculated with *G. mosseae* exhibited a significantly higher increase in available P than those inoculated with *R. irregularis*. However, there was no significant increase in soil available P by *R. irregularis* over the control treatment at harvest.

The highest available P was observed at 50% flowering followed by harvest and then pre-planting, but it is important to note that no fertilizer was added before planting. These observations could be attributed to a more intensive AMF and PSB activity during the growth and reproductive periods than at plant maturity. This agrees with the assertion by Mougel *et al.* (2006) that plants establish the highest level of mycorrhization and nodulation (symbiotic association) with AMF and rhizobia during the transition between vegetative and reproductive stages.

The treatments fertilized with TSP showed a higher available P in the soil over the control treatment pre-planting and at 50% flowering but not at harvest. The decline in available P at harvest after the addition of TSP can be attributed to the fixation of the phosphorus which is negatively charged (in the form of orthophosphate ions) and get precipitated by free aluminium (Al^{3+}) and iron (Fe^{3+}) cations in the soil solution or is adsorbed on the soil minerals as indicated by Sharma *et al.* (2013). It could also be explained by the very quick depletion by roots of the stock of Pi present in their immediate environment (Smith and Smith, 2011).

5.2.7 Mycorrhizal inoculation and phosphorus fertilizer application on plant height and stem girth of soybean under non-sterile soil

5.2.7.1 Plant height

The mycorrhizal inoculation significantly increased soybean plant height on the Haplic Acrisol during the minor and major cropping seasons 2016 and 2017, respectively

(Tables 4.15 and 4.16). This supports the findings of Alsamowal *et al.* (2013) who reported that AMF alone or in combination with Bradyrhizobium strains inoculated to groundnut performed significantly better than the bacteria alone in terms of root dry weight, shoot dry weight and plant height,. Plants inoculated with AMF were significantly taller than uninoculated treatments and the highest increases in plant height were brought about by *G. mosseae*. Furthermore, Meghvansi *et al.* (2008) reported among three AMF isolates inoculated with four soybean cultivars that *R. irregularis* produced the largest increase in shoot height, nodulation and grain yield.

Soybean plant height was significantly increased by P fertilization at 60 and 80 DAS during the minor cropping season and at 80 DAS during the major cropping season. Furthermore, there was a significant interaction between phosphorus rate and AMF isolate for plant height at 40 DAS during the minor cropping season (Appendix 15) and at 40 and 80 DAS during the major cropping season. For both cropping seasons, AMF inoculated soybean plants with or without TSP applied were significantly taller than uninoculated control plants, except when 30 kg P ha^{-1} was applied, where the difference in plant height between inoculated and uninoculated control was not significant. This followed the same trend as the shoot P uptake which significantly increased following the mycorrhizal inoculation. Taking into account the significant and positive correlations between the shoot P uptake and the plant height in sterile and non-sterile soil (Appendices 24 and 25) under greenhouse conditions, Wang *et al.* (2016) reported that, two soybean genotypes supplied with high P did not show any significant difference between non-mycorrhizal or mycorrhizal plants in terms of shoot P concentrations.

The increase in plant height was not consistent between the two soybean lines during the two cropping seasons. However, the significant interactive effects of soybean line

and AMF isolate for plant height at 40 DAS during the minor cropping season indicated tallest soybean plants with TGx 1989-48 FN inoculated with *G. mosseae* (Appendix 14). This is confirmed by the significant interaction between soybean line, P rate and AMF isolate for plant height during the major cropping season, where the tallest soybean plants (35.00 cm) were obtained with TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 15 kg P ha^{-1} (Appendix 16).

5.2.7.2 Stem girth

The mycorrhizal inoculation significantly increased stem girth of soybean under field conditions during the minor and major cropping seasons of 2016 and 2017, respectively (Tables 4.17 and 4.18). Plants inoculated with AMF had significantly greater stem girth than uninoculated treatments with highest increases in stem girth by inoculation with *R. irregularis* during the minor cropping season and by *G. mosseae* during the major cropping season. The inconsistency between the two AMF isolates in increasing the stem girth could be due to the time necessary for the two mycorrhizal species inoculated to be adapted to the non-native soil as described by Sruthilaxmi and Babu (2017). It could also result from the priority effects, meaning the influence of competition on the establishment of alternative stable AMF communities (Verbruggen *et al.*, 2013), or the functional differences among the two AMF isolates (Pellegrino *et al.*, 2011).

Stem girth of soybean was significantly increased by P fertilization only during the major cropping season and the increase was highest with the application of 30 kg P ha^{1}. This could partly be due to the decreased rainfall received during the vegetative and the reproductive stages in the minor cropping season. There were a two-week of drought that occurred between 5 and 19 DAS, and a three-week of drought between 54 and 74 DAS that might have affected expansion in stem girth (Appendix 27).

The increase in stem girth was also significant between the two soybean lines only during the major cropping season. The TGx 1989-48 FN increased in stem girth by 13.99% over TGx 1989-75 FN at 80 DAS. However, the significant interactive effects of soybean line and P rate for stem girth at 40 DAS during the minor cropping season indicated the highest stem girth was obtained with TGx 1989-48 FN inoculated with *G. mosseae* (Appendix 17). There was a significant interaction between soybean line, P rate and AMF isolate for stem girth during the major cropping season, where the greatest stem girth of soybean (15.43 mm) was obtained with TGx 1989-75 FN inoculated with *R. irregularis* and fertilized with 30 kg P ha^{-1} (Appendix 18). This corroborates the assertion by Köhl *et al.* (2016) that inoculation with *R. irregularis* can be successful despite a high soil P availability and an abundant native AMF communities.

5.2.8 Mycorrhizal inoculation and phosphorus fertilizer application on shoot biomass and grain yields of soybean on non-sterile soil

5.2.8.1 Shoot biomass yield

Soybean shoot biomass yield resulting from the non-sterile soil was higher with the treatments inoculated with *G. mosseae* than the plants inoculated with *R. irregularis* during the minor and major cropping seasons (Table 4.19); and the difference was significant during the major cropping season. This did not follow the trend observed with the shoot P uptake. However, shoot biomass yield response of soybean to inoculation with *R. irregularis* was significantly higher than that of uninoculated control during both cropping seasons. This observation is likely to adduce evidence that maximum shoot P uptake is not always synonymous to optimum shoot biomass production because a major proportion of polyphosphate (Pi) taken from the soil is accumulated in grains as phytase as reported by Richardson (1994). This is in

agreement with Grunwald *et al.* (2009) who asserted that the interaction of plantfungus may lead to either an increase in P levels in the plant tissues or to the production of biomass, or to both depending on the fungal and plant partners involved in the association.

During the minor cropping season of 2016, only 30 kg P ha^{-1} gave a significantly greater shoot biomass yield over the control treatment. However, both 15 and 30 kg P ha^{-1} during the major cropping season of 2017 resulted in a significantly higher shoot biomass yield over the control treatment with no significant difference in shoot biomass yield between the two rates. This is in accordance with Ofori (2016) who observed an increase in soybean shoot dry weight yield when 34.35 kg P_2O_5 ha^{-1} was increased to 68.70 kg P_2O_5 ha^{-1}.

Significant interaction between the soybean lines, phosphorus rates and AMF isolates for shoot biomass yield during the major cropping season of 2017 indicated that optimal yield was obtained with TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 15 kg P or 30 kg P with no significant difference between the two TSP rates. The findings agree with Sohrabi *et al.* (2015) who reported that inoculation with *G. mosseae* performed better than with *R. irregularis* in reducing the root rot disease of chickpea and improving its shoot and root dry weight.

5.2.8.2 Grain yield

Table 4.20 indicates that mycorrhizal inoculation significantly increased soybean grain yield under field conditions. Inoculation with *G. mosseae* resulted in significantly higher increase in grain yield than with *R. irregularis* over the uninoculated control. The increase over the control brought about by *G. mosseae* represented 39.11 and 56.03% during the minor and major cropping seasons, respectively. On the other hand,

R. irregularis increased grain yield by 23.26 and 45.56 % during the minor and major cropping seasons, respectively. A similar trend was observed for shoot biomass yield suggesting that higher shoot biomass yield lead to better ability of plants to exhibit higher grain yield inferring from the significant positive correlations (R^2 = 0.8031 and R^2 = 0.6140) between the shoot P uptake and grain yield during the minor and major cropping seasons, respectively (Tables 4.21 and 4.22). If that is the case, the major proportion of phosphorus absorbed by the shoot was likely translocated and accumulated in grains as phytase as reported by Richardson (1994).

Effect of AMF inoculation on soybean grain yield in this study supports the results of Alloush *et al.* (2000) who reported that shoot phosphorus content, shoot dry weight, number of nodules and grain yield were higher in chickpea plants inoculated with the mycorrhizal fungus *G. versiforme* than in uninoculated plants. Similarly, Cely *et al.* (2016) reported that 20% of soybean root colonization by *R. clarus* increased phosphorus and nitrogen content of vegetal tissues in inoculated plants which reflected in a higher yield. Alsamowal *et al.* (2013) also reported that AMF and Bradyrhizobium positively affected peanut nutrients uptake, plant growth and yield in greenhouse and field experiments, and that AM fungi alone or combined with Bradyrhizobium strains performed better than the bacteria alone in terms shoot dry weight, plant height and root dry weight. These findings suggest that plants act selectively on mycorrhizal symbiosis. Indeed, Meghvansi *et al.* (2008) reported that amongst three AMF isolates, inoculation with *R. irregularis* performed best in increasing plant growth, nodulation and grain yield of four soybean cultivars followed by *Acaulospora tuberculata* and *G. gigantea*.

The application of 30 kg P ha^{-1} significantly increased soybean grain yield over 15 kg P ha^{-1} and the control treatment during the minor and major cropping seasons (Table

4.20). Fertilisation with 15 kg P ha^{-1} also significantly increased grain yield over the control during the minor and major cropping seasons. The TGx 1989-75 FN had significantly higher grain yield than TGx 1989-48 FN during the minor cropping season but no significant difference during the major cropping season (Table 4.20). However, the significant positive interaction between the soybean line, phosphorus rate and AMF isolate for grain yield during the minor and major cropping seasons indicates that TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 15 kg P ha^{-1} exhibited the highest soybean grain yield during both cropping seasons (Appendix 20). A similar trend was observed for shoot biomass yield. It can thus be said that TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 15 kg P ha^{1} is potentially the best inoculum formulation for maintaining an acceptable yield under low P fertilizer regimes. This assertion is further buttressed by the significant positive correlation between the shoot phosphorus uptake and grain yield during the minor (R^2 = 0.6504) and major (R^2 = 0.8031) cropping seasons of 2016 and 2017 (Figures 4.4 and 4.5 Tables 4.21 and 4.22), and also by the significant positive correlation between the mycorrhizal inoculation effect (MIE) and grain yield during the major cropping season of 2017 (Figure 4.6, Table 4.22).

These findings corroborate the assertion by Chalk *et al.* (2006) that favourable responses to mycorrhizal inoculation might occur in soils with no fertilizer applied, but they are more probably to occur in soils where low phosphorus availability was corrected by superphosphate application. The findings also confirm the reports of Jie *et al.* (2013) who indicated that *G. mosseae* and *G.* spp. were the dominant AMF in the experimental fields of various soybean cultivars and under different continuous cropping regimes. Considering the initial characteristics of the Haplic Acrisol and particularly the available P which was medium according to the rating by Landon

(2014) (Table 4.8), these findings support the results of Herrera-peraza *et al.* (2011) who reported that *G. mosseae* and *G. manihotis* showed their best performances in soils with medium fertility. This also supports the assertion by Liu *et al.* (2016) that the recommended phosphate fertilizer rate could be reduced to approximately 80% via inoculation with AMF under certain conditions while maintaining similar crop yields. However, for the present study, mycorrhizal inoculation reduced up to 50% of the rate of TSP recommended by Lamptey *et al.* (2014).

5.2.9 Economic profitability following mycorrhizal inoculation and phosphorus fertilizer application for soybean production

5.2.9.1 Net benefits

The soybean grain yield considerably increased when changing from 0 kg P ha^{-1} to 15 kg P ha^{-1} during the minor and major cropping seasons of 2016 and 2017. There was an average increase of 457 kg ha^{-1} in grain yield for uninoculated control when changing from TSP applied at 0 kg P ha^{-1} to 15 kg P ha^{-1}, 500 kg ha^{-1} for soybean plants inoculated with *Glomus mosseae* and 562 kg ha^{-1} for soybean plants inoculated with *Rhizophagus irregularis* (Tables 4.23 and 4.24). The increase in yield is accompanied by rising net benefits (Figure 4.7), an average increase of GH ¢634 ha^{-1} for uninoculated control, GH ¢705 ha^{-1} for soybean plants inoculated with *G. mosseae* and GH ¢807 ha^{-1} for soybean plants inoculated with *R. irregularis*.

These findings suggest that the inoculation with AMF combined with the addition of 15 kg P ha^{-1} was economically more profitable for optimal soybean production and therefore higher net benefit. In fact, the change from 0 to 30 kg P ha^{-1} gave smaller increases in net benefit than those obtained by changing from 0 to 15 kg P ha^{-1} for

treatments with *G. mosseae* (GH ¢152 ha^{-1}) and for treatments with *R. irregularis* (GH ¢445 ha^{-1}), except for the uninoculated control which was highest (GH ¢1250 ha^{-1}).

Although the net benefit obtained in uninoculated control for the change from 0 to 30 kg P ha^{-1} was greater than that obtained in the transition from 0 to 15 kg P ha^{-1} for inoculated soybean plants, it is only the initial step to calculate the marginal rates of return which is the actual index to determine the profitability.

These results generally indicate that in addition of being detrimental to microbial activity, most especially the AMF as reported by several studies (Johnson *et al.*, 1997; Fernández *et al.,* 2011; Balzergue *et al.*, 2013; Berruti *et al.*, 2014), the addition of 30 kg P ha^{-1} is a waste of non-renewable resource in the presence of AMF inoculation. This supports the assertion by Drevon *et al.* (2015) that because soil chemistry converts the phosphatic fertilizer into less available forms, high phosphate fertilizer is inefficiently applied even in high-input systems where P deficiency is also a limitation. Furthermore, the excessive application of chemical P fertilizers, by disturbing microbial diversity, lead to the loss of soil fertility and as a consequence reduces yield of crops (Gyaneshwar *et al.*, 2002; Sharma *et al.*, 2013). In addition, Veneklaas *et al.* (2012) asserted that to improve farm economies and increase the nutritional value of grains, a greater effort to improve the efficiency of phosphorus use for crop production and to reduce P waste is needed.

5.2.9.2 Marginal rates of return

Tables 4.25 and 4.26 indicated that 30 kg P ha^{-1} had the highest variable costs during the minor and the major cropping seasons; and that the marginal net benefit obtained for the change from 0 to 15 kg P ha^{-1} was higher than that obtained for the change from 15 to 30 kg P ha^{-1} for all treatments during the minor cropping season. During the major

cropping season, the marginal net benefit obtained for the change from 0 to 15 kg P ha^{-1} was highest for treatments inoculated with AMF. In addition, the average of marginal rates of return of treatments with 15 kg P ha^{-1} (590%) were higher than 30 kg P ha^{-1} (-591%) over the two cropping seasons of 2016 and 2017.

For inoculated soybean plants with TSP applied at 15 kg P ha^{-1}, it meant that each GH ¢100 invested on average per hectare is recovered with a supplement of GH ¢590. In contrast, the negative sign observed on the marginal rate of return of the treatments with 30 kg P ha^{-1} represents the drop of this rate in relation to that of the treatments with 15 kg P ha^{-1}. It can be deduced that the addition of 15 kg P ha^{-1} combined with mycorrhizal inoculation is more profitable.

5.3 Residual effects after two seasons of inoculation with arbuscular mycorrhizal fungi on maize crop

5.3.1 Mycorrhizal inoculation and P fertilizer on maize root length colonization

After two successive years of inoculation with AMF under soybean cropping in the Haplic Acrisol, RLC by AMF ranged from 4.64 to 38.24% and was significantly higher relative to the control plots at 30, 50 and 70 DAS (Table 4.27). On average, the highest increase in RLC was observed in plots previously inoculated with *G. mosseae*. Osunde *et al.* (2003) reported that consecutive maize crops grown after soybean had significantly higher AMF colonization in the inoculated and N-treated soybean plots than the uninoculated and fallow plots.

The RLC of maize significantly increased in plots previously fertilized with 15 kg P ha^{-1} and decreased in 30 kg P ha^{-1} compared with 0 kg P ha^{-1} (Table 4.27). This could be explained by the persistence of the mycorrhizal isolates inoculated the previous cropping seasons which resulted in the establishment of stable colonies. These findings

are consistent with those of Pellegrino *et al.* (2011) who observed under field experiment in sandy loam that *G. mosseae* and *G. intraradices* inocula were still infective in maize roots and effective after two years following *Trifolium alexandrinum* cropping.

According to Berruti *et al.* (2016), if no detrimental practices are done after the first inoculation and before cultivation, the biodiverse AMF hyphal network will remain infective and unaltered in the future. During this research, only conservation tillage using hoe was carried out to remove the invasive weeds since the experimental field was inoculated. Similar observations were made by Pellegrino *et al.* (2012) who established the persistence of at least one of two *Funneliformis mosseae* (*G. mosseae*) isolates up to two years post inoculation.

5.3.2 Mycorrhizal inoculation and P fertilizer on maize nitrogen and phosphorus uptake

The increase in maize shoot N and P uptake was significantly ($P < 0.001$) highest in plots previously inoculated with *G. mosseae* and unexpectedly lowest in treatments with *R. irregularis* (Table 4.28). This could be attributed to the competition between introduced isolates or/and indigenous AMF which can lead to unpredictable results as indicated by Malusà *et al.* (2016). Maize grain P uptake in plots previously inoculated with AMF significantly increased in relation to uninoculated control plots, but grain N uptake did not significantly increase. This is likely related to the fact that a major proportion of Pi taken from the soil is accumulated in grains as phytase as reported by Richardson (1994). The plots with the isolate *G. mosseae* resulted in a significantly higher increase in grain P uptake than plots with the isolate *R. irregularis*.

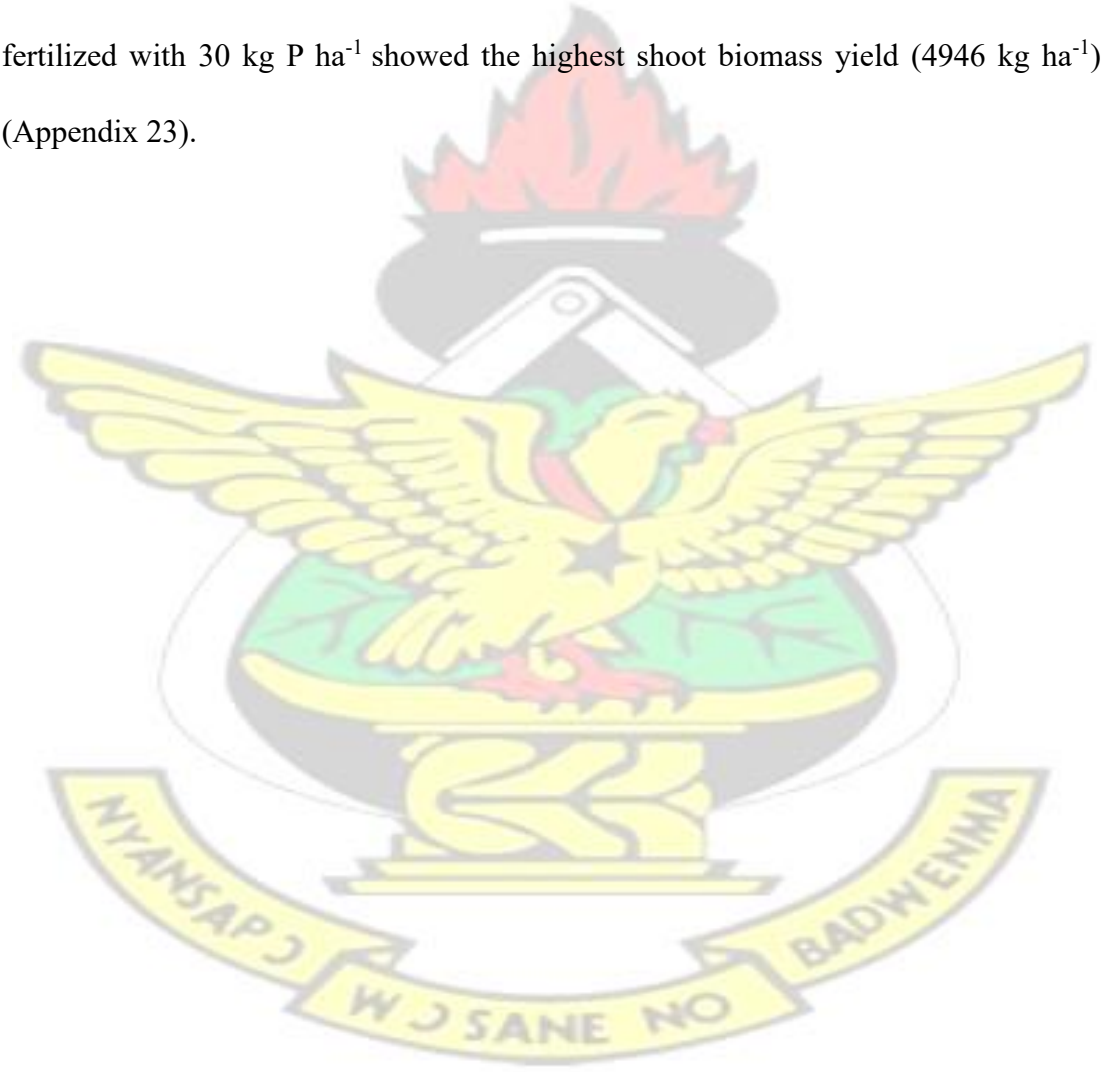
The highest shoot N and P uptake and grain N uptake were recorded in plots previously supplied with 30 kg P ha^{-1} followed by the control treatments which was unexpected. However, the significantly highest grain P uptake was observed in plots previously supplied with 15 kg P ha^{-1} (Table 4.28). This result corroborates the observations made on the PUE (Table 4.12) and confirms the assertion by Veneklaas *et al.* (2012) that crops growing in soils where no or little phosphate fertilizer is applied and with very low phosphorus content are expected to yield the largest benefits of improved PUE.

The significant interaction between phosphorus rate and AMF isolate for shoot N and shoot P uptake indicated that plots previously inoculated with *G. mosseae* and supplied with 30 kg P ha^{-1} had the highest shoot N and P uptake (Appendix 22). This corroborates the findings of Gosling *et al.* (2013) who indicated that high soil phosphorus supply is not always detrimental to AMF diversity under field conditions.

5.3.3 Mycorrhizal inoculation and P fertilizer on maize shoot biomass and grain yields

The data in Table 4.29 indicate that maize shoot biomass yield on the Haplic Acrisol was highest on plots previously inoculated with *G. mosseae* while plots previously inoculated with *R. irregularis* showed the lowest shoot biomass yield in relation to uninoculated control. This follows the same trend observed with the shoot N and P uptake and supports the assertion of Malusà *et al.* (2016) regarding the unpredictability of results after inoculation under field conditions. Plots previously fertilized with 30 kg P ha^{-1} gave a significantly greater maize shoot biomass yield over the control treatment whilst plots with 15 kg P ha^{-1} did not significantly differ with the control treatment in terms of shoot biomass yield. This is in accordance with Ofori (2016) who observed an increase in soybean shoot dry weight yield by increasing the P fertilizer rate from 34.35 kg P_2O_5 ha^{-1} to 68.70 kg P_2O_5 ha^{-1}.

The mean grain yield was significantly highest (2847 kg ha^{-1}) in plots previously inoculated with *G. mosseae* with an increase of 12.53% over the control treatment compared to 2.92% increase from *R. irregularis* which was not significant over the control. The grain yields obtained with plots previously fertilized with 15 kg P ha^{-1} and 30 kg P ha^{-1} treatments were statistically similar. The significant interaction between phosphorus rates and AMF isolates for maize shoot biomass yield followed the same trend as that of shoot N and P uptake. Plots previously inoculated with *G. mosseae* and fertilized with 30 kg P ha^{-1} showed the highest shoot biomass yield (4946 kg ha^{-1}) (Appendix 23).



CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS 6.1

Summary

Using arbuscular mycorrhizal fungi to increase phosphorus dissolution and availability in soil is a promising technology to remedy the rising cost of inorganic P fertilizers and at the same time develop a more sustainable agriculture system for smallholder farmers in Ghana. Overall, this study aimed at evaluating the influence of some AMF isolates in enhancing the phosphorus fertilizer use efficiency in soybean and its residual benefit on maize as a succeeding crop.

A greenhouse experiment was conducted from May - June 2016 and from December 2016 - January 2017 using sterile river sand and a non-sterile soil to assess the symbiotic potential of four AMF isolates on soybean. Field experiments were conducted during the minor and major cropping seasons of 2016 to assess the influence of AMF inoculation and triple superphosphate application on root colonization, P use efficiency, plant height, stem girth, shoot biomass and grain yield of soybean. The residual effect of AMF inoculation and TSP fertilizer application on root colonization, N and P uptake, shoot biomass and grain yields of maize were also evaluated during the minor cropping season of 2017.

Inoculation with *G. mosseae* showed a greater soybean root length colonization than all the other isolates in the sterile river sand and non-sterile soil. Under field conditions, plants inoculated with AMF showed higher RLC than the uninoculated control. Inoculation with *G. mosseae* coupled with the application of 15 kg P ha^{-1} resulted in the highest root length colonization of soybean. The highest mycorrhizal inoculation effect was obtained with *G. mosseae* plus 15 kg P ha^{-1}.

Rhizophagus irregularis supported the highest P uptake followed by *G. mosseae* in the sterile river sand and non-sterile soil. Under field conditions, inoculation with *G. mosseae* resulted in a significantly greater increase in grain P uptake over the control while inoculation with *R. irregularis* gave the greatest shoot P uptake. However, the interaction between soybean lines, TSP fertilizer and AMF isolates showed that the highest grain P uptake was obtained following inoculation of *G. mosseae* with 15 kg P ha^{-1} fertilization during the two cropping seasons.

In the greenhouse experiment, all AMF isolates produced plants taller than the uninoculated control treatment. Plants were tallest with *R. irregularis* in the sterile river sand and the non-sterile soil followed by *G. mosseae*. For stem girth, there were no significant differences among the different isolates, but each effect was greater than the uninoculated control. In the non-sterile soil, however, stem girth was greater in *G. mosseae* than all the other treatments. Treatment effect of *R. irregularis* was also greater than those of *G. etunicatum* and the control treatment. Shoot biomass yield was highest in *R. irregularis* in the sterile river sand and the non-sterile soil. In the field experiment, the AMF inoculation increased soybean plant height and stem girth over the uninoculated control. The difference between the two mycorrhizal isolates (*G. mosseae* and *R. irregularis*) was significant only at 40 DAS, where *G. mosseae* outperformed *R. irregularis*. The interaction between the soybean lines, phosphorus rates and AMF isolates showed that TGx 1989-48 FN inoculated with *G. mosseae* and fertilized with 15 kg P ha^{-1} produced the highest soybean shoot biomass yield, grain yield and phosphorus use efficiency. Mycorrhizal inoculation had no significant (P = 0.307) effect on microbial biomass P at 50% flowering, but inoculation with *G. mosseae* reduced it at harvest compared to the uninoculated control treatment. Soil available P at pre-planting, at 50% flowering and at harvest increased with AMF

inoculation over the uninoculated control. *G. mosseae* showed the greatest performance in enhancing P dissolution and availability.

The marginal rate of return for 15 kg P ha^{-1} was higher than 30 kg P ha^{-1}. It can be deduced that inoculation with AMF combined with the application of 15 kg P ha^{-1} is economically more profitable for soybean production, under the conditions of this study.

After two successive cropping seasons of inoculation with AMF on soybean, maize root length colonization by AMF was higher in previously inoculated plots relative to the control plots during the minor cropping season of 2017. Plots previously inoculated with *G. mosseae* and fertilized with 15 kg P ha^{-1} showed the highest RLC. Furthermore, shoot N uptake increased in plots previously inoculated with *G. mosseae* relative to the control treatment. However, plots previously inoculated with either *G. mosseae* or *R. irregularis* increased maize grain P uptake but not grain N uptake compared to uninoculated control plots. Similarly, shoot biomass and grain yields significantly increased only in plots previously inoculated with *G. mosseae* over the control.

6.2 Conclusions

The analyses and interpretation of data on the parameters used in evaluating the growth and yield of soybean as influenced by arbuscular mycorrhizal inoculation and triple superphosphate application, as well as, the residual effect of the treatments on succeeding maize crops led to the following conclusions:

i. *Rhizophagus irregularis* and *G. mosseae* were the most promising in growth improvement and phosphorus uptake of soybean relative to *G. etunicatum* and *G. fasciculatum*.

ii. inoculation with *G. mosseae* combined with 15 kg P ha^{-1} increased P use efficiency and shoot and grain P uptake, plant height, shoot biomass and grain yield values which were statistically at par with 30 kg P ha^{-1}.

iii. mycorrhizal inoculation combined with 15 kg P ha^{-1} increased plant available phosphorus and reduced microbial biomass P with *G. mosseae* being generally more effective than *R. irregularis*.

iv. inoculation with *R. irregularis* or *G. mosseae* combined with the application of 15 kg P ha^{-1} was economically more profitable than 30 kg P ha^{-1} with higher net benefit and marginal rates of return.

v. the top 50% mycorrhizal isolates (*G. mosseae* and *R. irregularis*) persisted after two years of inoculation. Maize root length colonization was higher in plots previously inoculated with *G. mosseae*, which consequently resulted in the highest maize shoot biomass N and P uptake, grain P uptake, shoot biomass and grain yields.

6.3 Recommendations

The findings of this study have demonstrated the potential of field inoculation with compatible and effective arbuscular mycorrhizal isolates in increasing phosphorus availability and use efficiency in soybean as well as shoot biomass and yield and succeeding maize crop with reduced phosphorus input. This study was limited to the assessment of the effect of the treatments on the phosphorus uptake and its impact on growth and yield of soybean as well as the residual effects on the succeeding maize crop. It is therefore recommended that further field studies should be undertaken to;

i. monitor the mycorrhizal community composition before and after inoculation in order to follow the evolution of species inoculated to the root system,

ii. conduct multi-location and large-scale trials and conduct economic analyses which will be a significant step towards the stable use of mycorrhizal inoculation in agricultural soils.

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APPENDICES

Appendix 1. Arbuscular mycorrhizal root colonization determination

Data record using the magnified intersection method

Root sample: _____ Data: _____

Slide=Microscope slide

N=Negative (crosshair did not cut through any hyphae nor arbuscules nor vesicles)

A= Arbuscules (crosshair cut through at least one arbuscule)

V=Vesicles (crosshair cut through at least one vesicle)

H=Hyphae only (crosshair cut through at least one hypha, but no arbuscules nor vesicles)

Slide	N	A	V	H		Slide	N	A	V	H	

Magnified intersections method (McGonigle *et al.*, 1990).

Appendix 2. Interactive effects of soybean and AMF on RLC under greenhouse conditions in sterile river sand and non-sterile soil

Soybean lines × AMF isolates	Root length colonization (%)	
	Sterile river sand	Non-sterile soil
Line TGx 1989-48 FN		
TGx 1989-48 FN × *Glomus mosseae*	89.90	35.90
TGx 1989-48 FN × *Rhizophagus irregularis*	76.82	15.40
TGx 1989-48 FN × *Glomus etunicatum*	69.72	14.20
TGx 1989-48 FN × *Glomus fasciculatum*	67.86	12.10
TGx 1989-48 FN × Control	0.00	0.00
Line TGx 1989-75 FN		
TGx 1989-75 FN × *Glomus mosseae*	77.77	30.20
TGx 1989-75 FN × *Rhizophagus irregularis*	70.88	24.70
TGx 1989-75 FN × *Glomus etunicatum*	64.30	28.80
TGx 1989-75 FN × *Glomus fasciculatum*	83.51	22.40
F pr.		< 0.001
LSD (5%)	2.99	2.89
CV (%)	0.7	5.7
	0.00	
TGx 1989-75 FN × Control	< 0.001	0.00

Appendix . Interactive effects of 3 soybean and AMF on relative mycorrhizal dependency under greenhouse conditions in sterile river sand and non-sterile soil

Soybean lines × AMF isolates	Relative mycorrhizal dependency (%)	
	Sterile river sand	Non-sterile soil
Line TGx 1989-48 FN		
TGx 1989-48 FN × *Glomus etunicatum*	92	104
TGx 1989-48 FN × *Glomus fasciculatum*	96	97
TGx 1989-48 FN × *Glomus mosseae*	113	111
TGx 1989-48 FN × *Rhizophagus irregularis*	119	109
Line TGx 1989-75 FN		
TGx 1989-75 FN × *Glomus etunicatum*	94	101
TGx 1989-75 FN × *Glomus fasciculatum*	95	88
TGx 1989-75 FN × *Glomus mosseae*	107	114
TGx 1989-75 FN × *Rhizophagus irregularis*	109	128
F pr.	< 0.001	< 0.001
LSD (5%)	3.60	9.90
CV (%)	1.3	2.3

Appendix 4. Interactive effects of soybean and AMF on shoot phosphorus uptake under greenhouse conditions in sterile river sand

AMF isolates	Shoot phosphorus uptake (kg ha^{-1})	
	Line TGx 1989-48 FN	Line TGx 1989-75 FN
Glomus etunicatum	2.01	1.47
Glomus fasciculatum	1.53	1.71
Glomus mosseae	2.20	1.98
Rhizophagus irregularis	2.74	2.11
Control	1.45	1.56

F pr. = 0.043, LSD (5%) = 0.43, CV (%) = 11.6.

Appendix 5. Interactive effects of soybean, TSP and AMF on soybean root length colonization under field conditions during the minor cropping season of 2016

P rates × AMF isolates	Root length colonization (%)

Appendix . Interactive effects of

	Line TGx 1989-48 FN	Line TGx 1989-75 FN
15 kg P ha^{-1} × *G. mosseae*	23.02	6.16
15 kg P ha^{-1} × *R. irregularis*	21.14	9.94
15 kg P ha^{-1} × Control	12.36	2.64
30 kg P ha^{-1} × *G. mosseae*	13.38	4.90
30 kg P ha^{-1} × *R. irregularis*	12.48	6.11
30 kg P ha^{-1} × Control	4.57	4.27
0 kg P ha^{-1} × *G. mosseae*	38.94	6.43
0 kg P ha^{-1} × *R. irregularis*	13.16	8.50
0 kg P ha^{-1} × Control	9.03	1.66

F pr. < 0.001, LSD (5%) = 3.96, CV (%) = 1.3. *G.* = *Glomus*, *R.* = *Rhizophagus*.

6 soybean, TSP and AMF on soybean root length colonization under field conditions during the major cropping season of 2017

P rates × AMF isolates	Root length colonization (%)	
	Line TGx 1989-48 FN	Line TGx 1989-75 FN
15 kg P ha^{-1} × *G. mosseae*	50.67	26.50
15 kg P ha^{-1} × *R. irregularis*	40.10	39.30
15 kg P ha^{-1} × Control	21.20	15.80
30 kg P ha^{-1} × *G. mosseae*	25.90	23.80
30 kg P ha^{-1} × *R. irregularis*	32.10	17.10
30 kg P ha^{-1} × Control	0.00	0.00
0 kg P ha^{-1} × *G. mosseae*	44.90	18.30
0 kg P ha^{-1} × *R. irregularis*	38.00	21.20
0 kg P ha^{-1} × Control	15.80	1.50

F pr. < 0.001, LSD (5%) = 5.10, CV (%) = 3.0. *G.* = *Glomus*, *R.* = *Rhizophagus*.

Appendix 7. Interactive effects of soybean, TSP and AMF on mycorrhizal inoculation effect under field conditions during the minor cropping season of 2016

P rates × AMF isolates	Mycorrhizal inoculation effect (%)	
	Line TGx 1989-48 FN	Line TGx 1989-75 FN
15 kg P ha^{-1} × *G. mosseae*	43	31
15 kg P ha^{-1} × *R. irregularis*	41	22
30 kg P ha^{-1} × *G. mosseae*	-7	24
30 kg P ha^{-1} × *R. irregularis*	-18	-5
0 kg P ha^{-1} × *G. mosseae*	57	17
0 kg P ha^{-1} × *R. irregularis*	54	3

F pr. < 0.001, LSD (5%) = 5.90, CV (%) = 7.7. *G.* = *Glomus*, *R.* = *Rhizophagus*.

Appendix . Interactive effects of

Appendix 8. Interactive effects of soybean, TSP and AMF on mycorrhizal inoculation effect under field conditions during the major cropping season of 2017

P rates × AMF isolates	Mycorrhizal inoculation effect (%)	
	Line TGx 1989-48 FN	Line TGx 1989-75 FN
15 kg P ha^{-1} × *G. mosseae*	16	38
15 kg P ha^{-1} × *R. irregularis*	10	30
30 kg P ha^{-1} × *G. mosseae*	24	24
30 kg P ha^{-1} × *R. irregularis*	14	12
0 kg P ha^{-1} × *G. mosseae*	32	14
0 kg P ha^{-1} × *R. irregularis*	7	24

F pr. < 0.001, LSD (5%) = 4.20, CV (%) = 3.5. *G.* = *Glomus*, *R.* = *Rhizophagus*.

9 soybean, TSP and AMF on P uptake in soybean grain and shoot under field conditions during the minor cropping season of 2016

Soybean lines × P rates × AMF isolates	Phosphorus uptake (kg ha_{-1})	
	Grain	Shoot
TGx 1989-48 FN × 15 kg P ha^{-1} × *G. mosseae*	18.56	18.97
TGx 1989-48 FN × 15 kg P ha^{-1} × *R. irregularis*	16.24	19.97
TGx 1989-48 FN × 15 kg P ha^{-1} × Control	8.00	14.94
TGx 1989-48 FN × 30 kg P ha^{-1} × *G. mosseae*	9.44	13.46
TGx 1989-48 FN × 30 kg P ha^{-1} × *R. irregularis*	9.37	16.27
TGx 1989-48 FN × 30 kg P ha^{-1} × Control	10.18	17.40
TGx 1989-48 FN × 0 kg P ha^{-1} × *G. mosseae*	10.31	13.69
TGx 1989-48 FN × 0 kg P ha^{-1} × *R. irregularis*	9.34	13.67
TGx 1989-48 FN × 0 kg P ha^{-1} × Control	3.79	6.72
TGx 1989-75 FN × 15 kg P ha^{-1} × *G. mosseae*	11.57	19.43
TGx 1989-75 FN × 15 kg P ha^{-1} × *R.irregularis*	10.25	17.17
TGx 1989-75 FN × 15 kg P ha^{-1} × Control	11.26	17.47
TGx 1989-75 FN × 30 kg P ha^{-1} × *G. mosseae*	16.37	14.42
TGx 1989-75 FN × 30 kg P ha^{-1} × *R.irregularis*	11.01	15.43
TGx 1989-75 FN × 30 kg P ha^{-1} × Control	13.56	20.08
TGx 1989-75 FN × 0 kg P ha^{-1} × *G. mosseae*	9.56	15.75
TGx 1989-75 FN × 0 kg P ha^{-1} × *R.irregularis*	7.55	13.54
TGx 1989-75 FN × 0 kg P ha^{-1} × Control	8.52	15.88
F pr.	< 0.001	0.029
LSD (5%)	1.65	1.86
CV (%)	3.3	1.8

G. = *Glomus*, *R.* = *Rhizophagus*.

Appendix . Interactive effects of

Appendix 10. Interactive effects of soybean, TSP and AMF on P uptake in soybean grain under field conditions during the major cropping season of 2017

P rates × AMF isolates	Grain P uptake (kg ha^{-1})	
	Line TGx 1989-48 FN	Line TGx 1989-75 FN
15 kg P ha^{-1} × *G. mosseae*	22.06	20.68
15 kg P ha^{-1} × *R. irregularis*	18.81	18.19
15 kg P ha^{-1} × Control	15.30	9.05
30 kg P ha^{-1} × *G. mosseae*	18.59	18.35
30 kg P ha^{-1} × *R. irregularis*	17.67	19.80
30 kg P ha^{-1} × Control	14.45	19.45
0 kg P ha^{-1} × *G. mosseae*	16.49	15.86
0 kg P ha^{-1} × *R. irregularis*	12.86	15.40
0 kg P ha^{-1} × Control	8.10	8.18

F pr. = 0.040, LSD (5%) = 2.98, CV (%) = 5.1. *G.* = *Glomus*, *R.* = *Rhizophagus*.

Appendix 11. Interactive effects of soybean, TSP and AMF on PUE under field conditions during the minor and major cropping seasons

Soybean lines × P rates × AMF isolates	PUE (kg kg^{-1})	
	Minor 2016	Major 2017
TGx 1989-48 FN × 15 kg P ha^{-1} × *G. mosseae*	63	61
TGx 1989-48 FN × 15 kg P ha^{-1} × *R. irregularis*	59	48
TGx 1989-48 FN × 15 kg P ha^{-1} × Control	37	34
TGx 1989-48 FN × 30 kg P ha^{-1} × *G. mosseae*	37	36
TGx 1989-48 FN × 30 kg P ha^{-1} × *R. irregularis*	33	30
TGx 1989-48 FN × 30 kg P ha^{-1} × Control	29	27
TGx 1989-75 FN × 15 kg P ha^{-1} × *G. mosseae*	37	26
TGx 1989-75 FN × 15 kg P ha^{-1} × *R. irregularis*	34	31
TGx 1989-75 FN × 15 kg P ha^{-1} × Control	29	20
TGx 1989-75 FN × 30 kg P ha^{-1} × *G. mosseae*	22	22
TGx 1989-75 FN × 30 kg P ha^{-1} × *R. irregularis*	22	20
TGx 1989-75 FN × 30 kg P ha^{-1} × Control	20	17
F pr.	0.014	0.013
LSD (5%)	6.8	5.2
CV (%)	6.6	1.7

Appendix 12. Interactive effects of soybean, TSP and AMF on microbial biomass P under field conditions during the major cropping season 2017

Soybean lines × P rates × AMF isolates	Microbial biomass phosphorus (mg kg$_{-1}$)	
	50% Flowering	Harvest
TGx 1989-48 FN × 15 kg P ha^{-1} × *G. mosseae*	37.03	38.32
TGx 1989-48 FN × 15 kg P ha^{-1} × *R. irregularis*	29.62	29.22
TGx 1989-48 FN × 15 kg P ha^{-1} × Control	18.22	21.36
TGx 1989-48 FN × 30 kg P ha^{-1} × *G. mosseae*	15.66	18.85
TGx 1989-48 FN × 30 kg P ha^{-1} × *R. irregularis*	33.86	29.42
TGx 1989-48 FN × 30 kg P ha^{-1} × Control	51.01	53.35
TGx 1989-48 FN × 0 kg P ha^{-1} × *G. mosseae*	15.55	36.37
TGx 1989-48 FN × 0 kg P ha^{-1} × *R. irregularis*	22.50	35.16
TGx 1989-48 FN × 0 kg P ha^{-1} × Control	14.86	27.82

Appendix . Interactive effects of soybean, TSP and AMF

TGx 1989-75 FN × 15 kg P ha^{-1} × *G. mosseae*	23.45	21.82
TGx 1989-75 FN × 15 kg P ha^{-1} × *R. irregularis*	24.90	44.79
TGx 1989-75 FN × 15 kg P ha^{-1} × Control	15.10	34.14
TGx 1989-75 FN × 30 kg P ha^{-1} × *G. mosseae*	27.82	24.21
TGx 1989-75 FN × 30 kg P ha^{-1} × *R. irregularis*	30.80	53.15
TGx 1989-75 FN × 30 kg P ha^{-1} × Control	39.77	38.97
TGx 1989-75 FN × 0 kg P ha^{-1} × *G. mosseae*	23.68	27.67
TGx 1989-75 FN × 0 kg P ha^{-1} × *R. irregularis*	15.60	19.50
TGx 1989-75 FN × 0 kg P ha^{-1} × Control	14.06	23.55
F pr.	0.001	< 0.001
LSD (5%)	7.72	8.13
CV (%)	2.6	5.3

G. = *Glomus*, *R.* = *Rhizophagus*.

13 and on available P under field conditions during the major cropping season 2017

P rates × AMF isolates	Available phosphorus at planting (mg kg^{-1})	
	Line TGx 1989-48 FN	Line TGx 1989-75 FN
15 kg P ha^{-1} × *G. mosseae*	86.75	90.73
15 kg P ha^{-1} × *R. irregularis*	80.81	83.02
15 kg P ha^{-1} × Control	65.56	69.22
30 kg P ha^{-1} × *G. mosseae*	84.40	103.76
30 kg P ha^{-1} × *R. irregularis*	79.33	71.25
30 kg P ha^{-1} × Control	70.49	76.52
0 kg P ha^{-1} × *G. mosseae*	63.90	86.18
0 kg P ha^{-1} × *R. irregularis*	64.35	88.78
0 kg P ha^{-1} × Control	56.82	62.37

F pr. < 0.001, LSD (5%) = 14.51, CV (%) = 3.6. *G.* = *Glomus*, *R.* = *Rhizophagus*.

Appendix 14. Interactive effects of soybean and AMF on plant height at 40 DAS under field conditions during the minor cropping season of 2016

AMF isolates	Plant height at 40 DAS (cm)	
	Line TGx 1989-48 FN	Line TGx 1989-75 FN
Glomus mosseae	52.60	47.30
Rhizophagus irregularis	47.00	50.40
Control	42.00	44.30

F pr. = 0.002, LSD (5%) = 3.91, CV (%) = 2.9.

Appendix . Interactive effects of soybean, TSP AMF

Appendix 15. Interactive effects of TSP and AMF on plant height at 40 DAS under field conditions during the minor cropping season of 2016

AMF isolates	Plant height at 40 DAS (cm)		
	15 kg P ha^{-1}	30 kg P ha^{-1}	0 kg P ha^{-1}
Glomus mosseae	54.50	44.80	50.50
Rhizophagus irregularis	47.50	49.90	48.80
Control	44.90	45.20	39.20

F pr. < 0.001, LSD (5%) = 4.84, CV (%) = 6.2.

16 on plant height at 40 DAS under field conditions during the major cropping season of 2017

P rates × AMF isolates	Plant height at 40 DAS (cm)	
	Line TGx 1989-48 FN	Line TGx 1989-75 FN
15 kg P ha^{-1} × *G. mosseae*	35.00	31.00
15 kg P ha^{-1} × *R. irregularis*	29.30	33.30
15 kg P ha^{-1} × Control	33.80	28.10
30 kg P ha^{-1} × *G. mosseae*	33.20	32.50
30 kg P ha^{-1} × *R. irregularis*	30.30	31.60
30 kg P ha^{-1} × Control	27.40	31.90
0 kg P ha^{-1} × *G. mosseae*	27.60	32.70
0 kg P ha^{-1} × *R. irregularis*	31.60	34.90
0 kg P ha^{-1} × Control	28.90	20.20

F pr. < 0.001, LSD (5%) = 7.42, CV (%) = 14.7.

Appendix 17. Interactive effects soybean of and TSP on stem girth at 40 DAS under field conditions during the minor cropping season of 2016

AMF isolates	Stem girth at 40 DAS (cm)

Appendix . Interactive effects of soybean, TSP and AMF

	15 kg P ha^{-1}	30 kg P ha^{-1}	0 kg P ha^{-1}
TGx 1989-48 FN TGx	5.09	5.83	5.74
1989-75 FN	5.73	5.41	5.34

F pr. = 0.004, LSD (5%) = 0.82, CV (%) = 3.8.

Appendix . Interactive effects of soybean, TSP and AMF 18 on stem girth under field conditions during the major cropping season of 2017

Soybean lines × P rates × AMF isolates	Stem girth (mm)	
	40 DAS	80 DAS
TGx 1989-48 FN × 15 kg P ha^{-1} × Gm	7.94	13.35
TGx 1989-48 FN × 15 kg P ha^{-1} × Ri	6.92	14.10
TGx 1989-48 FN × 15 kg P ha^{-1} × Control	7.32	11.68
TGx 1989-48 FN × 30 kg P ha^{-1} × Gm	7.68	14.26
TGx 1989-48 FN × 30 kg P ha^{-1} × Ri	7.78	13.96
TGx 1989-48 FN × 30 kg P ha^{-1} × Control	6.45	13.81
TGx 1989-48 FN × 0 kg P ha^{-1} × Gm	7.47	13.83
TGx 1989-48 FN × 0 kg P ha^{-1} × Ri	8.01	13.97
TGx 1989-48 FN × 0 kg P ha^{-1} × Control	5.63	12.08
TGx 1989-75 FN × 15 kg P ha^{-1} × Gm	6.25	14.72
TGx 1989-75 FN × 15 kg P ha^{-1} × Ri	6.61	14.43
TGx 1989-75 FN × 15 kg P ha^{-1} × Control	6.42	14.28
TGx 1989-75 FN × 30 kg P ha^{-1} × Gm	6.96	14.22
TGx 1989-75 FN × 30 kg P ha^{-1} × Ri	6.29	15.43
TGx 1989-75 FN × 30 kg P ha^{-1} × Control	5.91	14.38
TGx 1989-75 FN × 0 kg P ha^{-1} × Gm	6.76	14.55
TGx 1989-75 FN × 0 kg P ha^{-1} × Ri	6.23	13.39
TGx 1989-75 FN × 0 kg P ha^{-1} × Control	5.82	13.08
F pr.	0.043	0.014
LSD (5%)	0.93	1.41
CV (%)	1.5	1.7

Gm = *Glomus mosseae*, Ri = *Rhizophagus irregularis*.

Appendix 19. Interactive effects of soybean, TSP and AMF on shoot biomass yield under field conditions during the major cropping season of 2017

P rates × AMF isolates	Shoot biomass yield (kg ha^{-1})

Appendix . Interactive effects of soybean, TSP and AMF

	Line TGx 1989-48 FN	Line TGx 1989-75 FN
15 kg P ha^{-1} × *G. mosseae*	4392	3993
15 kg P ha^{-1} × *R. irregularis*	3364	3523
15 kg P ha^{-1} × Control	2819	1286
30 kg P ha^{-1} × *G. mosseae*	4486	3677
30 kg P ha^{-1} × *R. irregularis*	3941	3274
30 kg P ha^{-1} × Control	2618	2166
0 kg P ha^{-1} × *G. mosseae*	3826	1463
0 kg P ha^{-1} × *R. irregularis*	1589	2491
0 kg P ha^{-1} × Control	1319	877

F pr. < 0.001, LSD (5%) = 690.60, CV (%) = 5.6.

20 on grain yield under field conditions during the minor and major cropping seasons

Soybean lines × P rates × AMF isolates	Grain yield (kg ha^{-1})	
	Minor 2016	Major 2017
TGx 1989-48 FN × 15 kg P ha^{-1} × Gm	2369	2679
TGx 1989-48 FN × 15 kg P ha^{-1} × Ri	2313	2440
TGx 1989-48 FN × 15 kg P ha^{-1} × Control	1048	1957
TGx 1989-48 FN × 30 kg P ha^{-1} × Gm	1285	2445
TGx 1989-48 FN × 30 kg P ha^{-1} × Ri	1235	2213
TGx 1989-48 FN × 30 kg P ha^{-1} × Control	1338	1853
TGx 1989-48 FN × 0 kg P ha^{-1} × Gm	1378	2164
TGx 1989-48 FN × 0 kg P ha^{-1} × Ri	1320	1716
TGx 1989-48 FN × 0 kg P ha^{-1} × Control	499	1042
TGx 1989-75 FN × 15 kg P ha^{-1} × Gm	1400	2487
TGx 1989-75 FN × 15 kg P ha^{-1} × Ri	1261	2350
TGx 1989-75 FN × 15 kg P ha^{-1} × Control	1354	988
TGx 1989-75 FN × 30 kg P ha^{-1} × Gm	1906	2210
TGx 1989-75 FN × 30 kg P ha^{-1} × Ri	1313	2434
TGx 1989-75 FN × 30 kg P ha^{-1} × Control	1628	2267
TGx 1989-75 FN × 0 kg P ha^{-1} × Gm	1244	2150
TGx 1989-75 FN × 0 kg P ha^{-1} × Ri	1045	2033
TGx 1989-75 FN × 0 kg P ha^{-1} × Control	1023	954
F pr.	< 0.001	< 0.001
LSD (5%)	110.90	249.5

Appendix

CV (%)	1.5	2.9

Gm = *Glomus mosseae*, Ri = *Rhizophagus irregularis*.

Appendix 21. Interactive residual effects of mycorrhizal inoculation and TSP on maize root length colonization by AMF

P rates × AMF isolates	Root length colonization (%)		
	30 DAS	50 DAS	70 DAS
15 kg P ha^{-1} × *G. mosseae*	20.98	55.11	53.59
15 kg P ha^{-1} × *R. irregularis*	41.32 1.86	44.26	30.27
15 kg P ha^{-1} × Control	19.92	23.45	12.59
30 kg P ha^{-1} × *G. mosseae*	9.56	31.17	19.82
30 kg P ha^{-1} × *R. irregularis*	0.00	13.81	19.76
30 kg P ha^{-1} × Control	22.24	20.04	17.86
0 kg P ha^{-1} × *G. mosseae*	20.48	15.82	41.30
0 kg P ha^{-1} × *R. irregularis*	12.10	36.04	22.73
0 kg P ha^{-1} × Control		13.98	8.06
F pr.	< 0.001	< 0.001	< 0.001
LSD (5%)	1.457	0.785	1.162
CV (%)	10.0	7.7	8.0

G. = Glomus, *R. = Rhizophagus*.

22. Interactive residual effects of mycorrhizal inoculation and TSP on nitrogen and phosphorus uptake in maize shoot and grain

P rates × AMF isolates	Shoot		Grain	
	N uptake	P uptake	N uptake	P uptake
	(kg ha^{-1})			
15 kg P ha^{-1} × *G. mosseae*	26.16	11.39	45.52	2.60
15 kg P ha^{-1} × *R. irregularis*	20.71	7.92	40.98	2.46
15 kg P ha^{-1} × Control	25.49	9.73	42.19	1.79
30 kg P ha^{-1} × *G. mosseae*	43.37	13.19 9.61	49.89	2.32
30 kg P ha^{-1} × *R. irregularis*	31.58	12.57	42.50	2.03
30 kg P ha^{-1} × Control	38.06	10.78	48.55	1.98
0 kg P ha^{-1} × *G. mosseae*	27.16	10.41	46.24	2.49
0 kg P ha^{-1} × *R. irregularis*	23.13	8.47	43.35	2.12
0 kg P ha^{-1} × Control	25.55		40.32	1.94

Appendix . Interactive effects of soybean, TSP and AMF

F pr.	0.025	< 0.001	0.534	0.102
LSD (5%)	2.643	0.912	NS	NS
CV (%)	0.3	0.7	3.8	27.1

NS = Not Significant at P ≤ 0.05.

Appendix 23. Interactive residual effects of mycorrhizal inoculation and TSP on maize shoot biomass and grain yields

P rates × AMF isolates	Shoot biomass yield (kg ha^{-1})	Grain yield
15 kg P ha^{-1} × *G. mosseae*	3497	2909
15 kg P ha^{-1} × *R. irregularis*	2994	2652
15 kg P ha^{-1} × Control	3672	2474
30 kg P ha^{-1} × *G. mosseae*	4946	2776
30 kg P ha^{-1} × *R. irregularis*	3751	2558
30 kg P ha^{-1} × Control	4646	2717
0 kg P ha^{-1} × *G. mosseae*	3723	2856
0 kg P ha^{-1} × *R. irregularis*	3260	2602
0 kg P ha^{-1} × Control	3199	2399
F pr.	0.009	0.284
LSD (5%)	325.9	NS
CV (%)	0.8	1.6

NS = Not Significant at P ≤ 0.05.

Appendix 24. Pearson's correlation matrix for mycorrhizal root length colonization and soybean parameters in sterile river sand

	RLC (%)	H 20 DAS (cm)	H 40 DAS (cm)	SD 20 DAS (mm)	SD 40 DAS (mm)	SPU (kg ha^{-1})	SBY (kg ha^{-1})
RLC (%)	-						
H 20 DAS (cm)	0.5935***	-					
H 40 DAS (cm)	0.4919**	0.5772***	-				
SD 20 DAS (mm)	0.5484***	0.4209**	0.2183	-			
SD 40 DAS (mm)	0.4743**	0.3843*	0.1646	0.6246***	-		
SPU (kg ha^{-1})	0.4290**	0.5457***	0.3351*	0.3751*	0.2961	-	
SBY (kg ha^{-1})	0.4427**	0.4012*	0.3874*	0.5672***	0.3834*	0.7758***	-

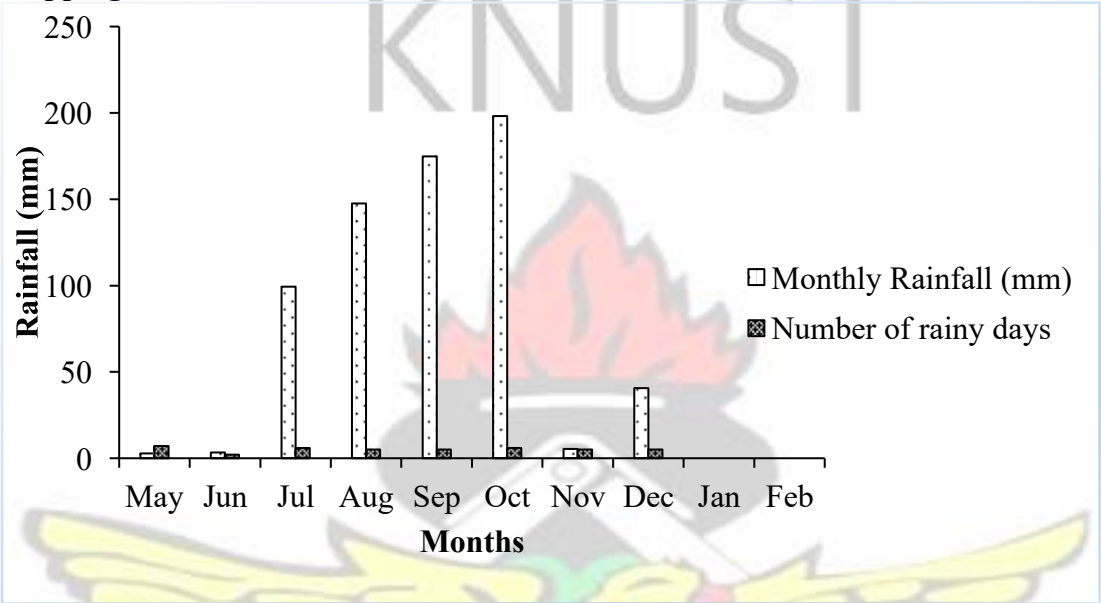
RLC = root length colonization, H = plant height, SD = Stem girth, SPU = shoot phosphorus uptake, SBY = Shoot biomass yield. Levels of significance: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Appendix 25. Pearson's correlation matrix for mycorrhizal root length colonization and soybean parameters in non-sterile soil

	RLC (%)	H 20 DAS (cm)	H 40 DAS (cm)	SD 20 DAS (mm)	SD 40 DAS (mm)	SPU (kg ha^{-1})	SBY (kg ha^{-1})
RLC (%)	-						
H 20 DAS (cm)	0.6733***	-					
H 40 DAS (cm)	0.6039***	0.6063***	-				
SD 20 DAS (mm)	0.6259***	0.6063***	0.4369**	-			
SD 40 DAS (mm)	0.6155***	0.6916***	0.5188***	0.7774***	-		
SPU (kg ha^{-1})	0.4649**	0.4604**	0.4708**	0.4641**	0.4224**	-	
SBY (kg ha^{-1})	0.3902*	0.4160**	0.4707**	0.4342**	0.4624**	0.7451***	-

RLC = root length colonization, H = plant height, SD = Stem girth, SPU = shoot phosphorus uptake, SBY = Shoot biomass yield. Levels of significance: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Appendix 26. Monthly rainfall distribution at the experiment site in minor cropping season of 2016



Appendix 27. Daily and cumulative rainfall distribution during growth period of

soybean in the minor cropping season of 2016