CELL BIOLOGY & DEVELOPMENT
Volume 4, Number 1, June 2020 E-ISSN: 2580-4499
Pages: 1-16 DOI: 10.13057/cellbioldev/v040101

Effects of mycorrhizal and *rhizobium* inoculation on soybean growth in acidic soils of Gatanga, Kenya

NAOMI NJERI KAMAU, JAMES B. KUNGU♥, DANIEL MUGENDI
Department of Environmental Sciences, Kenyatta University. Nairobi, Kenya. ♥email: kungu.james@ku.ac.ke, kungujames@gmail.com

Manuscript received: 21 July 2019. Revision accepted: 30 January 2020.

Abstract. *Kamau NN, Kungu JB, Mugendi D. 2020. Effects of mycorrhizal and* rhizobium *inoculation on soybean growth in acidic soils of Gatanga, Kenya. Cell Biol Dev 4: 1-16*. Central Kenya's farmers have found it challenging to appropriately conserve and replace soil nutrients due to small landholdings and poverty. The inevitable result has been soil erosion and nutrient leaching, resulting in soil acidity. The purpose of this study was to see how inoculating soybeans (*Glycine max* Merr.) with both *mycorrhiza* and *rhizobium* as a biological approach to enhancing soil fertility in acidic soils in Gatanga, Thika District, affected soil fertility. Field studies on sterilized and non-sterilized soils taken from Gatanga were conducted at Gatanga and Kenyatta University (on-station). The field studies used a complete randomized block design, whereas the on-station experiments used a complete randomized design. The Genestat for Windows Version 8.11 was used to analyze variance (ANOVA) on the data, with means separated using LSD at a 5% significance difference. As a result of the dual inoculation with *mycorrhiza* and *Rhizobium*, the growth parameters of height, root collar diameter, shoots, and root dry weight all increased. Higher nitrogen fixation by soybeans, as demonstrated by increased nodulation and grain yields, was also a result of dual inoculation. On the germination of soybeans, dual inoculation with mycorrhiza and *rhizobium* had no significant effect ($p<0.05$). In the long rains, the height of soybeans increased greatly over the control by 88 %, but in the short rains, the growth was not significant. In the on-station experiments, there was no significant difference in height between sterilized and non-sterilized soil. Dual inoculation improved root collar diameter in the long and short rains by 80% and 8.6%, respectively. Dual inoculation raised the dry weight of the shoots by 140 % in the on-farm long rains 2005 season, whereas the changes were not significant in the short rains season and on-station experiments. In the on-farm long rains 2005 season, dual inoculation improved grain yields by 356 %, while on-station experiments saw grain yields increase by 76 % and 107 % in sterilized and non-sterilized soils, respectively. Even though nodulation was poor in all the experiments, the number of nodules increased by 676 % over the control during the long rains of 2005. The control (S) had no nodules in the on-station experiments; maybe low precipitation caused the short rain crop to perform worse than the long rain. Finally, mycorrhiza and *rhizobium* biological organisms could boost the productivity of the legume soybean in acidic soils. However, technologies to make microorganisms available to farmers must be developed. The obligatory nature of mycorrhizal fungi makes cultivation and commercialization difficult, and the short shelf life of *rhizobium* at room temperature precludes its usage by resource-limited farmers.

Keywords: Acidic soils, growth performance, mycorrhiza, *rhizobium*, soybean

INTRODUCTION

Under continuous cultivation and in degraded soils, crop productivity in tropical countries is limited by the availability of one or more nutrients, with nitrogen and phosphorus being the most limiting (Giller 2002). Fertilization is thus required to boost crop yields. In Kenya, 7.5 million hectares (about one-third of the country) are acidic (Kanyanjua et al. 2002). These are deep, well-drained soils with low activity clay typical of an older environment with a lot of rain; because they are severely leached, they have a limited cation exchange capacity due to base leaching. The major clay fraction is kaolinite, which has a 1:1 silica:aluminum layer structure and has a lower negative charge than the 2:1 clay minerals (Giller 2002). When soils have been leached of other bases, aluminum could become the dominating cation if the original material has much of it. The proportion of cation exchange capacity occupied by cations that predominate in most soils, notably calcium, magnesium, and Potassium, can be as high as 80-90 %, and the base saturation, that is, the proportion of cation exchange capacity occupied by cations that predominate in most soils, is low (Giller 2002). For legume grain crop production, biological nitrogen fixation (BNF) by legumes provides an alternative and less expensive supply of nitrogen. Soil acidity, particularly in sub-humid countries, impedes nitrogen fixation by reducing phosphorus availability, limiting plant root development, and exacerbating the problem of insufficient nutrient input from the soil (Harter 2002). In acidic soils, a considerable part of applied phosphorus is bound to iron and aluminum oxides, rendering it unavailable to plants (Schroth et al. 2003).

In biological nitrogen fixation, phosphorus is essential for energy supply. Using arbuscular mycorrhiza symbiosis can solve the problem of plants' phosphorus (P) availability. First, AM (arbuscular mycorrhiza) inoculation boosts phosphorus uptake and nitrogen-fixing (Young 1997). Mycorrhiza is a fungus that feeds on carbohydrates from the host plant. In exchange, they boost nutrient extraction from the soil, causing the plant's root system to expand in size and surface area (Sieverding 1991). Next,

legume plants have a shallow root system and benefit substantially from this connection.

In deteriorated acidic soils, nitrogen availability to plants is also a limiting factor (Giller 2002). However, rhizobium, a soil bacteria, can create a symbiotic relationship with legumes. *Rhizobium* bacteria provide ammonia or amino acids to the plant through nitrogen fixation in exchange for organic acids as a carbon and energy source (Leigh 2002).

Most legumes simultaneously have symbiotic relationships with rhizobium and arbuscular mycorrhizae fungi (Barea et al. 2005). *Rhizobium* fixes nitrogen, which helps plants grow. The fungus helps the host by improving the efficiency of mineral and water uptake from the soil and changing host metabolisms and other physiological factors. Plant development is often significantly stronger in this tripartite interaction than when the plant is in a symbiotic relationship with just one bacteria (Arora et al. 1991). Inoculated legumes with mycorrhiza and rhizobia will reduce the requirement for mineral fertilizers, saving energy. It has been determined that the manufacture and use of nitrogen fertilizers absorb 50% of the total energy consumed in agricultural production in tropical regions (Chikowo 2004).

Plants with two mutualistic symbionts, such as *rhizobium* nodulated and mycorrhizal plants, are well adapted to environments with low nitrogen and phosphate availability (Bagyraj 1996). Unlike mineral fertilizers, both mycorrhiza and *Rhizobium* symbionts are likely to be self-replenishing once established. In highly cultivated areas, nitrates' presence in groundwater is a major health concern, as it can cause methemoglobinemia in babies, cancer, and respiratory sickness (Comly 1987). It is also a major source of pollution, resulting in issues such as eutrophication and stratospheric ozone depletion (Bohlool et al. 1992). BNF will provide a cost-effective and environmentally friendly method of crop production by minimizing external inputs while increasing internal inputs.

Glycine max is a legume that grows well in acidic soils; the general goal of this study was to investigate the effects of simultaneous inoculation with AM fungus and *rhizobium* on the growth performance of this legume. The research sought to determine (i) the effect of inoculating Glycine max with AM and *Rhizobium* bacteria on the establishment and germination of the plant in acidic soils, (ii) the effect of inoculating Glycine max with AM and *Rhizobium* bacteria on grain yield and biomass production in acidic soils, and (iii) the quantitative impact of inoculating Glycine max with AM and *Rhizobium* bacteria on nitrogen fixation in acidic soils.

MATERIALS AND METHODS

Site description

The on-farm experiment was carried out in the Gatanga division, Thika district, in the Central Province of Kenya. At an altitude of 1,680 meters above sea level, the region is located at 38º 58' 0" E and 0º 55' 59" S. The location is on the eastern slopes of the Nyandarua Ranges, and it receives 1,000 mm of rainfall yearly, which is split into two seasons that begin in mid-March and end in mid-October. The annual average temperature is 25ºC. According to FAO/UNESCO (1974), the soils are well-drained, extraordinarily deep dusky red friable clays with humic acid topsoil, characteristic humic Nitisols produced on tertiary basic igneous rocks. The soils are acidic and leached (Jaetzold and Schmidt 1983). The topography of the area is undulating and rolling. Gatanga has a population of 103,048 people, with a density of 410 people per square kilometer, according to the 1999 census. The average size of an agricultural holding is 0.25 hectares (Ministry of Agriculture, Thika district).

Kenyatta University is located in the Upper Midlands 4 district (UM4). It is located at 37º 10' 0" E, 0º 34' 0" S, and is 1650 meters above sea level. The annual average temperature is 25ºC. It is located in a semi-humid climatic zone, receiving an annual total of 750 mm of rainfall in two separate seasons: long rains (LR) from mid-March to June and short rains (SR) from mid-October to December.

Treatments and design of the study

The experiment took place on-farm in Gatanga as well as on-station at Kenyatta university. Soybean was used as a test crop (Glycine max). The studies in the on-farm study were designed using a complete randomized block design (CRBD). The plots were 7 m by 3.5 m in size and were divided into two blocks. The crop was cultivated for two seasons, 2005 long drops of rain and 2005 short drops of rain. Within the block, treatments included the following: (i) Soybeans inoculated with *Rhizobium* (S+R). (ii) Soybeans inoculated with both *rhizobium* and mycorrhiza (S+R+M). (iii) Soybeans inoculated with *Rhizobium* plus P fertilizer (S+P+R). (iv) Soybeans inoculated with mycorrhiza plus P fertilizers (S+P+M). (v) Soybeans inoculated with mycorrhiza (S+M). (vi) Soybeans Plus P fertilizers (S+P). (vii) Soybeans on their own (S) (Control).

Each treatment comprised a row of 25 plants, with each plant serving as a replication of the treatment. Planting occurred at the specified 45 x 15 cm spacing. Random sampling was used to decide the treatments assigned to the rows. A mixture of three AM species, *Glomus etunicatum*, *Glomus intraradices*, and *Gigaspora albida*, was used to inoculate the treatments S+R+M, S+P+M, and S+M by inserting 10 grams of it below the seeds in the planting hole. At a rate of 250 kg per hectare, triple super phosphate fertilizer containing 46 kg of P2O5 per 100 kg was given to the treatments S+P+R, S+P+M, and S+P. The *Rhizobium* inoculant for soybeans was obtained from the Kabete campus and applied at a rate of 50 grams per 15 kg of soybeans to the treatments S+R, S+R+M, and S+P+M. The control treatment consisted of soybeans grown in their natural state.

The on-station study employed a completely randomized design for the experiments (CRD). Each treatment comprised three 20 cm diameter half litter planting pots, one filled with sterilized soil and the other with non-sterilized soil gathered from Gatanga. For 48 hours, the soil was disinfected in an oven with hot air at 100°C. Seven treatments were used, the same as those used in on-farm

testing. Three times for each treatment, a total of 42 plots were created. Three soybeans seeds per pot were planted and then trimmed to one plant per pot. For four months, the plants were nurtured in a greenhouse. When germination occurred, plants were watered once daily for eight days and then once a week for the next three months.

Data collection

The pH, soil organic matter, accessible phosphorus, total nitrogen, exchangeable Potassium, magnesium, calcium, and cation exchange capacity of topsoil (0-20cm) obtained from Gatanga were all determined in a laboratory. At eight days, the germination percentage was assessed by counting the number of plants that had emerged. The height growth of the plants was determined every 15 days using a systematic sampling procedure by measuring the distance from the soil level to the growing apex of each plant, beginning one month after planting and ending at the onset of flowering, when plant height growth ended. The diameter of the root collar was measured three months after planting. Plants were picked and measured using a simple random sampling procedure with the help of vernier calipers. Five and one plants in the on-field and on-station studies were selected using destructive sampling with a hoe at the flowering and seed laying stages, respectively. Soil that had adhered to the root was rinsed away with a gentle trickle of tap water. After that, the nodules were separated and numbered. Four plants were randomly chosen at harvest, and their grain yields were determined following hand threshing. The identical plants' above- and below-ground biomass was dried in an oven at 50 °C until it reached a consistent weight.

Analyses of soils

Before planting, topsoil was collected from the Gatanga study site and evaluated for pH, organic matter content, total nitrogen, Potassium, and phosphorus. Five cores (diagonally and centrally) were sampled to a depth of 0-20 cm. After thoroughly mixing the soil and removing all visible plant debris to ensure homogeneity, it was wrapped in polythene sheets and sent to the University of Nairobi Kabete campus laboratory for analysis.

Soil pH was determined using a pH meter and a glass electrode in a 2.5:1 water-to-soil suspension ratio. Next, a dispenser was used to add 25 mL of deionized water to 10 mg of dirt obtained from Gatanga in a 60 mL bottle. After 10 minutes of stirring, the solution was allowed to stand for 20 minutes. Finally, a pH electrode was immersed after allowing the soil to settle, and readings were collected after pH stability.

Organic matter determination in soils

The Walkley-Black method (Okalebo et al. 2002) acquired soil organic matter. First, the organic carbon was oxidized using potassium dichromate in the presence of strong sulphuric acid. Next, to promote homogeneity and facilitate oxidation, a soil sample passed through a 2 mm sieve to remove the coarse fraction was ground to pass through a 0.5 mm sieve. Two (2) grams of this soil were placed in a conical flask, and 10 ml of 1 N potassium dichromate was added using a pipette and spun to oxidize the carbon in the soil, followed by the addition of 20 ml of concentrated sulphuric acid (36 N) in a constant stream. The heat of dilution produced by adding sulphuric acid provided a constant amount of heat to aid in the oxidation process. After that, the mixture was allowed to cool for 20 minutes. After adding 200ml of distilled water, 5.0 ml of 85 % orthophosphoric acid and 5.0 ml of diphenylamine sulphonate indicator were added.

Furthermore, the mixture was titrated with 0.1 N ferrous sulfates to decrease the residual dichromate. When the combination reached the endpoint, it changed color from turbid dark blue to light green. Carbon content in soil was determined using the following formula, which considers that 1 ml of dichromate oxidizes 0.39 mg of carbon (the average recovery rate of 77 % is taken into account).

$$\% \text{ Carbon} = \frac{(\text{m.e dichromate} - \text{m.e } FeSO4) \times 0.39}{\text{Weight of soil in grams}}$$

The amount of carbon obtained was then multiplied by 2 to obtain the percentage of soil organic matter (C forms an average of 58 % of soil organic matter).

Determination of available phosphorus

The Olsen technique was used to determine phosphorus levels (Okalebo et al. 2002). A polyethylene shaking container was filled with 2.5 grams of sieved soil and 50 ml Olsen extraction solution (0.5 M $NAHCO_3$ pH 8.5). The mixture was agitated for 5 minutes on a mechanical shaker before filtering through Whatman No 5 paper. Flasks were then filled with 10 ml of P standard solution, 10 ml of the sample, and 2 reagent blanks, with 5 ml of 0.8 boric acid added to each flask. After that, 10 mL of ascorbic acid reagent was added to each flask, followed by 50 mL of distilled water. The contents were sealed and thoroughly shaken. The absorbance of the solution was measured at a wavelength of 880 nm after one hour. The standard P calibration curve was then used to calculate the solution's parts per million (ppm). The phosphorus concentration in the sample was estimated as P mg kg^{-1} using the formula:

$$P = \frac{(a-b) \times v \times f \times 1000}{1000 \times w}$$

Where:

a = concentration of P in the sample b = concentration of P in the blank

v = volume of the extracting solution f = dilution factor

w = weight of the sample.

Determination of total nitrogen

Total nitrogen was quantified using a wet oxidation method based on Kjeldahl digestion with sulphuric acid and a catalyst (Anderson and Ingram 1993). First, a piece of the soil sample was crushed and sieved at 0.5 mm. Then, 0.3g of it was consumed and digested for 1 hour at 110ºC using a 2.5 ml digestion mixture (dissolved 3.2 g salicylic acid in 100 ml sulphuric acid – selenium mixture.) After

that, the solution was cooled, hydrogen peroxide was added, and the mixture was heated at 330ºC until colorless. Finally, 25 mL of distilled water was added until the sediments were completely dissolved. Total nitrogen was determined by calorimetry, with absorbency measured at 650nm and the following equation calculated:

$$\% \text{ N in soil sample} = \frac{(a - b) \times v \times 100}{1000 \times w \times al \times 1000}$$

Where:

a = Concentration of N in the solution b= Concentration of N in the blank

v = Total volume at the end of analysis procedure w = Weight of the dried soil sample taken

al = Aliquot of the solution taken for analysis.

Determination of exchangeable Potassium, sodium, calcium and magnesium, and cation exchange capacity

The exchangeable Potassium, sodium, calcium, and magnesium were removed by leaching the soil sample with neutral normal ammonium acetate. First, 5 grams of acid-washed sand was placed in numbered plastic funnels with an absorbent cotton wool stopper, followed by 5 grams of soil mixed with 5 ml sand. On top of the funnel, another layer of sand was added. Next, a funnel was filled with a Whatman filter paper No. 42 and inserted into the neck of a 250 ml flask. The funnel was then filled with a 10 aliquot of 20 mL ammonium acetate, which was allowed to drain through. The flask was then removed after being repeated several times. The ammonium ions (NH_4+) took the place of the soil's exchangeable cations. Titration with EDTA was used to quantify the exchangeable calcium and magnesium ions' presence in the ammonium acetate leachate, whereas flame photometry was used to determine Potassium and sodium.

The same soil and sand funnels were used to determine cation exchange capacity. The sample was repeatedly washed with methyl alcohol to eliminate leftover ammonium acetate. After transferring the alcohol-washed soil to a round-bottomed flask, 500 mL of water was added and connected to a Liebig condenser to create a 500 mL conical flask containing 20 mL of 2% boric acid and a few drops of mixed indicator (methyl red and methyl blue in methanol). The sample was then treated with three spatulas of magnesium oxide (to displace the NH_4 trapped in the soil). The contents of the flask were heated until 300 ml was distilled over and collected in the receiver. The liquid in the receiver turned green instead of blue. Next, to assess the amount of NH_4 in the distillate, the contents of the receiver were titrated with 0.1 N HCl to a pink endpoint. Because the initial soil sample weighed 5.0 g, each milliliter of 0.1 N HCl employed in the titration was equivalent to 2 m.e. per 100 g exchangeable capacity.

Determination of mycorrhiza spore count

The wet sieving and decanting procedure (Gerdemann and Nicolson 1963) were used first, followed by sucrose centrifugation (Daniels and Skipper 1982). The soil sample was suspended in water before being decanted using sieves (with 0.350 mm, 0.125 mm, and 0.045 mm). The contents of the medium and finest sieves were transferred separately with some water to 100 ml centrifuge tubes. Next, a gradient was produced by injecting a 40 ml sugar solution (70 g dissolved in 100 ml water) into the bottom of the tube. The sample was centrifuged for 2 minutes at 2000 rotations per minute. Soil particles settled to the bottom, and spores stayed on the surface during this process. Spores were removed with a syringe and deposited on a clean sieve with a 0.045 mm mesh hole, then rinsed in water for 3 minutes before being transferred to a petri dish. A stereomicroscope with a magnification of 40X was used to examine the sample. After that, the spores were counted.

Data analysis

Analysis of variance was performed on the data using the computer software Genstat for Windows version 8.11 (Genstat for Windows) (Genstat 2005). Next, the standard error of differences in means was employed to distinguish treatment means at a 95% confidence interval. Finally, mycorrhizal reliance was determined as the yield of inoculated plants minus the yields of non-inoculated plants divided by the yield of non-inoculated plants multiplied by 100.

RESULTS AND DISCUSSION

Soil chemical properties

The soil chemical properties for the Gatanga study site are shown in Table 1.

Soil pH

The soil in the area had a mean pH of 5.4 and was classed as mildly acidic (Table 1). That could have happened because most of the base cations were leached off, leaving clay colloids dominated by aluminum and hydrogen ions (Giller 2001). When large amounts of aluminum ions (Al3+) in the soil combine with water molecules at pH levels below 5.5, H+ is produced (aluminum hydrolysis). Aluminum ions may be concentrated to the point where root development is hindered or delayed, preventing plants from absorbing water or nutrients. As a result, they grow stunted and develop nutritional shortage syndromes (Ball 1999). One of the most critical soil variables that affect nutrient availability is pH. Macronutrients such as magnesium, calcium, and phosphorus are often less available in acidic soils. Still, micronutrients such as manganese, iron, boron, zinc, and copper are typically more available in high-pH soils but become less available as the pH increases above 8. (Muriuki and Quareshi 2001). The availability of molybdenum, on the other hand, diminishes as the pH of the soil decreases, which is especially true in the case of legume production. Nitrogenase, a molybdenum-rich enzyme, is found in the nodules of leguminous crops. As a result, if the soil pH is low and the available molybdenum is low, legumes will seem N strained, and leguminous crop output will decline. Because phosphorus mineralization is greater in near-neutral soils than in more acidic soils, the release of key elements from soil organic matter through mineralization could have been governed indirectly by soil pH through its influence on microbial activity (Muriuki and Quareshi 2001).

Table 1. Soil chemical properties for Gatanga site, Kenya

Soil parameter	Level of nutrient	Optimum level	remarks
pH 1:2.5 (soil: water)	5.4	5.5-7.0	Acidic
Phosphorus	8.0 ppm	15-25	Low
% Nitrogen	0.1%	0.2-0.5	Low
%Carbon	0.5%	10-30	Low
% Soil organic matter	1.0%	20-60	Low
Potassium	0.3 Cmol/kg	0.2-0.6	Adequate
Magnesium	0.4 Cmol/Kg	0.5-4	Adequate
Calcium	2.9 Cmol/kg	4-10	Low
Sodium	0.1 Cmol/kg	0.2-0.5	Low
CEC	15.5 Cmol/kg	15-25	Low

Available phosphorus

Compared with the optimal plant growth level, the available soil phosphorus level was low (8.0 ppm), which might be explained by its chemical linkage to iron and aluminum as a somewhat insoluble precipitate that makes it unavailable to plants (Giller 2001). Soil pH has a big impact on phosphorus availability. After reacting with free aluminum and iron ions in the soil at a low pH (pH 5.5), phosphates of limited solubility are generated, effectively "tying" it up (Wieldetholt and Johnson 2005). In addition, excess calcium in the soil solution due to basic soil conditions (pH > 7.5) might precipitate with P, reducing its availability. At pH 5.5-7.5, phosphorus could be the most accessible for plant uptake (Muriuki and Quareshi 2001). Phosphorus may have existed in organically or inorganically formed soils of Gatanga. Moreover, the soils of the Gatanga site contain little organic matter, an essential source of labile or quickly mineralized phosphorus; changes in soil organic matter (SOM) are likely to be accompanied by changes in plant availability of P (Frizano 1999).

Total nitrogen

Table 1 shows that the total nitrogen concentration of the soil was low (0.1 %). The low organic matter condition of the Gatanga site's soils could have contributed to this. The mineralization of soil organic matter is one of the sources of nitrogen. Nitrogen mineralization rates in the field are known to be affected by differences in the quantity and quality of soil organic nitrogen (Schroth et al., 2003). Microorganisms multiply and contribute to mineralization when they have enough food in the form of soil organic matter (Sande et al. 2001). Soil microorganisms play a key role in decomposing soil organic matter (SOM) and releasing inorganic nutrients into the soil by acting as a catalyst. The nutrients are then available for plant consumption (Smith 1994). Therefore, soil microorganisms must have been low in the Gatanga soils due to the low organic matter concentration, resulting in low organic nitrogen mineralization and, thus, a low total nitrogen level.

Organic matter in the soil

Table 1 shows that the soils at the Gatanga location have little soil organic matter (1 %). Living bacteria, partially decomposed plant debris, and hummus make up the organic matter in the soil (Griffin 2006). The soil's low level of organic matter may have been caused by crop removal and a lack of organic material in the form of manure in the area. Low crop yields, as a result of insufficient or non-use of fertilizers, could have resulted in little residue being returned to the soil, adding to the low levels of organic matter. Through the binding of microscopic soil particles into bigger aggregates by crop leftovers and some microorganisms like mycorrhiza, soil organic matter works as a revolving bank account for nutrients, improves soil structure, and reduces soil erosion (Perucci et al. 2000). The cation exchange capacity, and thus the soil's ability to retain nutrients in a plant-available form, in soils dominated by low activity clay, as is the situation at the Gatanga site, is highly influenced by its organic matter concentration (Schroth et al. 2003)

Potassium

Table 1 shows that the soils at the Gatanga site have appropriate potassium levels, which could be explained by the fact that the rocks that produced Gatanga's soil contained sufficient Potassium. The findings are consistent with those of other researchers who have found that Potassium is not a key nutrient-limiting factor in Central Kenya (Gikonyo et al. 2000). Potassium is a macronutrient and one of the most important minerals for plant growth (Muriuki and Quareshi 2001), which involves water, nutrient, and carbohydrate transport in plant tissue. Although Potassium (K) is not found in any major plant component, it is important for various physiological activities essential for plant growth, such as protein synthesis and maintaining plant water balance (Beegle 1990).

Magnesium

Magnesium levels in the soil were low but not insufficient, as indicated in Table 1, at 0.4 Cmol/kg, compared to the optimum for plant growth, which is 0.5-4 Cmol/kg. The poor organic matter content of the soil may have exacerbated the condition. As a result, the soil's ability to hold cations such as magnesium is limited, and the nutrient is easily leached. Magnesium shortage causes poor and stunted plant growth since it is the primary core of the chlorophyll molecule in plant tissue (Mayland 1983).

Calcium

Table 1 demonstrates that calcium levels in the soil were low. That could be due to a lack of organic matter in the soil, causing the soil to be unable to retain the cation and, as a result, leaching. Calcium is a necessary component of plant cell walls (Muriuki and Quareshi 2001).

Sodium

Sodium levels were low, at 0.1 %, compared to the ideal values for crop growth (see Table 1). Soil dispersion, poor water infiltration, and probable salt toxicity in plants would occur from sodium levels exceeding 0.5 % (Muriuki and Quareshi 2001).

Cation exchange capacity

The ability of a soil to hold cation nutrients is measured by its cation exchange capacity. Cations are elements that have a positive charge (Muriuki and Quareshi 2001). Calcium (Ca2+), magnesium (Mg2+), potassium (K+), sodium (Na+), and aluminum (Al3+) are the most prevalent exchangeable cations in soil. Clay and humus colloids have negatively charged particles that hold cations (Sachs, 1999). Table 1 demonstrates that the soils at the Gatanga experimental site had a poor cation exchange capacity (15.5 Cmol/kg). That could be due to the soil's poor organic matter content. The consequence is that the soil's nutrient and water storage capacity is insufficient. Because the cation reservoir (humus and clay) is low, soluble elements like potassium sulphate cannot be retained efficiently. Lack of water inhibits microorganisms like mycorrhiza, which are obligatory symbionts that rely on plant roots for existence. Because the cation nutrients are in the soil water, these soils are also prone to leaching. CEC can be improved by adding lime, which raises the pH, or by adding organic matter, which lowers the pH.

Mycorrhiza inoculant's spore count

The spore count was determined using a mycorrhiza inoculant obtained from the Kenya Forestry Research Institute (KEFRI) and utilized in on-station and on-field experiments. Table 2 summarizes the findings. *Glomus intraradices*, *Glomus etunicatum*, and *Gigaspora albida*, three mycorrhiza species, were employed to inoculate the soybeans. *Glomus etunicatum* had the largest spore count (205) in the long rain experiments, but *Glomus intraradices* and *Gigaspora albida* had the same number (140) of spores per 30 grams of inoculant. *Glomus etunicatum* had the largest number of spores (220) in short rains and the experiments at the screen house, followed by *Glomus intraradices* (180) and *Gigaspora albida* (135) per 30 grams, as indicated in Table 2. Mycorrhiza fungi species and strains have been shown to differ in their ability to improve nitrogen uptake and plant growth (Gracy and Bagyaraj 2005), necessitating the use of three different species in the trial.

Glomus leptotichum and *Glomus intraradices* were the best AM symbionts in boosting plant biomass compared to the others in an experiment to examine the efficacy of eleven mycorrhiza fungus on Kalmegh (*Andrographis paniculata*) (Chiramel et al. 2006). In addition, root colonization and sporulation were higher with the two symbionts, allowing for increased fungal-host contact and nutrient exchange and enhancing plant growth.

Effect of mycorrhiza and *Rhizobium* inoculation on soybeans germination

Table 3 demonstrates that the germination of soybeans was significantly different for the various treatments ($p<0.05$) in both the on-farm and on-station experiments. Only the dual inoculated, and mycorrhiza treated plants (S+R+M and S+M) demonstrated significantly greater germination rates than the control plants during the LR 2005 season (S). Soybeans infected with mycorrhiza (S+M) had the maximum germination percentage of 97.5%, whereas those inoculated with *rhizobium* and planted with phosphatic fertilizers (S+P+R) had the lowest germination percentage of 70%. The performance in decreasing order of the germination rate was S+M>S+R+M> S> S+P+M> S+R> S+P>S+P+R.

In the on-farm experiments 2005 LR, pairwise comparisons between the mycorrhizal plants and the control (soybeans alone with 80% germination) found that inoculation considerably enhanced germination in the S+M (97.5%) and S+R+M (87.5%) treatments, but not in the S+P+M treatment, where the rate of germination declined slightly to 77.5 %.

Table 2. Mycorrhiza spore count in the inoculant sourced from KEFRI used in the on-field and on-station experiments

Mycorrhiza species	Number of spores per 30 grams pure inoculum	
	Long rains 2005	Short rains 2005 and on-station
Glomus intraradices	140	180
Glomus etunicatum	205	220
Gigaspora albida	140	135

Table 3. Germination of soybeans on-field in Gatanga and on-station at Kenyatta University, Kenya, under different treatments

Treatments	On-farm		On-station	
	Long rains (%)	Short rains (%)	sterile soil (%)	Non-sterile soil (%)
Soybeans + *Rhizobium*	75.0cd	79.5a	77.7c	100.0a
Soybeans + *Rhizobium* + Mycorrhiza	87.5b	67.0d	100.0a	89.0b
Soybeans + P fertilizer + *Rhizobium*	70.0d	79.0a	77.7c	89.0b
Soybeans + P fertilizer + Mycorrhiza	77.5c	54.0d	100.0a	100.0a
Soybeans	80.0c	73.0b	89.00b	67.0c
Soybeans + Mycorrhiza	97.5a	77.0a	67.0d	56.0d
Soybeans + P fertilizer	70.5d	77.0a	67.0d	67.0c
SED	2.7	1.5	0.22	0.31

Note: Numbers in each column followed by the same letter are not significantly different at p = 0.05

Dual inoculation of soybeans with mycorrhiza and *Rhizobium* (S+R+M) resulted in significantly better germination (87%) than *Rhizobium*-treated plants (75%) but significantly lower germination than mycorrhiza-inoculated plants (S+M) (97.5%).

S+R had the highest germination rate of 79.5% in the second season, with short rainfall in 2005, whereas soybeans planted with phosphatic fertilizers and inoculated with mycorrhiza (S+P+M) had the lowest (54%). S+R, S+P+R, S+M, S+P, S, S+R+M, and S+P+M had the highest germination rates, followed by S, S+R+M, and S+P+M.

Treatments S+R, S+P+R, S+M, and S+P showed considerably higher germination rates than the control (S), whereas mycorrhizal treatments S+R+M and S+P+M had significantly lower rates than the control (S) ($p<0.05$). Furthermore, the single inoculated plants S+R (79.5%) and S+M (77%) germination rates were much greater than the dual inoculation plants S+R+M (67 %).

S+R+M and S+P+M had the highest germination rates of 100 % in the on-station sterile soils experiment, whereas S+P and S+M had the lowest at 67%. S+P+M=S+R+M>S> was the performance in decreasing order of germination. S+M=S+P> S+R=S+P+R> S+M=S+P. Only S+P+M and S+R+M (both mycorrhizal) germination rates were considerably greater than the control ($p<0.05$).

Dual inoculation with mycorrhiza and *rhizobium* improved germination rate in sterile soils, demonstrating that singly treated plants S+M and S+R had significantly lower germination rates than dual injected plants (S+R+M). S+P+R had a considerably greater germination percentage than S+P, indicating that *Rhizobium* inoculation may have boosted the germination rate in P-applied plants, whereas S+R had the same rate. The germination rate of S+P+M was much greater than that of S+P and S+M, indicating that mycorrhiza inoculation may have aided germination.

S+R and S+P+M had the highest germination rates of 100 % in non-sterile soil, while S+M had the lowest at 56%. S+R and S+P+M, S+R+M and S+P+R, S and S+P, and S+M were the top performers in decreasing the germination rate. When comparing the treatments to the control (S) plants, it was discovered that the S+R, S+R+M, S+P+M, and S+P+R treatments had considerably greater germination rates than the S+M and S+P treatments ($p<0.05$). The germination rates of dual inoculated plants (S+R+M) were significantly lower than the *rhizobium* inoculated (S+R) treatments but significantly higher than the mycorrhiza inoculated (S+M) treatments. That indicated that mycorrhiza, but not *Rhizobium*, might have had a role in germination.

The trial results were inconclusive, indicating that inoculation with mycorrhizal fungi and *Rhizobium* bacteria could boost soybean germination. Furthermore, this is because the plants had not formed roots at the time of germination and hence had not begun the symbiotic interaction with the two bacteria. Mycorrhiza hyphae respond to the presence of a root by growing towards it, establishing contact, and growing along its surface to form an association (Brundret et al. 1994). Many investigations have revealed a time lag between mycorrhizal inoculation and the time it takes for its effects to develop in the plant (Brandon and Shelton 1993). The fungus is at the lag stage during germination and is autotrophic (Sieverding 1991)

Those findings contrast with Kikuchi et al. (2007), who revealed that flavonoids detected in root exudates during plant germination operate as signaling molecules in symbiotic ectomycorrhizal fungi-woody plant partnerships. Similarly, the *Rhizobium* bacteria are in their saprophytic phase. Therefore, they will only infect and gain admission into the root (infective phase) and subsequently participate in the creation of a functional root nodule (symbiotic phase) in response to chemical signals (flavanoids) released by the germinating plant. Brandon and Shelton (1997) reached the same conclusion while studying the factors impacting the early growth of *Leucaena leucocephala*.

As AM fungus progressively expanded root colonization, phosphorus extraction efficiency improved, implying that high rates of P would be required to compensate for early delayed colonization. Large impacts of mycorrhiza inoculation were observed 41 days after sowing in their trials.

Germination is a process that involves the mobilization and use of food reserves (Howell 1960), and it is influenced by environmental elements like temperature, soil moisture, nutrients, and oxygen availability (Fagena et al. 1997). In addition, internal seed physiology, such as seed vitality, genetic potential, and seed maturation, as well as hormonal or chemical changes that occur as the seed is building its food stores, affect seed germination (Bennet 2004). One or all of these factors could explain the discrepancies in germination rates across the three treatments.

Effect of inoculating soybeans with mycorrhiza and *rhizobium* on height increment

In the on-field experiments, significant differences in height were seen between the various treatments at all three phases of height assessment, namely 30, 45, and 60 days after planting (Table 4).

After the 30th day of the LR 2005 season, only the treatments S+R+M and S+P+M (both mycorrhizal) had considerably larger height increments than the control S. In contrast, on the 45th day, S+R+M, S+P+R, S+P+M, and S+M all had significantly higher height increments than the control. S+R, S+R+M, S+P+R, and S+P+M levels were considerably higher on the 60th day than in control. The increased height increment in mycorrhizal (S+R+M) plants treated with *rhizobium* could be attributable to increased nutrient absorption and photosynthetic rates. It is well established that mycorrhizal colonization enhances plant development by boosting nutrient uptake and use (Marschner and Dell 1994, Clark and Zeto 2000). AM fungus may have shortened the distance nutrients diffused through soils to the roots via their hyphae.

Table 4. Height of soybeans during the long and short rains 2005 under different treatments at the on-field experiment at Gatanga, Kenya

Treatment	Long rains 2005 (cm)			Short rains 2005 (cm)		
	30 days	45 days	60 days	30 days	45 days	60 days
Soybeans + *Rhizobium*	9.20b	10.80d	19.90b	7.70c	10.70cd	14.20a
Soybeans + *Rhizobium* + Mycorrhiza	14.90a	18.50a	26.90a	8.85a	12.65a	15.25a
Soybeans + P fertilizer + *Rhizobium*	10.75b	14.55bc	21.95b	8.65ab	12.20ab	15.15a
Soybeans + P fertilizer + Mycorrhiza	15.45a	16.00b	23.50ab	8.00ba	10.60d	14.95a
Soybeans	7.90b	10.45d	14.30c	8.25abc	11.65bc	14.10a
Soybeans + Mycorrhiza	10.55b	14.03c	16.30c	7.90bc	11.70ab	14.35a
Soybeans + P fertilizer	9.00b	10.35d	15.75c	7.95bc	11.55bcd	13.75a
SED	1.90	1.24	1.71	0.39	0.50	0.84

Note: Numbers in each column followed by the same letter are not significantly different at p= 0.05

In the on-field LR 2005 season experiment, dual inoculation with mycorrhiza and *Rhizobium* (S+R+M) boosted soybeans' height significantly ($p<0.05$) over the control (S) at all three phases. At all stages, dual inoculation with mycorrhiza and *Rhizobium* (S+R+M) resulted in a significantly greater height increment than S+R and S+M (singly inoculated plants). Furthermore, this could have occurred due to the benefits derived from the tripartite symbiosis of legume-AM fungi-*Rhizobium* through host nutrition stimulation (Barea et al. 1992). The extraradical mycelium of the AM fungus extends beyond the zone of phosphate depletion, establishing a new source of soluble phosphates (Smith and Read 1997). Rhizobia's nitrogenase enzyme fixes atmospheric nitrogen in nodules, while fungal hyphae aid in the uptake of ions, primarily phosphates, via mycorrhizal roots (Postgate 1998; Leigh 2002). As a result, the photosynthesis rate increases, so the height increases.

Rhizobium inoculation plus the application of phosphatic fertilizer (S+P+R) resulted in a considerably greater height increase than *Rhizobium* inoculation alone (S+R) on the 30th day. On the 45th and 60th days, inoculating with *rhizobium* and applying phosphatic fertilizer (S+P+R) resulted in a considerably greater height increment than plants treated with phosphatic fertilizer (S+P). Increased height growth was facilitated by the addition of P fertilizer to soils that were deficient in P.

At all stages, inoculating plants with mycorrhizas and applying phosphatic fertilizers (S+P+M) resulted in considerably greater height increase than mycorrhiza-singly inoculated plants (S+M) and phosphatic fertilizer-applied plants (S+P). At all phases, the mycorrhizal inoculated plants (S+M) had the maximum height, indicating the mycorrhiza fungi's efficacy in extracting nutrients, particularly P, from the soil. Mycorrhizas have been shown to boost growth by boosting nutrient intake, particularly P and other critical elements (Marschner and Dell 1994; Clark and Zeto 2000). However, this was accomplished by mycorrhizal fungus physically exploring the soil more extensively than the roots. The ratio of hyphae to root length has been determined to be between 300 and over 8000. (Read and Boyd 1986; Jones et al. 2001). The discrepancies between the three treatments were significantly different ($p<0.05$) at all stages during the short rains of the 2005 season, as well as during the long rains. At all stages, all treatments except the dual inoculation plants (S+R+M) on the 45th day did not perform substantially better than the control (S). On the 30th and 45th days, dual inoculation with mycorrhizal fungi and *Rhizobium* bacteria (S+R+M) resulted in a considerably greater growth increment than *Rhizobium* singly infected plants (S+R), but not on the 60th day. Inoculating with mycorrhiza and *Rhizobium* (S+R+M) resulted in a considerable height increase above (S+M) on the 30th day, but not at the other stages.

The data indicate that the soybeans-Rhizobium-mycorrhiza symbiosis did not benefit the short rains crop, which would have increased photosynthesis and, thus, height increment. During the 2005 long drops of rain, when moisture was not a constraint, the *Rhizobium* bacteria transformed atmospheric nitrogen to ammonia, which the plants might have ingested and converted to amino acids, resulting in a growth boost. Moisture stress during the 2005 season's short rains may have harmed these bacteria's ability to fix atmospheric nitrogen, as P and other nutrients can only be absorbed in solution. In field experiments, soybeans planted with P alone (S+P) grew at a non-significant rate relative to the control (S). Although P was supplied, plants' roots could not absorb it properly without the benefit of the increased surface area caused by the mycorrhizal connection. Inoculation with mycorrhiza and application of P fertilizer (S+P+M) resulted in a non-significant height increment above S+M and S+P at any stage during the brief rains. Inoculating soybeans with *rhizobium* and applying P fertilizer (S+P+R) resulted in a little height increase over planting with P alone (S+P) during the short rains.

In the on-station studies, the differences between the various treatments were significant ($p<0.05$) in both sterile and non-sterile soils, except on the 60th day in the sterile soils (Table 5). Dual inoculation with mycorrhiza and *Rhizobium* (S+R+M) resulted in a significantly greater height increase ($p<0.05$) than the control (S) on the 30th and 45th days, but not on the 60th day, in sterile soil. Only on the 30th day did the dual inoculation plants (S+R+M) significantly outgrow the control (S) in the non-sterile soil. Indigenous microbes could have contributed to increased nutrient absorption, resulting in height increment in non-sterile soils. On the 30th and 45th days, dual inoculation with *rhizobium* and mycorrhiza (S+R+M) resulted in a considerably greater height increment than inoculating with *rhizobium* alone (S+R), but not on the 60th day. In non-sterile conditions, dual inoculation (S+R+M) resulted in a considerably greater height increment than inoculating with

rhizobium alone (S+R) on the 30th day.

Dual inoculation with *rhizobium* and mycorrhiza (S+R+M) in sterile soils resulted in a much greater height than S+M on the 30th day. A considerable plant height increased on the 30th observed day in non-sterile soil but significantly reduced on the 45th and 60th days. The observation showed no increase in height above single-inoculated plants (S+R) and (S+M) due to the dual inoculation with mycorrhizal fungi and *rhizobium*. The benefits of legume *Rhizobium* mycorrhiza symbiosis were not reflected in these experiments. Without the benefits of mycorrhiza, phosphorus and other generally stable elements of the soil may not have been accessed by plants. The inadequate delivery of these nutrients impeded the BNF process.

The difference between the (S+P) and the mycorrhiza plants (S+P+M) was considerably higher in all tests (S+P+M) (sterile and non-sterile). Mycorrhizal fungi could have played a key role in acquiring additional P and other nutrients from distances beyond phosphorus depletion. Nutrients for BNF must eventually lead to greater photosynthesis.

In an experiment to assess rock phosphates and mycorrhizal impacts on growth and nutrient uptake of F albida seedlings in alkaline soil, even without rock phosphates, mycorrhiza inoculated plants achieved a higher biomass result. The dependency on mycorrhiza decreased as the phosphates increased (Ba and Guissou 1996).

In the case of most tropical soils, the soil at the experimental location was heavily weathered, acidic, and base-leached, resulting in low fertility (Giller 2001). Phosphorus is known to be rapidly reduced within a few millimeters of the growing root (Sieverding 1991). This zone cannot be fully refilled with P due to the exceedingly slow diffusion rate. External mycorrhizal mycelium extends far beyond this zone, increasing the amount of soil available for P absorption (Sieverding 1991). Acidic soils promote increased organic acid excretion, which increases the solubility and uptake of the mineral nutrient P and the micronutrients Zn, Fe, and Mn in particular (Marschner 1992). Phosphorus is a crucial nutrient that is deficient in nitrogen fixation. *Rhizobium* is extremely susceptible to the mycorrhizal association and requires it to meet the high P requirements for nodulation and nitrogen fixation (Bhatia et al. 1998). Dual inoculation with micorrhiza, *G. caledonius*, and *rhizobium* improved the performance of transplanted *P. juliflora* in a semiarid wasteland.

Similarly, *Leucaena leucocephala* injected with three *Rhizobium* strains greatly boosted height growth in a field experiment in Jammu, India (Dutt and Palhania 1983). Marques et al. (1999) concluded after inoculating *Cetrolobium tomentosum*, a woody legume, with both mycorrhizal and *rhizobium* bacteria. *Rhizobium*-inoculated plants increased in height only when connected with a mycorrhizal fungus.

Effect of mycorrhiza and *Rhizobium* inoculation of soybeans on root collar diameter

In the on-farm experiments, there was a significant difference ($p<0.05$) between the various treatments during the long and short rains of the 2005 seasons, but not in the on-station experiments, as shown in Table 6.

Table 6 indicates a significant increase in root collar diameter over the control and the other treatments in the on-farm LR 2005 studies. For example, inoculating soybeans with mycorrhiza (S+M) or *Rhizobium* (S+R) enhanced root collar diameter by 54.8% and 30%, respectively, above the control.

Table 5. Height of soybeans in the on-station sterile and non-sterile soils under different treatments at Kenyatta University, Kenya

Sterile soil (cm)				Non-sterile soil (cm)		
Treatment	**30 days**	**45 days**	**60 days**	**30 days**	**45 days**	**60 days**
Soybeans + *Rhizobium*	16.2cd	41.7bc	58.7	27.9c	51.0b	75.7b
Soybeans + *Rhizobium* + Mycorrhiza	25.4a	49.3a	61.7	34.7a	53.0b	73.7b
Soybeans + P fertilizer + *Rhizobium*	14.0de	20.0d	59.7	12.1d	25.0c	56.7c
Soybeans + P fertilizer + Mycorrhiza	23.7ab	53.0a	75.3	35.0a	54.0b	74.7b
Soybeans	15.7d	36.0c	56.7	28.9c	46.3b	62.bc
Soybeans + Mycorrhiza	21.7b	52.3a	68.3	30.3bc	65.7a	88.7a
Soybeans + *Rhizobium*	11.3e	41.3bc	62.7	8.9d	23.7c	62.0bc
SED	1.5	3.6	4.6	2.1	5.0	6.4

Note: Numbers in each column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 6. Effect of mycorrhiza and *Rhizobium* inoculation of soybeans on root collar diameter in Gatanga and Kenyatta University, Kenya

Treatments	On-farm (cm)		On-station (cm)	
	Long rains	**Short rains**	**Sterile**	**Non-sterile soil**
Soybeans + *Rhizobium*	3.2d	2.3d	2	2
Soybeans + *Rhizobium* + Mycorrhiza	4.5ab	3.8ab	2	3
Soybeans + P fertilizer + *Rhizobium*	4.1abc	3.4c	1	2
Soybeans + P fertilizer + Mycorrhiza	4.6a	3.9a	2	2
Soybeans	2.5 e	3.5bc	2	2
Soybeans + Mycorrhiza	3.9acd	3.8ab	1	2
Soybeans + *Rhizobium*	3.5cd	3.5bc	2	2
SED	0.4	0.2	0	0

Note: Numbers in each column followed by the same letter are not significantly different at $p=0.05$.

Dual mycorrhizal inoculation of soybeans (S+R+M) resulted in a root collar diameter gain of 80% above the control (much more than S+R), which indicated that mycorrhizal fungus may have contributed to the root collar's growth.

Although the increase was not significant, the treatment S+R+M had a bigger root diameter than S+M. Thus, when soybeans were planted with only one microorganism inoculant (S+R and S+M), the dual inoculated plants (S+R+M) had a higher root collar diameter. The increased diameter of the root collar in mycorrhizal plants may result from increased inorganic nutrient absorption and photosynthesis rates (Marschner 1992). Increased photosynthetic rates resulting from the availability of P and other nutrients via wider mycorrhiza exploration of soils and provision of plant accessible N through biological nitrogen fixation by the *Rhizobium* bacteria could have resulted in larger diameters in the dual inoculation plants (S+R+M) (Allen et al. 1981). In the long rains, the treatment S+P+R showed an increase that was not substantially greater than S+P and similar to S+R. Providing P and inoculating with *rhizobium* may have resulted in increased photosynthesis rates and, thus, a bigger root collar diameter. Dual inoculated plants (S+R+M) had the same root collar diameter as mycorrhiza solely treated plants (S+M) during the short rain drops but were substantially larger than *rhizobium* inoculated plants (S+R). Dual inoculation improved the diameter of the root collar by 8.6% compared to the control S. Due to dryness, mycorrhizal fungi could not efficiently perform their nutrient acquisition duty.

Though the differences between treatments were not significant in the on-station experiments, soybeans inoculated with both mycorrhiza and *Rhizobium* (S+R+M) exhibited the largest root collar diameter (3 mm) in both the sterile and non-sterilized soil, as indicated in Table 6. Mycorrhiza inoculation has been found to increase plant root collar diameter. Ghosh and Verma (2006) inoculated *Acacia mangium* with three VA-mycorrhiza fungi (*Glomus occultum, Glomus aggregatum*, and *Glomus mosseae*). They discovered that all inoculations increased shoot, height, root collar diameter, chlorophyll, and biomass compared to uninoculated control seedlings. Inoculation with mycorrhizal fungus and rhizobial bacteria does not lead to steadily increased plant development. In a study of the effect of dual inoculation with *rhizobium* and mycorrhiza on the growth of *Calliandra calothyrsus*, it was discovered that, while plants inoculated with both symbionts grew better than controls, the results were not statistically significant. Inoculation did not have a long-term effect on tree growth, even when most nodules were inoculated (Leisueur and Sarr 2008).

Effect of mycorrhiza and *Rhizobium* inoculation of soybeans on root dry weight

Some significant differences among the treatments were shown in the root dry weight ($p<0.05$) on-farm 2005 long drops of rain season experiments. Table 7 reveals that the differences between the on-farm short rains 2005 season and the on-station experiments were not significant. The differences between control (S) and the other treatments were significant in the on-field long rains 2005 season experiment, except for S+R and S+P. Soybeans planted with phosphorus and mycorrhizal fungi (S+P+M) had the highest roots dry weight, followed by soybeans planted with phosphorus and *Rhizobium* fungi (S+P+R). As seen in Table 7, the control (S) had the least root mass. The higher weight in mycorrhizal plants could be related to the ability of mycorrhizal fungi to improve nutrient intake via increased surface area for absorption. Enhanced nutrient uptake must increase photosynthetic rate, resulting in increased plant growth. Therefore, the roots grew faster, implying an enhanced flow of photosynthates to the roots and fungal hyphae. The association of plant roots with mycorrhizal fungi may have increased the amount of phosphorus available to the plants, increasing root biomass.

The results of these experiments suggest that higher photosynthates to the roots increased mycorrhizal plants' root biomass due to increased phosphorus nutrition caused by mycorrhizal fungi. Gueye (1990) reached the same conclusion after inoculating Bambara groundnuts with *rhizobium* and mycorrhiza (*Glomulus mosseae*). Mycorrhizal infection was reported to always result in a considerable rise in root weight. In a greenhouse experiment in Belgium, micropropagated bananas inoculated with mycorrhizal *Glomus interradices* exhibited significantly larger shoots, dry root weight, and P content than non-mycorrhizal plants (Declerck et al. 2002).

Table 7. Effect of mycorrhiza and *Rhizobium* inoculation of soybeans on dry rot weight in On-farm and On-station Experiments in Gatanga, Kenya

Treatments	On-farm (g/plant)		On-station(g/plant)	
	Long rains	Short rains	Sterile	Non-sterile soil
Soybeans + *Rhizobium*	0.522d	0.345	0.04	0.09
Soybeans + *Rhizobium* + Mycorrhiza	1.146bc	0.761	0.5	0.21
Soybeans + P fertilizer + *Rhizobium*	1.44abc	0.459	0.05	0.08
Soybeans + P fertilizer + Mycorrhiza	1.96a	0.470	0.32	0.12
Soybeans	0.477d	0.414	0.07	0.11
Soybeans + Mycorrhiza	1.034c	0.705	0.16	0.2
Soybeans + *Rhizobium*	0.781d	0514	0.08	0.05
SED	0.3114	0.1693	0	0

Note: Numbers in each column followed by the same letter are not significantly different at p=0.05.

During the SR season, the discrepancies in root dry weight between treatments were not significant. Drought stress was applied to the crop during the flowering stage, resulting in growth retardation. Moreover, this is in contrast to an experiment in which drought stress raised the percentage of mycorrhizal infection in *G. sepium* and *Albizia lebbeck* by an average of 8-41 % (Awatonye et el. 1992).

The root dry weight differences across the four treatments were not significant ($p<0.05$) in the on-station studies. That was true for both sterilized and non-sterilized soils, albeit mycorrhizal plants had a larger diameter than non-mycorrhizal plants, as shown in Table 7. effect of Mycorrhiza and *Rhizobium* inoculation on soybeans.

Shoot dry weight

As Table 8 indicates, there was a significant difference in dry shoot weight (p0.05) between the different treatments only during the 2005 long drops of rain season on the farm, but not during the 2005 short drops of rain season or on-station experiments. During the 2005 long drops of the rain season, except for the S+R and S+P treatments, differences between the control and each of the other treatments were significant. The order of performance was S+P+R >S+M> S+R+M> S+P+M> S+R>S and S+P.

The dual inoculated plants (S+R+M) increased their shoot dry weight by 172 % compared to the control S, but the single inoculated plants (S+R and S+M) increased by 103 and 185 %, respectively. Furthermore, this could be attributable to increased inorganic nutrient absorption, particularly P, and increased photosynthetic rates caused by mycorrhiza and *Rhizobium* inoculation (Jia and Gray 2004). As a result, there was a little increase in the shoot dry weight of the dual inoculation plants (S+R+M) compared to S+R. However, the other mycorrhizal plants, S+M and S+R+M, performed better than the non-mycorrhizal plants, except for treatment S+P+M.

The dual inoculated plants could be attributed to increased inorganic nutrient absorption, particularly of P, and increased photosynthetic rates in inoculated plants. Extensive mycorrhizal hyphal networks may have enabled soybeans to collect phosphorus from sources outside the roots' nutrient depletion zone and to solubilize phosphorus from inaccessible sources (Marschner 1992). Mycorrhizal plants may also have obtained P from ordinarily unavailable sources, both inorganic and organic (Koide and Kabir 2000; Feng et al. 2003). The additional phosphorus may have been used as an energy source in biological nitrogen fixation, a process in which atmospheric nitrogen is converted to ammonia (NH3), which the plant absorbs and converts to amino acids, resulting in increased leaf weight (Hogberg 1986).

Increases in stem and leaf biomass were closely linked with increases in P uptake in an experiment to determine the effect of mycorrhiza inoculation in an alley cropping trial, indicating that the improvement was ascribed to mycorrhiza inoculation (Atayese et al. 1992). Without mycorrhizal colonization, *Faidherbia albida* seedlings developed slowly, producing more biomass when colonized (Ba and Guissoui 1996). At the end of drought stress, inoculating *Faidherbia albida* and *Acacia nilotica* with mycorrhiza and *rhizobium* in barren soil boosted the plant biomass of the two tree species (Onsubi et al. 1992).

Similar results were achieved in an experiment to determine the effect of dual inoculation of black locust (*Robinia pseudoacacia* L.) with rhizobia and glomus on desurfaced soil. Again, a synergistic impact was discovered, as dual inoculation resulted in a 93% increase in shoot mass compared to single inoculation (Ferrali et al. 2008). The authors concluded that mycorrhizal colonization enables nodulated plants to meet their P requirements in soils deficient in P.

Combining S+R+M was the most effective treatment in the 2005 short drops of rain season experiment, followed by S+P+R, S+P+M, S+P, S+R, S+M, and S. Dual inoculation (S+R+M) resulted in greater soybean dry weight. However, the difference was not statistically significant when each microbe was used alone (S+R and S+M). Due to the drought during this experiment, multiple treatments may have failed to demonstrate a meaningful difference over the control and among themselves. The number of rhizobia in the soil may have decreased significantly due to soil drying, as illustrated in Table 8. The rate of nitrogen fixation and the transfer of nitrogen fixation products to shoots may have been slowed down by lowering the soil water content (Giller 2001). Awatonye et al. (1992) concluded the same thing about *Acacia auriculiformis* in an experiment to assess the response of certain tropical nitrogen-fixing woody legumes to drought and mycorrhiza inoculation in sterile soil. They concluded that mycorrhiza-inoculated plants outlived uninoculated plants and had a higher dry matter, nutritional content, and larger leaf surface area.

Table 8. Effect of mycorrhiza and *Rhizobium* inoculation of soybeans on shoot dry weight in Gatanga and Kenyatta University, Kenya, Kenya

Treatments	On-farm (g/plant)		On-station (g/plant)	
	Long rains	Short rains	sterile	Non-sterile soil
Soybeans + *Rhizobium*	3.35bcd	0.7	0.76	0.6
Soybeans + *Rhizobium* + Mycorrhiza	4.49b	1.2	1.92	1.2
Soybeans + P fertilizer + *Rhizobium*	7.94a	1.0	0.65	0.6
Soybeans + P fertilizer + Mycorrhiza	3.92b	0.8	1.39	1.3
Soybeans	1.65cd	0.4	0.51	0.5
Soybeans + Mycorrhiza	4.71b	0.6	1.42	1.2
Soybeans + *Rhizobium*	1.17d	0.8	0.63	0.6
SED	1.097	0.2	0	0

Note: Numbers in each column followed by the same letter are not significantly different at p=0.05

Although the results were not statistically significant, the mycorrhizal plants had greater shoot weights than the non-mycorrhizal plants in the on-station experiments. The presence of indigenous rhizobia or mycorrhiza in unsterilized soil may account for the experiment's findings. Indigenous strains may have competed for nodule occupancy with introduced strains, reducing their efficacy (Marques et al. 1999).

Effects of mycorrhiza and *Rhizobium* inoculation on soybeans grain yields

Table 9 shows inoculating soybeans with mycorrhiza and *rhizobium* resulted in a significant difference ($p<0.05$) between the different treatments on-farm (long rains 2005 season) and on-station sterilized and non-sterilized soils. Due to dryness, no grain yields were realized in the second season (short rains 2005).

Soybeans inoculated with mycorrhiza and *Rhizobium* (S+R+M) produced the highest grain yields during the 2005 long drops of the rain season, followed by soybeans planted with phosphorus and inoculated with mycorrhiza (S+P+M). The remainder of the sequence was; S+P+R, S+M, S+P, and S+R and S in that order. Except for soybeans treated with *Rhizobium* (S+R), all treatments increased grain yields much more than the control (S).

Dual inoculation of soybeans with mycorrhiza and *Rhizobium* (S+R+M) resulted in a 356% increase in grain production compared to the control (S). By contrast, inoculation with either of the microorganisms alone resulted in a 71 percent rise in *Rhizobium* (S+R) and a 189 % increase in mycorrhiza (S+M) growth. Dual inoculation improved grain yield by 82 % and 166 % above singly inoculated plants S+M and S+R, respectively. Thus, mycorrhizal fungi and *rhizobium* operated synergistically since simultaneous inoculation increased grain yield more than either mycorrhizal fungus or *rhizobium* alone (Table 9). Inoculation with mycorrhiza may have expanded the volume of soil to be examined for nutrient intake, therefore improving the efficiency of soil nutrient absorption. Nutrients such as phosphorus, which were in short supply in the site soil, have a far slower diffusion rate in soil than the rate at which growing roots absorb them and, therefore, rapidly deplete the root zone (Busman et al. 2002). The extraradical mycelium of mycorrhizal fungi must have expanded well outside the depletion zone, establishing a new source of soluble phosphates (Smith and Read 1997). Thus, phosphorus was made available to plants and used in nodules for biological nitrogen fixation, where it provided the energy necessary to convert nitrogen to ammonia. As a result of the conversion of ammonia to amino acids and proteins, soybean grain yields were boosted.

Along with providing P, mycorrhiza fungus may have helped to improve nutrient uptake by increasing the absorbent surface (Marschner 1992; Marschner and Dell 1994). The overall consequence was an increase in photosynthetic rate and, as a result, in yields. Mridha et al. (1992) discovered that dual inoculation with *rhizobium* and micorrhiza (*Glomus clarum*) significantly enhanced the growth, yield, and nutrient content of yard-long beans (*Vigna unguiculata* sesquipedalis) compared to the non-inoculated control. Sieverding (1991) conducted over 50 field tests inoculating cassava cultivars with mycorrhiza in acidic soils of varying fertility levels and reported an increase in tuber yields of 20-25 %. Applying P fertilizer to mycorrhiza inoculated Soybeans (S+P+M) resulted in a grain production increase that was not statistically significantly greater than plants grown with fertilizer alone (S+P) or with mycorrhiza alone (S+M). Inoculating soybeans with *rhizobium* and fertilizing with P (S+P+R) increased grain production much more than *Rhizobium*-treated plants (S+R) but not mycorrhiza-treated plants (S+M). Mycorrhiza could have enhanced the volume of soils from which the plant roots obtained P.

Soybeans inoculated with mycorrhizal fungi (S+M) produced the maximum yields in sterilized soils. S+M> S+P+M> S+R+M> S+R> S>S+P and S+P+R, in decreasing order of grain yield. Each therapy was qualitatively distinct from the others. For example, in the 2005 long drops of rain experiment, plants treated with mycorrhiza produced more than non-mycorrhizal plants. In non-sterilized soils, grain yields decreased in the following order: S+R+M> S+P+M> S+M, S+P> S+P+R> S, and S+R. Mycorrhizal plants fared much better than non-mycorrhizal plants in both sets of on-station studies. Dual inoculation of soybeans with mycorrhiza and *Rhizobium* (S+R+M) increased grain yield statistically more than when soybeans were planted with *rhizobium* alone (S+R) but resulted in a gradual decrease when soybeans were planted with mycorrhiza alone (S+M). Dual inoculation (S+RM) increased grain production much more than S+R or S+M in non-sterile soils. The synergistic interactions between the elements of the tripartite symbiotic association (legume-*Rhizobium*-mycorrhiza) have been proven to increase legume productivity.

Table 9. Effect of mycorrhiza and *Rhizobium* inoculation on soybeans grain yield (g/plant) in on-farm and on-station experiments

Treatment	On-farm	On-station	
	Long rains 2005	Sterile	No-sterile soil
Soybeans + *Rhizobium*	0.84cd	0.52d	0.37g
Soybeans + *Rhizobium* + Mycorrhiza	2.24a	0.86c	0.89a
Soybeans + P fertilizer + *Rhizobium*	1.71ab	0.23g	0.62e
Soybeans + P fertilizer + Mycorrhiza	1.98ab	0.9b	0.76b
Soybeans	0.49d	0.49e	0.43f
Soybeans + Mycorrhiza	1.42bc	1.59a	0.74c
Soybeans + *Rhizobium*	1.39bc	0.33f	0.67d
SED	0.32	0.20	0.15

Note: Numbers in each column followed by the same letter are not significantly different at $p=0.05$.

Jia et al. (2004) discovered that plants having symbiotic associations with *rhizobium* and arbuscular mycorrhizal fungi have better photosynthetic rates per unit leaf area. Lizzy (1999) discovered that particular mycorrhizal fungi and *Rhizobium* combinations boosted plant growth and yield when used on peas and lentils. According to Xavier and Germida (2003), pea yield and nitrogen nutrition inoculated with mycorrhizal fungi and *rhizobium* differed depending on the mycorrhizal fungi-*Rhizobium* strain combination used. Inoculating peas with a superior *Rhizobium* strain and a suitable mycorrhiza fungal species increased yield and N nutrition.

Mycorrhiza dependency of soybeans inoculated with mycorrhiza and *rhizobium*

Mycorrhiza dependency, defined as a plant's reliance on mycorrhiza to achieve maximal growth or production at a given level of soil fertility (Brundett et al. 1994), was high (248.9 % to 312.3 %) for all plants treated with mycorrhiza in the on-farm experiment (Table 10). In on-station experiments, mycorrhizal dependence ranged from 75.5 to 224.5 % in sterilized treatments and from 72.1 to 107.6 % in non-sterilized treatments. The effect of competition in non-sterilized soil from indigenous mycorrhiza species implies the low mycorrhiza dependence in this experiment. In general, soybeans are legumes and hence have a coarse root system with a high phosphorus need for biological nitrogen fixation (Brundett et al. 1994).

Due to the low fertility level of the soils at the experimental site (Table 1), mycorrhizal fungi may have played a significant role in supporting the plant in obtaining nutrients, particularly phosphorus, by depleting their large hyphal network and making them available for plant absorption and utilization (Bagyara 1996; Arola et al. 2004), furthermore, this resulted in higher production. Ghosh and Verma (2006) discovered that when *Acacia mangium* was inoculated with three VA-mycorrhiza fungi (*Glomus occultum*, *G. aggregatum*, and *G. mosseae*), its growth was 57 % dependent on *G. occultum*, 47 % dependent on *G. mosseae*, and 46 % dependent on *G. aggregatum*. Ba and Gissou (1996) found that increasing the quantities of rock phosphate and mycorrhiza fungi had a negative effect on the growth and nutrient uptake of *Faidherbia albida* seedlings in alkaline soil. Mycorrhizal-inoculated plants absorbed significantly more P from the soil and rock phosphate than non-mycorrhizal plants.

Effect of inoculating soybeans with micorrhiza and *rhizobium* on root nodule numbers

As indicated in Table 11, the number of nodules in the various treatments differed significantly, that is, (P0.05) in both the on-farm experiments (LR and SR 2005 seasons) and the on-station experiments on sterile and non-sterile soils. In the on-farm experiment (LR), all treatments except S+R and S+P had considerably more root nodules than the control S. The following treatments were performed in decreasing order of the number of nodules: S+M>S+R+M>S+P+M>S+P>S+R=S+P+R>S.

Table 10. Effect of inoculating soybean with mycorrhiza and *rhizobium* on mycorrhiza dependency in on-farm and on-station experiments in Gatanga, Kenya

Treatment		Mean yield (Kg/Ha)	Mycorrhiza dependency (%)
Soybeans	Long rains 2005 (on-field)	299.3	312.3
+*Rhizobium*	Sterilized soil	127.4	75.5
+Mycorrhiza	Non-Sterilized soil	131.9	107.6
Soybeans + mycorrhiza	Long rains 2005 (on-field)	253.3	248.9
	Sterilized soil	235.6	224.5
	Non-Sterilized soil	109.6	72.1
Soybeans + P fertilizer + Mycorrhiza	Long rains 2005 (on-field)	293.3	303.9
	Sterilized soil	133.3	83.6
	Non-Sterilized soil	112.6	76.8
Soybeans (CONTROL)	Long rains 2005 (on-field)	72.6	
	Sterilized soil	72.6	
	Non-Sterilized soil	63.7	

Table 11. Number of soybean nodules under different treatments in the on-farm and on-station experiments in Gatanga, Kenya

Treatments	On-farm (no/plant) 2005		On-station (no/plant) 2005	
	Long rains	Short rains	Sterile soil	Non-sterile soil
Soybeans + *Rhizobium*	6.0b	3bc	3d	4a
Soybeans + *Rhizobium* + Mycorrhiza	26.4a	14.4a	7b	4a
Soybeans + P fertilizer + *Rhizobium*	19.5b	2.0bc	0f	1c
Soybeans + P fertilizer + Mycorrhiza	29.4a	6.3b	8a	4a
Soybeans	3.4b	0.4c	0f	0d
Soybeans + Mycorrhiza	16.6a	6.3b	5c	4a
Soybeans + P fertilizer	6.1b	1.6c	1e	2b
SED	6.40	2.20	0.32	0.40

Note: Numbers in each column followed by the same letter are not significantly different at p=0.05.

In comparison to the individually inoculated plants with *Rhizobium* (S+R) and mycorrhiza (S+M), dual inoculation (S+R+M) resulted in a substantially larger increase in the number of nodules than the control (S).

Dual inoculation with mycorrhiza and *Rhizobium* (S+R+M) significantly increased the number of nodules compared to *rhizobium* inoculated soybeans (S+R), but not to mycorrhiza inoculated soybeans (S+M). The number of nodules produced by *rhizobium* inoculated soybeans (S+P+R) did not differ significantly from *rhizobium* inoculated (S+R), or P applied soybeans (S+P).

This experiment demonstrated that plant roots could not properly acquire P from the soil without the advantage of mycorrhiza. Phosphorus application to mycorrhiza-inoculated soybeans (S+P+M) had no significant effect on the number of nodules compared to mycorrhiza-only inoculated soybeans (S+M). Only the mycorrhizal plants S+R+M, S+P+M, and S+M, exhibited significantly larger nodule numbers than the control in the short rains experiment. The following was the order of the performance in decreasing order of the number of nodules: S+R+M>S+P=M>S+M>S+R>S+P+R>S+P and S. Dual inoculation with both microorganisms (S+R+M) significantly enhanced root nodules compared to single inoculation with *Rhizobium* (S+R) and mycorrhiza (S+M). Phosphorus fertilization of *Rhizobium*-inoculated soybeans (S+P+R) had no significant effect on the number of nodules compared to soybeans inoculated with *rhizobium* alone (S+R). In contrast, P fertilization of mycorrhiza-inoculated plants (S+P+M) significantly increased the number of nodules compared to S+P but not S+M. The results of the LR and SR experiments on the number of nodules on mycorrhizal plants might be related to the role of mycorrhizal fungus in nutrient sourcing, which was observed in both experiments. Soybean roots that have been inoculated with mycorrhizae have been shown to increase nodulation and nitrogen fixation, especially in soils low in accessible nitrogen.

A similar conclusion was reached by P. Olsen and Habte (1995) when they investigated the influence of mycorrhizal infection on nodulation and nitrogen buildup in the plant *Cajanus cajan*. The incorporation of P explained the increased nodulation observed at low soil P concentrations by mycorrhizae. The application of phosphorus fertilizer to soybeans has drastically minimized mycorrhizal infection in the crop (Hicks and Loynachan 1987). Inoculated plants could meet the high P demand for nitrogen fixation by *rhizobium*, resulting in a rise in nodulation and, as a result, an increase in nitrogen fixation. An inoculation of mycorrhizal fungi and *rhizobium* produced similar results when *Leucaenia leucocephala* was inoculated with *Rhizobium* (Punj and Gupta 1988). Compared to single inoculation of either organism, dual inoculation resulted in better growth, nodulation, and nitrogen fixation. In a similar vein, inoculation of *Acacia auriculiformis* with both mycorrhiza (*Glomus fasciculatum*) and *rhizobium* resulted in the highest number of nodules and other growth parameters, as well as the highest number of nodules and other growth parameters (Chang et al. 1986). Nodules are where biological nitrogen fixation occurs, and a rise in their number indicates an increase in biological nitrogen fixation during the experiment. In their investigation of the interaction of *Cajanus cajan* with *Rhizobium* and vesicular-arbuscular mycorrhiza *Glomus aggregatum*, Olsen and Habte (1995) discovered that, at low soil P levels, mycorrhiza inoculation significantly increased nodule numbers and shoot dry weight. They concluded that the increased nodulation was caused by mycorrhiza-mediated P uptake. The same conclusion was reached by Kumar et al. (1998), who investigated the effects of mycorrhizal fungi, *rhizobium*, and phosphate on nodulation in chickpea and reached the same conclusion. Nodulation was dramatically higher in the dual inoculation plants compared to the non - inoculated plants. In the legume *Faidherbia albida*, inoculating the seedlings with *G. mosseae* and Brady *Rhizobium* resulted in profuse nodulation of the legume seedlings (Diop et al. 2002). Infected soybeans with Brady *Rhizobium* and *Glomulus clarum* produced 30 % more nodules than soybeans inoculated with *rhizobium* alone, according to the results (Antunes et al. 2006). Compared to single inoculation, Stanchaveva et al. (2006) found that dual inoculation of pea (*Pisum sativa*) with mycorrhiza and *rhizobium* boosted plant biomass, nodulation parameters, and nitrogen fixation activity.

Dual inoculation with rhizobia and mycorrhiza (S+R+M) produced the same number of nodules as when each of the microorganisms was treated alone (S+R and S+M) in the non-sterile soil treatments, indicating that there was no statistically significant difference. Inoculating with mycorrhiza and applying P fertilizers (S+P+M) resulted in a significant increase in nodule increment compared to S+P alone, but not to S+M alone.

Mycorrhizal plants (S+R+M, S+P+M, S+M) produced more nodules in sterile soils than non-mycorrhizal plants. When the number of nodules was counted in decreasing order, the results were as follows: S+1PM > S+1PM > S+1PM > S+1PM > S+1PM > S+1PM > R+1PM > R+1PM. S, the control, did not have any nodules.

All control trials (S) had reduced nodulation, which could have been caused by a lack of compatible and effective rhizobia, nutritional shortages, and an inadequate inoculum of mycorrhizal fungi, among other factors. Houngnandan et al. (2000) researched Mucuna pruriens as a fallow plant to restore soil fertility and manage the invasive grass *Imperata cylindrica* in the Beninese descended savanna. They came to the same conclusion as they did in their earlier research. Because of low quantities of functional rhizobia, the rate of nitrogen fixation by the plant was frequently limited, and this rate may be increased by rhizobial inoculation, except in extremely P-poor soils. The author concluded that farmer's management approaches that encourage the growth of mycorrhizal fungi would alleviate the P deficit and, as a result, boost nitrogen fixation.

In summary, with this study, the major goal was to determine the effect of dual inoculation of mycorrhizal fungi and *rhizobium* on the growth performance of the legume *Glycine max* grown in acidic soils. In particular, the study sought to determine the impact of inoculating

Glycine max with mycorrhizal and *rhizobium* bacteria on the germination, grain yield, and biomass production of the plant in acidic soils. A secondary goal of the study was to measure the influence of inoculating *Glycine max* with mycorrhizae and *rhizobium* on the amount of nitrogen fixed in acidic soils. The study's findings reveal that all growth parameters, except germination, are greatly improved when soybeans are inoculated with mycorrhizae and rhizobium in acidic soils. Plant height, root collar diameter, biomass (shoots and roots), and yields rose due to the dual inoculation of mycorrhiza and *Rhizobium* bacteria on the same plant, according to the results. The effect of inoculation differed depending on the crop's growth stage under investigation. According to the study, inoculating soybeans with mycorrhizae and *rhizobium* did not influence seed germination. However, it is possible because mycorrhizal fungi and *Rhizobium* bacteria colonization of the roots was still at a low level shortly after planting. At the time of planting, the mycorrhiza *Rhizobium* symbiosis in soybeans had not yet been established.

The study discovered that inoculating soybeans with mycorrhizal fungi and *rhizobium* enhanced plant height when moisture was not a constraint. Growth in height is not greatly increased when soybeans are planted with simply phosphate fertilizers and *rhizobium* or mycorrhizae. Mycorrhizal-inoculated plants' root collar diameter, roots, and shoot dry weights were greater than those of non-mycorrhizal plants during the long rains season when moisture was plentiful. During the short rains and the on-station experiments, this was not the case, however. A combination of mycorrhizal fungi and *Rhizobium* bacteria inoculated into soybeans resulted in a considerable increase in grain yield when growing circumstances were perfect. The number of soybean nodules was significantly higher in the treatments that contained mycorrhiza. Still, it was highest in the dual inoculated plants, indicating that dual inoculation with mycorrhiza and *rhizobium* increased the rate of nitrogen fixation, with nodules being the sites where this activity takes place. Dual inoculation with mycorrhiza and *rhizobium* increased the rate of nitrogen fixation, with nodules being the sites.

REFERENCES

Anderson JM, Ingram JSL. 1993. Tropical Soil Biology and Fertility: A Handbook of Methods. CAB International, Wallingford.

Antunes PM, Deaville D, Gross MJ. 2006. Effect of two AMF life strategies on the tripartite symbiosis between brady *Rhizobium japonicum* and soybean. Mycorrhiza 16 (3): 167-173. DOI: 10.1007/s00572-005-0028-3

Arora DK, Bharat R, Mukerji KG, Marth EH. 1991. Handbook of Applied Mycology. Revista do Instituto de Medicina Tropical de São Paulo 35 (4): 322-322. DOI: 10.1590/S0036-46651993000400018

Atayese MO, Awotonye OO, Osonubi O, Mulongoy K. 1992. Comparisons of the influence of VAM fungi on the productivity of hedgerow woody legume and cassava at the top and the base of a hill slope in alley cropping systems. Biol Fertil Soils 16: 198-204. DOI: 10.1007/BF00361408

Awatonye OO, Atayese MO, Osanubi O, Mulongoy K, Okali OOO. 1992. Response of some tropical nitrogen fixing woody legume to drought and inoculation with mycorrhiza. Biological Nitrogen Fixation and Sustainability of Tropical Agriculture, 24-28 September 1990. Ibadan, Nigeria.

Ba AM, Guissoui T. 1996. Rock phosphate and vesicular-arbuscular mycorrhiza effects on growth and nutrient uptake of *Faidherbia albida* seedlings in an alkaline sandy soil. Agrofor Syst 34 (2): 129-137. DOI: 10.1007/BF00148157

Bagyara DJ. 1996. Vesicular-arbuscular mycorrhiza fungi, nitrogen fixing bacteria and their interactions in improving plant productivity. Use of Mycorrhiza and nitrogen fixing symbionts in revegetating Degraded sites FORSPA Publication 14: 63-64.

Ball J. 1999. Understanding and Correcting Soil Acidity. Nobile Research Institute.

Barea JM, Azcon R, Azcon-Anguilar C. 1992. Versicular arbuscular mycorrhizal fungi in nitrogen fixing systems. In Noris JR, Read DJ, Varma AK, eds. Methods in Microbiology.

Barea JM, Werner D, Azcon-Anguilar C, Azcon R. 2005. Interactions of arbuscular mycorrhiza and nitrogen fixing symbiosis in sustainable agriculture. In Werner D, Newton WE, eds. Agriculture, Forestry, Ecology and The Environment.

Beegle D. 1990. The Role of Potassium. The Agronomy Guide 1989-1990.

Bennet MA. 2004. Seed and agronomic factors associated with germination under temperature and water stress. In Roberto L Benech, Rodolfo A Sanchez, (eds). Handbook of Seed Physiology Applications to Agriculture.

Bhatia NP, Adholeya A, Sharma A. 1998. Biomass production and changes in soil productivity during long term cultivation of *Prosopis juliflora* (Swartz) DC inoculated with VA mycorrhiza and *rhizobium* spp. in a semiarid wasteland. Biol Fertil Soils 26(3): 208-214. DOI: 10.1007/s003740050369

Bohlool BB, Ladha JK, Garrily DP, George T. 1992. Biological nitrogen fixation for sustainable agriculture: A perspective. In Ladha et al Editors, Biological Nitrogen Fixation for Sustainable Agriculture.

Brandon NJ, Shelton HM. 1993. Role of vesicular arbuscular mycorrhizae in *Leucaenia* establishment. Proceedings of the XV11 International Grassland Congress.

Brandon NJ, Shelton HM. 1997. Factors affecting the early growth of *Leucaena leucocephala*. Role of indigenous arbuscular mycorrhizal fungi and its importance in determining yield of *Leucaena* in pots and in the field. Australian J Exp Agric 37 (1): 45-50.

Brundett M, Bougher N, Dell B, Grove T, Malajczuk N. 1994. Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research.

Busman L, Lamb L, Randall G, Rehm G, Schmitt M. 2002. The Nature of Phosphorus in Soils.

Chang KP, Hu HT, Kao PC. 1996. Effect of endomycorrhiza fungi and *Rhizobium* inoculation on growth of *Acacia auriculiformis* in macdicken inoculation of forest rees: The use of *rhizobium*, frankia and micorrhiza. FORSPA Publication.

Chikowo R. 2004. Nitrogen Cycling in Agroforestry Systems of Sub Humid Zimbabwe: Closing The Loop.

Chiramel T, Bagyaraj DJ, Patil CSP. 2006. Response of *Andrographis paniculata* to different arbuscular mycorrhiza fungi. J Agric Technol 2 (2): 221-228.

Clark RB, Zeto SK. 2000. Mineral acquisition by arbuscular mycorrhiza plants. J Plant Nutr 23: 867-902. DOI: 10.1080/01904160009382068

Comly HH. 1987. Cyanosis in infants caused by nitrates in well water. J Am Med Assoc 257: 2788-2792. DOI: 10.1001/jama.1987.03390200128027

Daniels BA, Skipper HD. 1982. Methods for the recovery and quantitative estimation of propagules from soil. In: Methods and Principles of Mycorrhizal Research. The American Phytopathological Society, St. Paul.

Declerck S, Risede JR, Delvaux B. 2002. Greenhouse response of micropropagated bananas inoculated with in vitro monoxenically produced arbuscular mycorrhiza fungi. Sci Hortic 93 (4): 301-309. DOI: 10.1016/S0304-4238(01)00347-8

Diop TA, Gueye M, Plinchette C, Erya M, Dreyful BL, strullu DG. 2002. Growth and nutrient mineral status of *faidherbia albida* seedlings as influenced by inoculation with *Glomus mosseae*, brady *Rhizobium* and fertilization.

Dutt AK, Palhania U. 1983. Response of *Leuceana leucecocephala* to *Rhizobium* inoculation 7-10.

Fageria NK, Baligar VC, Jones CA. 1997. Growth and mineral nutrition of field crops. Exp Agric 47 (3): 574. DOI: 10.1017/S0014479711000263

Feng GS, Li XL, Christie P. 2003. Contributions of arbuscular mycorrhiza fungi to utilization of organic sources of phosphorus by red clover in

a calcareous soil. Appl Soil Ecol 22:139-148. DOI: 10.1016/S0929-1393(02)00133-6

Frizano J. 1999. Nature, distribution and biological significance of phosphorus in forests soils from Puerto Rico, Chile, and Brazil. [Dissertations]. University of Pennsylvania.

Ferrali, Alejandro E, Wall, Luis G. 2008. Coinoculation of black jack with *rhizobium* and glomus on a desurfaced soil. Soil Sci 173 (3): 195-202. DOI: 10.1097/ss.0b013e318163a9e6

Genstat. 2005. Genstat 8.11 Release for Windows XP. Lawes Agricultural Trust, Rothamstead Experimental Station, UK.

Gerdemann JW, Nicolson TH. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet-sieving and decanting. Trans Br Mycol Soc 46: 235- 244. DOI: 10.1016/S0007-1536(63)80079-0

Ghosh S, Verma NK. 2006. Growth and mycorrhiza dependency of *Acacia mangium* Willd inoculated with three vesicular arbuscular mycorrhiza fungi in lateritic soil. New For 31 (1): 75-81. DOI: 10.1007/s11056-004-4763-7

Gikonyo EW, Smithson PC, Nandwa SM, Lijzenga M, Jansen M. 2000. Effects of potassium fertilizers on some major food crops in Kenya. Soil Science Society of East Africa, Kenya.

Giller KE. 2001. Nitrogen fixation in tropical systems: Environmental constraints to nitrogen fixation. CABI Publishing.

Griffin T. 2006. Soil Organic Matter, Know Your Soils. University of Maine Extension Bulletin.

Gracy LS, Bagyaraj DJ. 2005. Influence of different AM fungi on growth nutrition and forskholin content of *Coleus forskohlii*. Mycol Res 109: 383-387. DOI: 10.1017/S0953756205002832

Gueye M. 1990. Effect of *Rhizobium* and *Rhizobium*/glomus inoculation on nitrogen fixation in bambara groundnuts. Biological Nitrogen Fixation and Sustainability of Tropical Agriculture 34: 283-287.

Harter DR. 2002. Acid Soils of The Tropics. Echo Technical Note.

Hicks PM, Loynachan TE. 1987. Phosphorus mycorrhiza fertilization reduces versicular arbuscular mycorrhiza infection and changes nodule occupancy of field grown soybean. Agronom J 79: 841-844. DOI: 10.2134/agronj1987.00021962007900050018x

Hogberg P. 1986. Mycorrhiza and Nitrogen Fixing Root Nodules in Trees in East and South-Central Africa. Umeaa (Sweden) Swedish Univ. of Agricultural Science.

Howell RW. 1960. Physiology of the soybean. Adv Agronom 12: 265-310. DOI: 10.1016/S0065-2113(08)60085-1

Jaetzold R, Schmidt H. 1983. Farm management handbook of Kenya volume II: Natural conditions and farm management information. Part Central Kenya: Ministry of agriculture/GTZ.

Jia Y, Gray VM, Straker CJ. 2004. The influence of *rhizobium* and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *Vicia faba*. Ann Bot 94:251-258. DOI: 10.1093/aob/mch135

Jia YS, Gray VM. 2004. Interrelationships between nitrogen supply and photosynthetic parameters in *Ficia faba* L. Photosynthetica 41: 605-610. DOI: 10.1023/B:PHOT.0000027527.08220.2c

Jones MD, Durall DM, Tinker PB. 1990. Phosphorus relationships and production of extrametrical by two types of willow ectomycorrhizas at different soil phosphorus levels. New Phytologist 115: 259-267. DOI: 10.1111/j.1469-8137.1990.tb00451.x

Kanyanjua SM, Ireri L, Wambua S, Nandwa SM. 2002. Acidic soils in Kenya: Constraint and Remedial Options. South Eastern Kenya University.

Kikuchi K, Matsushita N, Suzuki K, Hogestu T. 2007. Flavonoids induce germination of basidiospores of the ectomycorrhizal fungus *Suillus bovinus*. Mycorrhiza 17 (70): 563-570. DOI: 10.1007/s00572-007-0131-8

Koide RT, Kabir Z. 2000. Extraradicle hyphae of the mycorrhizal fungus *glomus intraradices* can hydrolyse organic phosphate. New Phytol 148: 511-517. DOI: 10.1046/j.1469-8137.2000.00776.x

Kumar M, Yadav K, Thakur SK, Mandal K. 1998. Effect of vesicular – arbuscular mycorrhiza fungi and *Rhizobium* inoculation, root colonization, nitrogen fixation and yield of chick pea. J Indian Soc Soil Sci 46: 375-378.

Leigh JG. 2002. Nitrogen Fixation at The Millennium. Elservier Science, London.

Leisueur D, Sarr A. 2008. Effect of single and dual inoculation with selected microsymbionts (rhizobia and arbuscular mycorrhiza) on field growth and nitrogen fixation of *Calliandra calothyrsus*. Agrofor Syst 73 (1): 37-45. DOI: 10.1007/s10457-008-9111-z

Lizzy JL. 1999. The effect of interactions between arbuscular mycorrhiza fungi and *Rhizobium* legumenosarum on pea and lentil (tripartite symbiosis, *Pisum sativum*, *Lens esculenta*). Dai 60-07B: 3027.

Mayland HF. 1983. Role of magnesium in plants. In: Fontenot JP. (eds.) Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Marques MS, Pagano M, Scotti MRMM. 1999. Dual inoculation of a woody legume (*Centrolobium tomentosum*) with rhizobia and mycorrhiza fungi in South-Eastern Brazil. Agrofor Syst 52: 107-117. DOI: 10.1023/A:1010637401475

Marschner H. 1992. Nutrient dynamics at the soil root interface (Rhizosphere). Mycorrhizas in Ecosystems. CAB International, Wallingford.

Marschner H, Dell B. 1994. Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159: 89-102. DOI: 10.1007/BF00000098

Mridha MAU, Mitra M, Purveen Z. 1992. *Rhizobium* interactions in productivity and nutrient content of yard long bean (*Vigna unguiculata sesquipedalis*). Mycorrhizas in Ecosystems, CAB International, Wallingford.

Muriuki AW, Quareshi JN. 2001. Fertilizer Use Manual. KARI.

Okalebo JR, Gathua KW, Woomer PL. 2002. Laboratory Method of Soil Analysis. A Working Manual, 2nd Edition.

Olsen T, Habte M. 1995. Mycorrhizal inoculation effect on nodulation and N accumulation in *Cajanus cajan* at soil P concentrations sufficient or inadequate for mycorrhiza-free growth. Mycorrhiza 5 (6): 395-398. DOI: 10.1007/BF00213438

Onsubi O, Bukare ON, Mulongoy K. 1992. Interactions between drought stress and V-AM on the growth of *Faidherbia albida* and *Acacia nilotica* in sterile and non-sterile soils. Biol Fertil Soils 14 (3): 159-265. DOI: 10.1007/BF00346056

Perucci P, Dumont S, Buffo SA, Masseur A, Caducei C. 2000. Effects of organic amendment and herbicide treatment on microbial biomass. Biol Fertil Soils 32:17-23. DOI: 10.1007/s003740000207

Postgate JR. 1998. Nitrogen Fixation. Cambridge University Press, Cambridge.

Punj V, Gupta RP. 1988. Mycorrhiza fungi and *rhizobium* as biological fertilizers for *Leucaena leucocephala*. In Macdicken KG, Inoculation of Forest Trees: The Use of *Rhizobium*, frankia and mycorrhiza. FORSPA Publishers.

Read DJ, Boyd R. 1986. Water relations of mycorrhizal fungi and their host Plants. In P Ayres, L Boddy (eds) Water Fungi and Plants. Cambridge University Press, Cambridge.

Sachs PD. 1999. Edaphos: Dynamics of A Natural Soil System.

Sande S, Ogol CKPO, Woomer PL. 2001. Effect of some common soil management practices on soil fauna populations in central Kenya. Africa Crop Science Conference Proceedings 5: 833-840.

Schroth G, Leymann J, Barrios E. 2003. Trees, Crops and Soil Fertility Concepts and Research Methods.

Sieverding E. 1991.Vesicular Arbuscular Mycorrhiza Management in Tropical Agrosystems. German Technical Cooperation.

Smith JL. 1994. Cycling of nitrogen through microbial activity. In: Hatfield JL and Stewart BA. (eds) Soil Biology; Effects on Soil Quality; Advance in Soil Science. Lewis Publishers, London.

Smith SE, Read DJ. 1997. Mycorrhiza Symbiosis. Academic Press, London.

Stanchaveva M, Geneva G, Zehirov M, Hristozkova, Georgiev G. 2006. Effect of combined inoculation of pea plants with arbuscular mycorrhiza fungi and *rhizobium* on nodule formation and nitrogen fixing activity. Gen Appl Plant Physiol Special Issue 61-66.

Xavier LJC, Gemida JJ. 2003. Selective interaction between arbuscular mycorrhiza fungi and *Rhizobium* legumenosarum bv. Viceae enhanced pea yield nutrition. Biol Fertil Soils 37: 267-267. DOI: 10.1007/s00374-003-0605-6

Young A. 1997. Agroforestry for Soil Management. CAB International, Wallingford.