Does Rhizobial Inoculation Functionality Vary With Host Plant Genotype? A Case Study of Common Bean *Phaseolus Vulgaris l.* Germplasms Grown by Smallholder Farmers In Eastern Kenya.

Ephraim Motaroki Menge,Ezekiel Mugendi Njeru, John Maingi,
Department of Microbiology,
Kenyatta University,
ephraimmenge@gmail.com

ABSTRACT

Rhizobia inoculants are soil bacteria that promote biological nitrogen fixation (BNF). Understanding of rhizobia-host genotype association is a critical step in enhancing legume productivity. Questionnaires were used to identify the common bean varieties grown in Eastern Kenya. The native rhizobia were obtained from the root nodules of MAC 13 and MAC 64 bean varieties, which were used for trapping. Afterwards, a greenhouse bioassay was set up in a complete randomized design with three replications. Four weeks later, beans were sampled and examined for nodule number (NNO), nodule dry weight (NDW), shoot dry weight (SDW), root dry weight (RDW), shoot nitrogen (%N) and phosphorus (P). Results demonstrated that highest and significant ($p<0.001$) NDW, SDW and shoot %N content were achieved in a mix of native consortium+exotic rhizobia (Biofix), while the highest and significant ($p<0.001$) P content was realized in a consortium of native rhizobia inoculation. Moreover, there was a significant interaction ($p=0.001$) between rhizobia and bean varieties with Kabuu recording the highest NDW, SDW, %N and P contents in a mix of native consortium+exotic rhizobia. Gacere recorded the highest NDW and SDW when inoculated with exotic rhizobia. Native rhizobia inoculation recorded the highest shoot %N variability in all bean varieties when compared to exotic and a mixture of native+exotic rhizobia. These results show the mutual preference that exists between rhizobia and bean varieties and the multistrain synergism between native and exotic rhizobia. Further studies should explicate the performance of diverse native rhizobia inoculants used in this study under field conditions.

Key words: Biological nitrogen fixation, Rhizobia inoculation, Common bean, Eastern Kenya

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important nutritious food legumes in the Sub-Saharan Africa (SSA) region (Hillocks, 2011). The crop plays a significant dietary function of supplying proteins, essential vitamins and carbohydrates to both urban and rural communities (Hamdani and Wani, 2017). According to Thornton *et al*. (2010), the crop is estimated to contribute to more than 50% of the dietary protein to the households in the entire SSA with the annual highest consumption per capita being among the low-income people. The consumption of common beans varies in the different regions of the globe. For instance, in Latin America, the consumption of the crop per capita lies between 4 and 17 kg year^{-1} (Leterme and Muñoz, 2002) and corresponds to their production per acreage. Contrarily, in Eastern Africa, the bean consumption per capita is

about 50 to 60 kg year^{-1} with the consumption of the crop being relatively higher in Kenya, Rwanda, and Uganda (Canfield *et al*., 2010).Hence, there is a need to enhance the productivity of common beans in Eastern Africa to meet the high consumption demand.

Despite the importance of common bean crop, its yield potential has not yet been maximized especially, in the resource-limited regions such as SSA, where the average production is still less than the consumer demand (National Research Council, 2009). The low yield of common bean in SSA has been associated partly to the low nitrogen and phosphorus content in the soil. Soil acidic conditions exacerbated by phosphorus (P) fixation, aluminum toxicity and drought stress brought by climate change are also to blame (Bekunda *et al*., 2010). Efforts by the smallholder farmers to enrich soil using synthetic chemical fertilizers has been derailed by the rising cost of living, high cost of farm inputs and limited knowledge on modern agronomic practices (Pretty, 2008).

Common beans in association with plant growth promoting rhizosphere bacteria such as rhizobia carry out a significant role in biological nitrogen fixation (BNF). BNF occurs through a high energy driven complex reaction where nitrogen from the atmosphere is transformed to ammonia by the enzyme known as nitrogenase in the reaction ($N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2$). The reduction of nitrogen into ammonia requires energy in the form of ATP for oxidation of sugars and other compounds (Peters and Boyd, 2015). The host plant through the process of photosynthesis synthesizes carbohydrates that undergo oxidation and are utilized by the bacteria as substrates.

The potential of BNF in enhancing common beans productivity in SSA is limited by unfavorable soil and climatic conditions. Soil pH, insufficient nutrient content, temperature and water stress being the most restraining factors in bean production (Wekesa *et al*., 2017). Bio-inoculants containing complex formulations of plant growth promoting bacteria have been designed to improve early growth, BNF and plant yields. However, most inoculant formulations contain exotic bacterial isolates, which may not survive or perform efficiently in SSA due to unfavorable edaphic conditions and negative microbial interactions. This necessitates the use of native rhizobia strains well adapted to the local ecological conditions and that are effective in nodulation and N-fixation with different common bean cultivars grown in that particular ecological niche (Sessitsch*et al*., 2002). Common beans □nlike other leg□mes are poor nitrogen fixers; however, some common bean genotypes and specific rhizobia strains exhibit high potential for N-fixation (Fageria, 2002). In some instances, increasing the diversity of rhizobia strains could enhance N-fixation ability of the plants (Remans*et al*., 2008).

Currently, the research and developmental efforts have consistently focused on overcoming the limiting conditions of the production constraints of common bean. The emphasis is mainly focusing on abiotic factors while giving minimal regard to biotic factors like rhizobia bacteria and local bean germplasms, which have a high potential in developing an effective symbiotic nitrogen fixation native rhizobia strains. In addition, rhizobia inoculants can be used to produce ecofriendly bio-fertilizers. Besides, the efficiency of BNF in selected common bean varieties grown in Eastern Kenya has not been evaluated. Interestingly, native rhizobia isolated from the tropical areas of the SSA were found to increase BNF in soil relative to the available commercial exotic strains (Chianu*et al*., 2011). According to the study conducted by Overbeck *et al*. (2007), the BNF

between the native rhizobia and native legume plants of genus *Desmodium* increased the level of nitrogen in the soil.

A study by Mburu *et al*. (2016) on crop diversity in Eastern Kenya indicated that farmers maintain a wide diversity of common bean cultivars due to the significant role they play in BNF and food security. Previous research has shown the variability of diverse bean lines to form nodules and fix nitrogen through BNF. For instance, Subbarao *et al*. (1995) and Valentine *et al*. (2010) have reported common bean genetic variability in N-fixation activity as a possible reason explaining why some bean cultivars accumulate high nitrogen contents under water deficit and phosphorus deficient conditions. In this case, it is essential to determine the performance of different common bean cultivars grown by smallholder farmers in regards to nitrogen fixation and their yield potential in different contrasting environments. The bean lines that show high nitrogen fixing potential and improved yield potential could be used for cultivar development.

In this study, we hypothesized that nodulation and BNF effectiveness of a diverse group of native rhizobia, exotic rhizobia (Biofix) and their functionality in promoting BNF vary with bean genotypes grown by smallholder farmers in Eastern Kenya. The specific objectives of this study were; (1) to determine and compare the symbiotic nitrogen fixation effectiveness of native and exotic rhizobia nodulating different common bean varieties grown by smallholder farmers in Eastern Kenya, (2) to determine whether increasing *Rhizobium* isolate diversity would enhance nodulation and nitrogen fixation in common beans, and (3) to determine the effect of rhizobia inoculation on nodulation, growth and shoot nutrition (N and P) content of common bean varieties grown in Eastern Kenya.

MATERIALS AND METHODS

Bean variety identification and collection of germplasm

Ten common bean cultivars were identified by conducting an in-person interview with farmers in Eastern Kenya. Sixty households were interviewed, 32 households in Embu County and 28 in Tharaka Nithi County. The two counties were chosen due to their high production potential of diverse common bean cultivars in both upper and lower midland agro-ecological zones. Based on the interviews, the preferred bean varieties were; Kasango, Mwitemania brown, Mwitemania white, Karoyo, Muviki and Gacere and four non-climbers; Rosecoco, Get□r□, Kab□□and Kayiero. Confirmation of these bean varieties was done at Kenya Agricultural Livestock and Research Organization (KALRO) in Embu, Kenya. A half a kilogram of healthy untreated seeds of each bean variety, kept by the interviewed farmers in the previous season before the interview, were collected randomly for use in the greenhouse bioassays.

Soil characterization and analysis

Soil samples from fo□r farms; two in each Co□nty, were collected before the start of long rainy season of March 2015. The sampling of soil was done on diagonal transects from 20 locations in each of the selected farms by making cores of 5-20 cm deep using a spade. The spade was sterilized

before making each core with 5 % sodium hypochlorite solution and then rinsed in three changes of sterile water after which it was dried with a sterile cloth. The soil samples were mixed thoroughly to make a composite sample, which after drying was sieved through a 2 mm diameter strainer to make a homogenous composite soil that was used in the greenhouse. One-kilogram sample of the composite soil was analyzed for soluble salts in the laboratory by use of both physical and chemical methods. Walkley-Black technique was used to establish the carbon content. Both Ca and Mg ions were assessed by the use of atomic absorption spectrophotometry while Bray-I technique was used to determine soil phosphorus (Robert, 1993; Okalebo *et al*., 2002).

Preparation of yeast mannitol broth

The yeast mannitol broth was made by combining 1 gram of baker's yeast, 0.5 g K_2HPO_4, 10 g of Mannitol, 0.2 g $MgSO_4.7H_2O$, 0.1 grams of NaCl, as well as 1 gram of $CaCO_3$ so as to give the broth. The ingredients were suspended in a liter of distilled water, heated to boil, and mixed thoroughly. The Yeast Mannitol media were then autoclaved at a temperature of 121 °C and pressure of 15 atmospheres for 15 minutes (Tomaszewska *et al*., 2012).

Field trap cultures and isolation of native rhizobia

The trap cultures of native rhizobia were set in four selected farms, with no previous rhizobia inoculation, in both Counties of Embu and Tharaka Nithi using MAC 64 and MAC 13 bean varieties obtained from Kenya Seed Company Limited (Nairobi, Kenya). These two varieties were selected because they are compatible with the native rhizobia in Eastern Kenya (Koskey*et al*., 2017). Quality seeds of both MAC 13 and MAC 64 were selected and planted in each farm after tilling. The bean varieties were supplied with phosphorus by applying Triple Superphosphate (46.0% P_2O_5) fertilizer at a rate of 50 kg ha^{-1}. Thirty days after emerging, ten bean plants from each farm were randomly sampled and harvested by making a 15 cm radius circle around the plant with a cut out section of 20 cm deep using a spade. The clump was then lifted slowly and soil carefully removed from the plant roots. The root nodules were detached and washed in sterile water to remove soil particles. Nodules were packed in sampling vials containing desiccated silica gel and cotton wool and transported to the Microbiology Research Laboratory at Kenyatta University, Nairobi, Kenya, for isolation of rhizobia.

In the laboratory, rhizobia were isolated from the root nodules following the procedures described by Somasegaran and Hoben (1994). Nodules were surface sterilized by wrapping them in a muslin cloth containing 90% alcohol for 1 minute. The nodules were then thoroughly washed using six changes of sterilized water after which the small roots were removed. The obtained nodules were crushed using a sterile glass rod in a watch glass containing 0.5 ml of sterile water. To ensure that proper sterilization was performed, the plates containing Yeast Extract Mannitol Agar (YEMA) complemented with Congo red (CR) were used to test the sterility (Vincent, 1970). One plate was streaked with a loop full of sterile water (control), the second plate was streaked with water used for the sixth change of the root nodules and the third plate was streaked by a loop full of the nodule exudates. The plates that had colonies were picked and subjected to further purification. If a culture

had many colonies, the colonies were aseptically transferred to separate plates of YEMA-CR and treated as separate isolates.

Verification of isolated rhizobia

Typical rhizobia were recognized by cultural and morphological appearance, Gram staining, biochemical tests such as the production of acidity or alkalinity in YEMA with Bromothymol blue and growth on peptone glucose agar (Vincent, 1970). Gram staining was carried out following the procedures outlined by Claus (1992). The identity of rhizobia was recognized by weak absorption of the Congo-red dye, a characteristic that is not found in any other agro-bacteria. Other distinct features such as colony shape, elevation, margin, exo-polysaccharide (EPS) production were used to identify rhizobia (Maingi *et al*., 2001). Upon using YEMA supplemented with Bromothymol blue indicator, production of yellow coloration was detected, an indication of fast growing acidifying rhizobia bacteria (Workalemahu and Assefa, 2007). In the last method, rhizobia bacteria were cultured in plates containing peptone glucose agar, then incubated at 28 ^{0}C for 48 hours. The absence of bacteria growth was a clear indication of the presence of *Rhizobium* bacteria (Hotter and Scott, 1991). Based on morpho-cultural and biochemical features, the native rhizobia isolates were grouped in to nine distinct morphotypes. Verification of isolates used in this study as rhizobia bacteria was based on the descriptions outlined by Somasegaran and Hoben (1994).

Rhizobia inoculum preparation

Nine pure isolates of native rhizobia (obtained from each distinct morphotype group), exotic rhizobia (Biofix) and a mix of all the nine native isolates and exotic rhizobia were aseptically transferred into three different identical conical flasks containing 100 ml of YEM broth. The consortium of native rhizobia was prepared by mixing all the nine native isolates in equal volumes of 1 ml each. Similarly, a mixture of native consortium and exotic isolates were prepared by mixing an already prepared consortium of native rhizobia with the exotic isolates in equal volume ratio of 1:1. The rhizobia isolates were then incubated in a rotary shaker at 28 ^{0}C, 7 days prior to planting. After 5 days of incubation, the bacteria isolates revealed moderate turbidity in YEMB a clear characteristic of viable rhizobia (Workalemahu and Assefa, 2007).

Greenhouse bioassays

Experimental design

The greenhouse experiment was set using a completely randomized design (CRD) with 10 common bean varieties as the main treatments and four rhizobia inoculants as the sub-treatments. The ten bean varieties included Kabuu, Gacere, Geturu, Muviki, Mwitemania brown, Mwitemania white, Kasango, Kayiero, Karoyo and Rose coco. Rhizobia inoculants included a consortium of native rhizobia (NTV), exotic rhizobia (EXT) - Biofix from MEA limited, Nakuru, Kenya, and a mix of native consortium + exotic rhizobia (MIX).

Soil sterilization, seed preparation and pre-germination

A kilogram of homogenously mixed soil (section 2.2) containing sand (1:1 by volume) was sterilized and aseptically transferred to sterile pots. Uniform seeds of the ten common bean varieties were surface disinfected by submerging them in 95% ethanol for 15 seconds to eliminate air and waxy material after which they were submerged in a sterile flask containing 3 % NaClO for 3 minutes. The seeds were then washed in six changes of sterile water. The bean seeds were left in the last change of sterile water for four hours until they were completely soaked up. The bean seeds were again washed in two changes of sterile water. A short time later, they were aseptically transferred with a sterilized forceps onto the surface of a 2 % water agar petri-dish and incubated at a constant temperature of 25 °C until they developed a radical of about 1 cm long (Elfeel, 2012).

Planting and inoculation of the seedlings

Three holes were made in the soil medium each one centimeter deep. The water agar pre-germinated seedlings were aseptically picked with a sterilized forceps and sown one seed for every hole. After 5 days of planting, the young seedlings were thinned to two uniform plants per pot and inoculated with an exact quantity of 1 ml of broth having 10^9*Rhizobium* microbes using a micro-pippete. The seedlings were inoculated with a consortium of native rhizobia (NTV), exotic strain (EXT), a mix of native + exotic rhizobia (MIX), and a control, with no rhizobia inoculation (CONTROL). Each treatment was then replicated 3 times with 2 plants in each pot.

Crop maintenance and harvesting

Plants were irrigated 2 times a week with sterile water until sampling time. Throughout this time, the plant growth and leaf color were frequently noted for any abnormality. During the study period, the highest daytime temperature recorded was 30 ^{0}C while the lowest was 24 ^{0}C. After 28 days, the three replicates of bean varieties from each treatment were randomly selected, removed from pots and separated into shoots, roots, and nodules with each treatment being kept in separate sampling bags. The sampled plants were then dried in an oven at a temperature of 70 ^{0}C until a constant weight was attained. The shoot samples were then analyzed for nitrogen (N) and phosphorus (P) (Jensen *et al*., 2010).

Biomass measurements and determination of shoot nutrients (N and P) content

The dry weights of roots, nodules and shoots were measured and recorded using digital weighing balance. Kjedahl procedure was used to determined shoot nitrogen (%N) content (Justes *et al*., 1994). Shoot phosphorus was determined by using colorimetric and photometric procedure, which involved sulphuric-perchloric acid digestion (Leidi and Rodriguez-Navarro, 2000).

Data analyses

The greenhouse data were tested for homogeneity of variance using Bartlett test before analyses. The percentage data were arcsine ($\sqrt{x}$) transformed, whereas other data were log (x+1) transformed

wherever it was necessary to achieve the expectations of ANOVA. The data reported in tables and graphs was as well back transformed. Two-way ANOVA was used to analyze data obtained from the greenhouse experiment based on a completely randomized design. Pearson correlation coefficient was used to find out the relationship between growth parameters and nitrogen fixation. Wherever applicable, post hoc test was executed using Tukey's HSD test ($P< 0.05$). All statistical analyses were performed using the general linear model (GLM) procedure of the Statistical Analysis System (version 9.0) (SAS Institute Inc., Cary, NC, USA).

RESULTS

The soil used in the greenhouse experiment were slightly acidic with a pH of 5.93 (Table 1). The soil had moderate amounts of organic carbon (2.8%), total nitrogen (0.24%), potassium (2.7 cmol/kg) and calcium ions (9.1 cmol/kg). The available phosphorus and magnesium ions were slightly higher above the critical limits described by Okalebo *et al*. (2002). The soil texture was sandy clay loam (Table 1).

Forty-one isolates of nodule occupants with morphological, cultural and biochemical characteristics of common bean rhizobia as described by Herridge (1982) and Somasegaran and Hoben (1994) were isolated from MAC 13 and MAC 64 bean varieties.The 41 isolates were grouped into nine distinct morphotypes based on their morpho-cultural and biochemical features. All the isolates were Gram-negative rods, fast growers and turned YEMA-BTB from green to yellow. The isolates did not absorb Congo red dye upon culturing in YEMA-CR media and did not show any growth in peptone-glucose agar media. The isolates showed varied morpho-cultural features including exhibiting white, milky, creamy, firm gummy, soft gummy or watery colonies with domed, convex or raised elevations.

From the greenhouse bioassays, inoculation with a mixture of native and exotic rhizobia isolates significantly ($P< 0.001$) enhanced common bean nodulation, recording the highest NNO (68.87±6.28 plant^{-1}) and NDW (0.0835±0.007 g plant^{-1}) (Table 2). Bean inoculation with a consortium of native rhizobia performed relatively better recording an average of 58.73 ±6.18 g plant^{-1} and NDW of 0.0603±0.006 g plant^{-1} compared to the inoculation with exotic rhizobia alone which recorded an average of 44.20±4.65 nodules plant^{-1} and NDW of 0.0484 ±0.005 g plant^{-1}. There was a significant ($P< 0.001$) nodulation difference observed across the ten bean varieties with Kabuu bean variety recording the highest NNO and NDW (74.42±14.94 plant^{-1}, 0.0757±0.014 g plant^{-1} respectively) while Rose cocoa recorded the lowest NNO and NDW (17.83±4.54 plant^{-1}, 0.0218±0.006 g plant^{-1} respectively). As expected, the controls without rhizobia inoculation did not show any nodulation. A significant ($P = 0.001$) interaction between rhizobia isolate inoculation and common bean variety was shown with Kabuu bean variety recording the highest nodulation (NDW=0.126±0.086 g plant^{-1}) when inoculated with a mixture of native + exotic rhizobia (Figure 1). Gacere bean variety recorded the highest NDW when inoculated with native rhizobia (0.121±0.019 g plant^{-1}) and exotic rhizobia (0.100±0.005 g plant^{-1}).

There was a significant ($P< 0.001$) difference in shoot dry weight (SDW) of the common bean

varieties tested upon inoculation with rhizobia, with a mixture of native consortium + exotic rhizobia recording the highest SDW (2.365±0.089 g plant^{-1}) when compared with exotic (2.082±0.068 g plant^{-1}) and native rhizobia (2.146±0.079 g plant^{-1}) inoculations (Table 2). The control, with no rhizobia inoculation, was the least, producing an average SDW of 1.666±0.048 g plant^{-1}. There was a significant (P< 0.001) SDW difference observed across the ten bean varieties with Kabuu recording the highest SDW at an average of 2.715±0.159 g plant^{-1} while Kayiero had the least SDW at an average of 1.765±0.114 g plant^{-1}. A significant (P = 0.001) interaction between rhizobia inoculation and common bean varieties was shown with Kabuu producing the highest SDW in a mix of native + exotic rhizobia (3.257±0.014 g plant^{-1}) (Figure 2).There was a significant (P< 0.001) difference in RDW of the ten bean varieties with Muviki recording the highest RDW of 0.721±0.0614 g plant^{-1}, while Mwitemania white recorded the lowest RDW with an average of 0.401±0.0558 g plant^{-1} (Table 2).

Results of shoot nitrogen indicated that different rhizobia isolates significantly (P< 0.001) enhanced shoot nitrogen in inoculated common beans when compared with the un-inoculated controls (Table 3). The multi-strain mixture of both native consortium + exotic rhizobia recorded the highest shoot nitrogen at an average of 3.398±0.08 percent; the least shoot percentage nitrogen was recorded by the un-inoculated controls at an average of 2.114±0.06 percent. Likewise, a significant difference (P< 0.001) in percentage shoot nitrogen of bean varieties tested was revealed with Kabuu bean variety producing the highest percentage nitrogen at an average of 3.216±0.20 percent (Table 3). Kayiero produced the least shoot percentage nitrogen at an average of 2.187±0.12 percent. Moreover, there was a significant (P = 0.006) interaction between rhizobia inoculation and bean varieties with Kabuu recording the highest percentage nitrogen (3.973±0.067 g plant^{-1}) in a mixture of both the native consortium + exotic rhizobia (Figure 3). Muviki bean variety recorded the highest percentage nitrogen (3.753±0.090 g plant^{-1}) when inoculated with native rhizobia, while Gacere bean variety recorded the highest percentage nitrogen (2.863±0.071 g plant^{-1}) when inoculated with exotic rhizobia.

Rhizobia inoculation with different isolates revealed a significant difference (P< 0.001) in shoot phosphorus compared to un-inoculated controls with native rhizobia showing the highest amount of shoot phosphorus at an average of 9293.70±291.12 ppm (Table 3). Inoculation with a mixture of native consortium + exotic rhizobia recorded an average of 9000.13±288.92 ppm while exotic rhizobia showed an average shoot phosphorus of 8704.27±196 ppm. A significant difference (P< 0.001) was also revealed among the ten bean varieties with Kabuu recording the highest amount of shoot phosphorus at an average of 11740.92±671.99 ppm. The least amount of shoot phosphorus was recorded in Kayiero with an average of 6654.25±219.27 ppm (Table 3). In addition, there was a significant (P = 0.003) interaction between rhizobia inoculation and bean varieties with Kabuu bean variety recording the highest amount of shoot phosphorus in a mixture of both the native consortium + exotic rhizobia (14635.33±19.33 g plant^{-1}) and when inoculated with exotic rhizobia (10685±15.81 g plant^{-1}) (Figure 4). Muviki bean variety recorded the highest amount of shoot phosphorus (12816.67±17.93 g plant^{-1}) when inoculated with native rhizobia.

The analysis on the relative increase in shoot dry weight (SDW) as influenced by different rhizobia inoculants revealed a significant difference (P< 0.001) among the ten common bean genotypes

(Figure 5). However, the relative increase in SDW of Geturu and Karoyo varieties was non-significant (P=0.09) compared to that of other varieties upon inoculation with all the rhizobia inoculants. Similarly, the relative increase in SDW of Kabuu variety was non-significant (P=0.106) compared to that of other varieties upon inoculation with exotic rhizobia inoculant (Figure 5).

The shoot nitrogen analysis of different bean varieties revealed a significant difference (P<0.001) upon rhizobia inoculation when compared to the non-inoculated controls (Figure 6). There was a greater shoot percentage nitrogen variability observed in most of the bean varieties inoculated with native rhizobia when compared to those inoculated with exotic and a mixture of native and exotic rhizobia. The ten bean varieties produced the highest relative increase in percentage nitrogen content due to inoculation with a mixture of both native consortium + exotic rhizobia while the least relative increase in percentage nitrogen was observed in exotic rhizobia inoculation (Figure 6).

Finally, there was a positive and a significant correlation (R^2 = 0.7361, P<0.0001*)* between nodule dry weight and shoot dry weight where an increase in nodule dry weight resulted to an increase in shoot dry weight (Figure 7). Similarly, a positive and a significant correlation (R^2 = 0.4702*,* P<0.0001) between nodule dry weight and percentage nitrogen was observed (Figure 8).

DISCUSSION

The physic-chemical analysis revealed that the soil from Eastern Kenya was slightly acidic and rich in phosphorus content. Phosphorus is crucial to proper development of leaves and dry matter in legumes. The slightly acidic nature of the soil could interfere with the maximal functioning of nitrogen-fixing rhizobia strains. Kawaka *et al*. (2014) suggests that bean plants require neutral soil for appropriate growth especially when they rely exclusively on symbiotic nitrogen fixation for nitrogen acquisition.The presence of microelements such as calcium and magnesium in the soil used in this study is an indication that the soil were suitable for use in the greenhouse since most plant tissues require micro-nutrients for their development (Hart *et al*., 2003).

Generally, the morpho-cultural, biochemical and other phenotypic properties of the 41 rhizobia isolates obtained from the field trapping using MAC 13 and MAC 64 climbing bean varieties showed a large variation and grouped into nine distinct morphotypes. The morpho-cultural, biochemical and Gram staining characteristics confirmed the isolates used in this study as common bean rhizobia as described by Somasegaran and Hoben (1994), Hungria (2000) and Kawaka *et al*. (2014).

From the greenhouse study, the inoculation of different common bean cultivars with a multi-strain mixture of both native consortium and exotic rhizobia revealed a significant increase in bean nodulation, shoot biomass and N-fixation. This could be attributed to the multi-strain synergistic effect caused by the diverse strains of native and exotic rhizobia applied to the beans during inoculation. A proper combination of different infective and effective *Rhizobium* strains enhances nodule occupancy, biological fixation of nitrogen and common bean development. These results are similar to those of Hungria *et al*. (2000) who noted that a combination of specific rhizobia

strains, which are well adapted to the local ecological conditions, performs better in promoting N-fixation and growth of different bean cultivars as compared to the use of individual rhizobia strains.

Among the ten common bean genotypes studied, inoculation with a mixture of both native consortium + exotic rhizobia produced the highest NDW in Kabuu bean variety, while inoculation with exotic rhizobia produced the highest NDW in Gacere bean variety. This NDW variability as influenced by different rhizobia inoculations could be associated with the fact that there exists rhizobia-bean preference. This could further relate to the genetic variability and the type of exudates that the plant produce to attract a specific rhizobia. These results relate to that of Triplett and Sadowsky (1992) and Mhamdi *et al.* (2002), who found that different bean genotypes prefer certain rhizobia strains for nodulation and nitrogen fixation. Notably, bean inoculation with the consortium of native rhizobia showed a significant increase in root nodulation producing a relatively higher NDWthan that of exotic strains. This could be because native rhizobia are well adapted to the local agro-climatic and edaphic conditions and moreover, native rhizobia have developed a long-term symbiotic association with the existing native bean plants. These results relate to the work done by Romdhane *et al.* (2007) who found out that native rhizobia are well adapted to the native bean genotypes and can compete more effectively in root colonization when compared to the exotic rhizobia strains.

For shoot dry weight, all rhizobia inoculations revealed a significant difference with a combination of native consortium + exotic rhizobia producing the highest SDW and this was likely because of the synergistic effects of native and exotic rhizobia isolates in symbiotic nitrogen fixation. Common bean (*Phaseolus vulgaris* L.) is a non-selective plant host and can perceive signals for nodulation from different strains of homologous and non-homologous rhizobia and this may promote nitrogen fixation, plant growth and development (Mitchell-Olds *et al*., 1998). These findings relate to the study carried out by Zablotowicz *et al*. (1991) who found that increasing rhizobia diversity enhances shoot dry weight in bean plants.There was significant SDW differences observed across the ten bean varieties and upon inoculation with the three rhizobia inoculants. Additionally, due to the interaction between bean varieties and rhizobia inoculation, Kabuu variety recorded the highest SDW upon inoculation with a mixture of native + exotic rhizobia. Inoculation with exotic rhizobia produced the highest SDW in Gacere bean variety. These results could suggest that increasing rhizobia diversity increases SDW of bean plants. Interestingly, in some bean varieties such as Gacere and Geturu, inoculation with native rhizobia was not as efficient in shoot biomass accumulation as that of exotic rhizobia and thus there could be a need to introduce other compatible rhizobia strains for maximum bean development to be achieved. In support of this, Hungria *et al*. (2003) observed that for high shoot dry weight to be achieved in bean plants, proper combination of *Rhizobium* strains hasto be identified to enhance more competitiveness in nodule occupancy, nitrogen fixation and production of shoot biomass. The root biomass of the ten bean varieties varied significantly. The difference could be attributed to the bean genotype, which could affect root development (Aguilar *et al*., 2001). On the other hand, rhizobia inoculation did not significantly affect the RDW of the 10 bean varieties studied. These findings are similar to those reported by Koskey *et al*. (2017) who reported non-significant difference in RDW of MAC 13 and MAC 64 climbing beans inoculated with different rhizobia isolates.

Shoot nitrogen analysis revealed that all rhizobia inoculants enhanced significantly the shoot nitrogen when compared with the non-inoculated controls, with the combination of native consortium + exotic rhizobia producing the highest percentage nitrogen due to the multi-strain synergism between the native and exotic rhizobia. This result suggests that an increase in rhizobia diversity could enhance the shoot percentage nitrogen. This also indicates that to achieve higher shoot nitrogen content in common bean, the use of native rhizobia alone may not be satisfactorily effective and thus there is a necessity to introduce other effective rhizobia strains that could offer synergistic benefits to the plants. This study relates to the work done by Maingi *et al*. (2001) who observed that for high shoot nitrogen content in bean plants to be achieved, proper combination of *Rhizobium* strains have to be identified to enhance more effective fixation of nitrogen. Similarly, a significant difference in percentage shoot nitrogen of bean varieties tested revealed that Kabuu variety recorded the highest percentage nitrogen while Kayiero produced the least shoot percentage nitrogen. This variation in percentage nitrogen accumulation in plant shoots could be explained by the genetic variation of the beans, which indirectly affects the symbiotic association with the rhizobia bacteria found in the plant rhizosphere. This relates to the work done by Ramaekers *et al*. (2010) who noted that for high shoot nitrogen content to be accumulated by the bean plants, compatible bean varieties and effective *Rhizobium* strains have to be identified.

The relative increase in SDW and shoot percentage nitrogen of the ten bean varieties as influenced by the three rhizobia inoculants varied significantly. Bean genetic variation and the difference in chemical exudates signalling rhizobia during root infection could affect bean-rhizobia compatibility and consequently affecting the performance of BNF process (Ramaekers *et al*., 2010). A greater shoot percentage nitrogen variability observed in most of the bean varieties inoculated with native rhizobia could be explained by the fact that native rhizobia are well adapted to the local edaphic conditions and have developed a long term symbiotic association with the existing local bean varieties.

The positive and significant associations between NDW and SDW, NDW and shoot nitrogen confirms the dependence of bean shoot biomass accumulation and nitrogen fixation on nodulation. These results support the assertions made by Kawaka *et al*. (2014) and Koskey *et al*. (2017), that there is a direct association among nodulation, plant growth and nitrogen accumulation in legume plants.

The shoot phosphorus analysis showed that all plants inoculated with rhizobia showed a significant increase with native rhizobia producing the highest phosphorus content. These findings are similar to the study by Neila*et al*. (2012) who observed that native rhizobia increase shoot phosphorus in bean plants. Native rhizobia together with other localized plant growth promoting bacteria are known to form synergistic associations that would lead to phosphate solubilization in the soil and hence availing phosphorus for plant uptake (Leidi and Rodriguez-Navarro, 2000). Among the bean genotypes, Kabuu bean variety accumulated the highest shoot phosphorus content upon inoculation with all the three rhizobia inoculants. This result suggests that specific bean varieties respond well to rhizobia inoculants regardless of the diversity and hence such varieties are suitable for cultivation. Therefore, for higher shoot phosphorus content to be achieved in common beans, variety response against inoculants should be screened and compatible genotypes should be

identified. Ramaekers *et al*. (2010) observed that for high shoot phosphorus content in bean plants to be achieved, compatible bean varieties and effective *Rhizobium* strains have to be identified.

CONCLUSION

In this study, it was established that Kabuu bean variety responded better to inoculation than any other bean varieties. Kabuu bean variety should therefore be considered for further screening for other beneficial properties such as yield in different agroecologizal zones in Kenya. The rhizobia inoculation functionality varied significantly in nodulation, plant growth parameters and nitrogen fixation with the ten common bean varieties tested in the greenhouse. It was evident that a mixture of the consortium of native + exotic rhizobia enhanced nodulation, shoot biomass and shoot percentage nitrogen content in common bean varieties grown by smallholder farmers in Eastern Kenya. Thus, diversification of rhizobia isolates should be considered when developing affordable rhizobia biofertilizer inoculants for use by resource limited smallholder farmers in bean production. These results demonstrate a key performance of different common bean varieties grown by smallholder farmers in regard to nitrogen fixation and form an important step towards the selection and development of bean cultivars with high biological nitrogen fixation potential. Further studies should elucidate the performance of the various rhizobia inoculants used here under field conditions.

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