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Efficacy of different rhizobia strains with specific common beans landraces in biological nitrogen fixation

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Abstract

Bean production in Tanzania barely meets half of the domestic demand because of low yield attributed to low fertility, nitrogen being the most common deficient. The use of rhizobia inoculants can substantially increase bean yields. However, less than 1% of the farming population is aware of inoculants. The aim of the study was to evaluate the efficacy of different rhizobia strains with specific common beans landraces in biological nitrogen fixation and yields. Four experiments were conducted during in a screen house at Sokoine University of Agriculture (SUA). Two experiment consisted Leonard jar and the other two were pot experiments in which the soil used were either sterilized or unsterilized. The landraces collected were Kasuka Nywele, Msafiri and Manjano from Mbeya; Nyayo, Soya and Kachumbaa from Arusha; and Kabungu, Nyamungu and Karanga from Morogoro. Two sets of Leonard jars were arranged in completely randomized block design in triplicate and the other two pot experiments were arranged in split split plot design. Inoculants in pot experiments used were Nitro-SUA, Underwood Biostacked as commercial and native. Landrace were the main plot, soil sterilization as a sub plot and inoculants as sub sub plot replicated three times. Data were subjected to analysis of variance using GENSTAT software. Significance differences in N_2 fixation were observed among all landraces in all experiments, promising results were observed from Nyayo, Nyamungu, Msafiri and Kabungu. Native rhizobia had comparable symbiotic effectiveness to commercial inoculants in unsterilized soils and vice versa in sterilized soils conditions though soil sterilization was not significant. The findings on this study provide rooms for further research especially on the promising landraces to nodulation and explore their effectiveness.

Keywords: Common beans; Rhizobia; Nitrogen fixation; Landrace; Seed inoculations

1. Introduction

Common beans (*Phaseolus vulgaris L*) belongs to the tribe *Phaseoleae*, subfamily *Papilionoideae*, Family *Leguminosa*e, or Rosales (CIAT, 2006). Plants of common beans may be either bushy or trailing. Bushy beans are determinate in growth habit and stem elongation ceases when the terminal flower racemes have developed. Most cultivars complete their life cycle (germination to seed maturity) in 100 to 130 days. Beans are important crop that needs to be assessed for its capacity to fix nitrogen (N_2) as source of N for increasing its yields, since many people depend on the crop for food and nutrition. In Tanzania, like in many other African countries, the crop is used not only as the less expensive source of dietary protein to both rural and urban communities, but also as a cash crop. Production systems for common beans are characterized by extensive use of different varieties or landraces by the farmers. These landraces present a potential source of germplasm for improvement of the bean crop (Martin and Adams, 1987), both in yields and in N_2 fixation.

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Improve productivity in common bean cultivation is the exploitation of Symbiotic Nitrogen Fixation (SNF) which involves a symbiotic interaction between leguminous plants and *Rhizobium* bacteria Lara (2011). Ahmet & Halime(2023) reported that many studies show that the use of rhizobium as a bio fertilizer (inoculant) is effective in cowpea. Kumar & Kumar, (2020) reported that Since ancient times, it has been understood that legumes have had a positive impact on increasing soil fertility because of the specialized structures, such as nodules produced by Rhizobium species, legumes compensate for mineral fertilizer by fixing nitrogen. Ayalew *et al*. (2021) reveled that due to inoculation the number of pods per plant, 100-seed weight, and seed yield of cowpea was significantly improved. Borges *et al*. (2023) have revealed that rhizobium inoculation improves the growth and nodulation of cowpea.

However, in present day commercial bean varieties are poor nitrogen fixers and research results in this area are quite variable. Yet under optimal conditions, estimates of SNF of up to 73% of total plant nitrogen have been obtained for specific genotypes and in beans with longer growing seasons, amounts of up to 125 kg/ha have been recorded (Giller, 2001) showing that the potential for improvement of SNF in common bean is present.

Due to high cost of inorganic fertilizers relative to the resources available to small scale farmers in developing countries, it has been suggested that biological nitrogen fixation (BNF) is probably the cheapest and most effective approach for increasing bean yields in African agriculture (Abebe *et al.,* 2005; Nandwa, 2003; Mafohngoya *et al.,* 2003). For example, researchers in Kenya have exploited the legume - *Rhizobium* symbiosis as a substitute for the expensive N fertilizers (Maingi *et al.,* 2001; Shisanya, 2003).

Nitrogen is the most commonly deficient nutrient in soils, contributing to low bean yields throughout the world (Chemining'wa *et al*., 2011). BNF can contribute the N needed for sustaining high bean yields (Chemining'wa *et al*., 2011). Different bean cultivars may differ in capacity for BNF due to differential performance of the symbiosis of different cultivars/landraces with different rhizobia strains (Vance and Heichel, 1991). The capacities of different Tanzanian local landraces of beans for BNF have not been evaluated and thus are not known. Promiscuity for rhizobia large ranges of N_2-fixing capabilities have been documented among bean cultivars and current commercial bean lines have the lowest value among legume crops (Martínez-Romero, 2003). In contrast to other legumes including soybean, common bean is highly promiscuous for many *Rhizobium* strains, which is demonstrated by the large number of *Rhizobium* species capable of nodulating common bean. This promiscuity complicates establishment of an efficient symbiotic interaction in the field due to severe competition with locally adapted indigenous strains often less efficient in nitrogen fixation. Variation among bean cultivars or landraces in favoring more efficient nitrogen fixing rhizobia has been described and demonstrates the potential to improve SNF Lara, (2011). A study by Alemayu, (2009) reported that, diversity among rhizobia strains and bean landraces imply possibility of obtaining potentially effective rhizobia strains that can enhance faba bean productivity. The same can be said of common beans, hence the need for evaluating different bean landraces for BNF.

2. Material and methods

2.1. Description of the study sites

The study was conducted in different villages of Arusha, Mbeya, Mvomero and Morogoro rural districts. Soils and bean landrace seeds were collected. The relevant information of each area was described according to ecological zonation of Tanzania by De pauw (1984) in which Arusha rural falls in ecological zone 14, at high altitude (>1 500 m a.s.l), fertility status is moderate with high available water capacity, dominant soils are eutric nitrosols – FAO Udic Paleustalf – Taxonomy), rainfall pattern is bimodal ranges from 600 – 1 000 mm/annum. Morogoro rural falls in ecological zone number 6 at high altitude, fertility status is moderate with medium to high available water holding capacity, dominant soils are rhodic ferralsols – FAO (Typic Haplustax – Taxonomy) on the plateau summits and Ferric Luvisol – FAO, rainfall pattern is bimodal with evarage of 1 000mm/ per annum. Mvomero district falls in ecological zone number 8, with low to medium altitude, fertility status is low with medium available water holding capacity, rainfall pattern is monomodal, and low dominant soils are Rhodic Ferrasols – FAO (Typic Haplustax – Taxonomy) on the plateau summits and Ferric Luvisols – FAO (Oxic Rhodulstalt – Taxonomy) on the slope. Mbeya rural falls in agro ecological zone number 3 at high altitude, fertility status is moderate with high to medium water holding capacity, rainfall pattern is monomodal with average of >1 000 mm per year, the dominant soils are Eutric Nitosols – FAO (Paleustalfs – Taxonomy).

2.2. Collection of seeds of different landraces

The seeds of different bean landraces collected were Nyayo, Soya and Kachumbaa from Arusha rural district at Ilkuishin, Ekenywa and Selian villages respectively. Nyamungu landrace from Mvomero district at Chenzema village, Kabungu landrace and Karanga from Morogoro rural district at Kibungo Juu and Tawa villages respectively and Msafiri, Manjano and Kasuka Nywele from Inyala, Itewe and Mwashoma villages respectively. The bean landraces were selected on the basis of seed color, size, and plant form. The landraces/plants were identified in the field and 6 - 10 plants from each landrace were selected for nodule sampling. Landraces collected are shown on (Table 1), below.

Table 1 Landraces used in the screening experiment

Treatment	Local name	Photograph	Seed coat colour
1	NYAYO		Dark red with white spots
2	NYAMUNGO		Pale red with white dots
3	SOYA		Grey
4	KICHUMBAA		Light red
5	MSAFIRI		Dark red
6	KARANGA		Cream with brown patches
7	KASUKA NYWELE		Brown with white strips
8	MANJANO		Light yellow
9	KABUNGU		Red with creamy dots

2.3. Sampling of nodules

Nodules were collected from healthy and succulent plants that were deep green in color. Ten to fifteen medium to large nodules with pink red interiors, which indicates active fixation, were sampled per plant. A spade was used to excavate the plant to a depth of at least 20 cm. The soil clump was lifted and soil removed carefully by hand from the roots. Secondary roots from the plant were not detached as some of the big nodules were found on lateral roots as well as the tap root. Whole plants were carefully placed into plastic bags and labeled. In the laboratory roots were washed carefully to remove adhering soil, and nodules were collected from each plant. Nodules were removed from the roots by cutting the root about 0.5 cm on each side of the nodule. The root nodules collected were preserved in vials with silica gel beads.

2.4. Isolation of Rhizobia from nodules collected from farmer's fields

Undamaged nodules were surface-sterilized by immersing them intact for 5-10 seconds in 95% ethanol and then were transferred to a 2.5-3% (v/v) solution of sodium hypochlorite and soaked for 2-4 minutes. They were rinsed in five changes of sterile water using sterile forceps for transferring. Forceps were sterilized quickly by dipping in alcohol and flaming. Then the sterilized nodules were crushed with a pair of sterilized blunt tipped forceps in a large drop of sterile water in a petri dish.

One loopful of nodule suspension was streaked on yeast mannitol agar (YMA) plates containing bromothymol blue (BTB) (Beck *et al.,* 2010). The plates were incubated for 7 days, and representative colonies purified by transferring to fresh media. Pure colonies were streaked in agar slants for storage at 4ºC in a refrigerator.

2.5. Screening different bean landraces for BNF using different Rhizobia strains

2.5.1. Rhizobia culture initiation

The rhizobia used in this experiment were those collected and isolated from farmers' fields (section 3.2) and stored in agar slants. Initiation of culture was necessary before inoculation. Few drops of culture suspension of rhizobia strain isolated from each bean landrace were placed in a 40 ml bottle containing sterilized Yeast Mannitol Broth medium and placed on a reciprocating shaker for seven days. The Yeast Mannitol broth medium was made by mixing 0.5 g K_2HPO_4, 0.2g $MgSO_4$, 10 g mannitol and 0.8 g yeast extract dissolved in one little of distilled water, shaken using magnetic stirrer to uniformly dissolve all particles, sterilized by autoclaving for 20 minutes at 103 x 10^3 Pascal (15 lb/in^2) pressure (Somasegaran and Hoben, 1994; Atlas, 1997), and left to cool.

2.5.2. Setting of Leonard jar

Two sets of experiments, sown at different dates, were conducted in the screen house at SUA using Leonard jars. Leonard jars were filled with sterilized sand and N- free nutrient solution. The Leonard jar assembly consisted of a bottle with the lower portion cut off and another bottle of 500 ml capacity with its upper portion cut off to act as reservoir as into which the previous bottle was inverted. The perlite in the upper inverted bottle was connected to the reservoir bottle by a cotton wick. The reservoir was filled with solution containing all essential nutrients with exception of nitrogen i.e. nitrogen was the limiting factor. Then nitrogen free solution was prepared from four stock solutions (Table 4), according to Broughton and Dilworth (Woomer *et al*., 2011) For each 10 liters of full strength N - free culture solution, 5.0 ml each of stock solution were added to 5.0 liters of water, then diluted to 10 liters using distilled water, and filled into the Leonard jar bottoms.

2.5.3. Seed inoculation

Seed inoculation was performed thoroughly by mixing pre – measured liquid rhizobial inoculants at the rate of 1 ml per seed, providing 10^3 rhizobia per seed with the seeds held in a plastic bottle immediately before seed sowing (Table 2). The seeds inoculated with the same rhizobia strain were sown immediately, and the instruments used were sterilized before processing other batches of seeds with different rhizobia strains. The rhizobia used were those isolated from nodules from farmers' fields.

2.5.4. Sowing of bean seeds into Leonard jars

Three bean seeds for each landrace (Table 2) were planted in each Leonard jar and later thinned to two plants at 15 days after planting. The jars were arranged in a Completely Randomized Design, replicated three times. Whole plants were harvested at flowering (i.e 40 days after planting) and various data were recorded as described in the next section (3.7.5).

Table 2 Composition of the Broughton and Dilworth N-free plant nutrient solution

Stock solution	Element	M	Form	g/l	M
1	Ca	1000	$CaCl.2H_2O$	294.1	2.0
2	P	500	KH_2PO_4	136.1	1.0
3	Fe	10	Fe- citrate	6.7	0.02
	Mg	250	$MgSo_4.7H_2O$	123.4	0.5
	K	250	K_2SO_4	87.0	0.5

	Mn	1	$MnSO_4.H_2O$	0.338	0.02
4	B	2	H_3BO_3	0.247	0.004
	Zn	0.5	$ZnSO_4.7H_2O$	0.288	0.001
	Cu	0.2	$CuSO_4.7H_2O$	0.056	0.0004
	Co	0.1	$CoSO_4.7H_2O$	0.056	0.0002
	Mo	0.1	$Na_2MoO_2.2H_2O$	0.048	0.0002

Source: Woomer *et al.* (2011)

2.6. Data collection

2.6.1. Days to 50% crop emergence

This was done by visual observation of number of days from one day after planting (DAP) to the time when half of the total number of seeds of each landrace planted had emerged. A seed was considered emerged when the plumule broke through the surface of the sand.

2.6.2. Days to 50% flowering

Days to 50% flowering were recorded as DAP to the time coinciding with the plant development stage when about 50% of the plants had at least one or more flowers.

2.6.3. Number of nodules per plant

These were obtained by carefully uprooting the plants, detaching, counting and recording as number of nodules from tap root and lateral roots in each plant.

2.6.4. Dry weight of nodules

The nodules collected were dried at 70°C to constant weight, and weights, were recorded as gram nodule weight per plant.

2.6.5. Effectiveness of nodules

This was done by detaching randomly four nodules per landrace and observing the pink coloration and recording as percent effective. A scale of 1 - 2 was used where 1= very pink (very effective) and 2 = not pink (other color) (not effective) (Beck *et al*., 2010).

2.6.6. Nitrogen fixation determination

Nitrogen was determined by assessing the total plant N using the Kjeldahl method.

2.6.7. Plant biomass

The entire uprooted plant in section 3.7.4 with fallen leaves retrieved and included was oven dried at 70°C for three days and weighed at the end of the third day and recorded as dry weight in gram per plant.

2.6.8. Data analysis

Data were subjected to analysis of variance for each parameter after completely randomized design using the procedure given by Gomez and Gomez (1984), using the GENSTAT software. The statistical model was:

$$Y_{ijk} = \mu + A_i + Bj + A_iBj + e_{ijk} \qquad (1)$$

Whereby:

Y_{ijk} = The measurement obtained for the unit in i^{th} landrace of the k^{th} replicate of the J^{th} observation

μ = Overall mean

A_i = Effect of i^{th} level factor A (bean landrace)

B_j = Effect of j^{th} level of factor B (rhizobia strain)

A_iB_j = The interaction of strain and landrace

e_{ijk} = Random error

Multiple comparisons (mean separation test) was performed by using Duncan's New Multiple Range Test.

Evaluation of biological nitrogen fixation in pots

Plants were grown in 3L pots filled with 4 kg soil. The composite soils for pot experiment were collected from SUA crop museum and analyzed for its physical and chemical characteristics.

The sample with low total N was selected, which was 0.2%. The soil was used to set two experiments, in which the soils were either sterilized or unsterilized. Soils were sterilized by autoclave for 15 minutes at 103 x 10^3 Pascal (15 lb /in^2) pressure and cooled. Three seeds were sown per pot and later thinned to two 15 DAP in each set. The pots were arranged split – split in a completely randomized design replicated three times and placed in a screen house at SUA which is located at altitude of 525 m above sea level, latitude 60 52'S and 37035'E (East the department of crop science and production). Bean landraces was the main plot. The landraces used were the same used in first experiment, i,e. Leonard jar experiment.

Rhizobia inoculation was the sub-plot treatment with three levels, namely soil with its native rhizobia (non - inoculated), soil with native rhizobia inoculated with Bio stacked (BS) and soil with native rhizobia and the inoculating rhizobial strain locally developed Nitro SUA (NS), for both unsterilized and sterilized soils with all other treatments.

3. Results and discussion

3.1. Effects of soil sterilization on nitrogen fixation variables under pots experiments

Soil sterilization did not result in any significant ($p \leq 0.05$) differences in nitrogen fixation variables as compared to unsterilized soils (Table 3). Therefore, data for unsterilized and sterilized soils have been pooled together because of this. The lack of significant effect of sterilization implies that even in the unsterilized soils the native rhizobia did not offer much competition with the rhizobia introduced as inoculant, and the vice versa. Such lack of competition has also been observed by Mkwachu (2011). Thus, the search for more competitive rhizobia for inoculants needs to be continued.

3.2. Effects of inoculation on nodulation and N_2 fixation by different bean landraces

The effects of rhizobial inoculation on parameters of nitrogen fixation are presented in Table 3. There were significant ($p \leq 0.005$) differences between some inoculated bean landraces in the parameters of nitrogen fixation.

Table 3 Means of variables for effect of landraces and sterilization on nodulation

Landraces/ Inoculant	**Number of nodules/plant**			**Dry weight of nodules (mg)**			**% Effective nodule**			**% Plant total Nitrogen**		
	N	BS	NS	N	BS	NS	N	BS	NS	N	BS	NS
Nyayo	248	278	120	259.3	267.3	211.5	86.83	86.83	91.33	1.93	1.96	1.97
Nyamungu	125	102	99	202.5	200.7	166.7	87.83	87.50	86.33	1.20	1.21	1.23
Soya	95	93	69	136.2	133.3	84.3	84.00	84.00	88.67	1.95	1.87	1.70
Kachumbaa	59	61	45	20.8	22.8	13.8	57.17	57.33	54.50	1.74	1.67	1.67

Landraces/ Inoculant	Number of nodules/plant			Dry weight of nodules (mg)			% Effective nodule			% Plant total Nitrogen		
	N	BS	NS	N	BS	NS	N	BS	NS	N	BS	NS
Msafiri	193	200	131	300.2	325.7	223.5	86.83	87.00	86.50	1.64	1.67	1.51
Karanga	103	103	84	351.0	309.8	245.8	84.00	84.17	85.33	2.00	2.00	1.90
Kasuka Nywele	112	111	81	68.7	65.7	53.7	84.17	84.17	85.67	2.13	2.14	2.00
Njano	46	48	85	46.8	34.2	109.2	80.67	79.50	83.17	1.69	1.83	1.60
Kabungu	138	139	97	241.0	230.5	147.8	82.67	82.67	86.67	2.16	2.29	2.00
P- value	<0.001			<0.001			<0.001			<0.001		
LSD (P≤0.05)	17.27			31.66			2.64			0.26		
Sterilization												
Unsterilized	165	199	87	207.8	149.9	133.7	81.89	81.22	82.89	1.96	1.67	1.67
Sterilized	144	172	93	153.6	170.8	145.4	81.26	81.70	83.37	1.70	2.03	1.78
P-value	<0.001			<0.001			0.016			0.033		
LSD (P≤0.05)	32.05			59.13			4.0			0.4		
%CV	18.2			23.0			2.8			12.1		

Key: N = Native rhizobia; BS = Seeds inoculated with Biostacked; NS = Seeds inoculated with Nitro SUA

3.3. Number of nodules per plant

The number of nodules per plant were generally significantly (p≤0.05) different among the landraces (Table 3). The numbers of nodules were highest in Nyayo landrace inoculated with Biostacked inoculant (278) and lowest in Kachumbaa landrace inoculated with Nitro-SUA (45). These results reveal probably that Nyayo landrace has high compatibility with the Biostacked inoculant than with the other inoculants, thus producing with Biostacked inoculant more nodules than the rest of landraces under observation. These results in the present study underscore the need to identify the more compartible rhizobia – landrace combination to maximize nodulation (nodule numbers), with the prospect of maximizing nitrogen fixation. In this connection the Biostacked inoculant also resulted in high nodule numbers with the landrace Msafiri. Nyayo, Msafiri and Kabungu landraces were nodulated more by Biostacked inoculant (respectively 278, 200, and 139 nodules) than by the Nitro SUA inoculant (respectively 120,131 and 97 nodules). However, the Biostacked inoculant was not superior to the native strains in all bean landraces except Nyayo and Nyamungu. On some occasions, native rhizobia even surpassed Nitro SUA in nodule numbers, as in in the case of landraces Nyayo, Msafiri, Kabungu and Nyamungu. Similar findings were reported by Gicharu *et al.* (2013) in the study of effects of inoculating selected climbing bean varieties with different rhizobia strains on nitrogen fixation whereby different treatments (rhizobia strains) within cultivars differed significantly (P≤0.05) in nodule numbers. Chemining'wa *et al.* (2011) also reported significantly different numbers of nodules between bean landraces.

3.4. Dry weight of nodules per plant

The weights of nodules per plant were generally significantly (p≤0.05) different among the landraces (Table 3). The mean of dry weight of nodules per plants were highest in Karanga landrace inoculated with Natives strains (351.0 mg) and lowest in Kachumbaa landrace inoculated with Nitro - SUA (13.8 mg). These results reveal probably that Karanga landrace has high compatibility with the Natives strains than other inoculants, thus results to high dry weight of nodules per plant than the rest of landraces. Again these results in the present study emphasizes the need to explore potential of rhizobia strains for improving symbiotic performance aiming at maximizing biological nitrogen fixation.

The Native strains also resulted in high nodules dry weight with the landrace Karanga. Nyayo, Msafiri, and Kabungu landraces resulted to high dry weight of nodules when inoculated Native strains (respectively 259.3, 300.2, and 241.0 mg of dry weight of nodules) than by the Nitro SUA inoculant (respectively 211.5, 223.5 and147.8 mg). However, as for nodule numbers, the Native strains were not superior to the Biostacked inoculants in all bean landraces except Nyayo, Nyamungu, Msafiri and Kachumbaa landraces. Nitro SUA inoculant also exceeded Biostacked inoculant and Native strains in nodule dry weight per plant in in the case of landrace Njano. These findings are in close conformity with Mungai and Karubiu (2011) who reported that rhizobia inoculation did not improve dry weight of nodules over non-inoculated treatment in common beans. Buttery *et al*. (1997) also showed significantly ($p \leq 0.05$) different effects between cultivars and *Rhizobium* strains dry weights of nodules in common beans.

3.5. Proportion of effective nodules per plant

The proportion % of effective nodules per plant were generally significantly ($p \leq 0.05$) different among the landraces (Table 3). Although there were some significant differences, these were very fewer, which implies that the per cent effective nodulation was almost similar in most of the landraces under comparison.

Means for the percentage proportion of effective nodules per plant were highest in Msafiri landrace inoculated with Biostacked inoculant (87.0%) and lowest in Kachumbaa landrace inoculated with Native strains (57.17%). These results reveal probably almost all landraces under comparison have the same compatibility with inoculants under comparison, with exception of Kachumbaa landrace. The means for the percentage proportion of effective nodules per plant generally were significantly ($p \leq 0.05$) different among the inoculants, which implies probably all three inoculants tested were almost the same in % proportion of effective for the effectiveness of nodules with exception of Nitro SUA inoculant which was superior to Native and Biostacked inoculant in Nyayo landrace.

These results are consistent with the findings of Zarrin *et al.* (2007) who reported nodulation phenotypic parameters as nodule weight per plant, percent effective nodules and N_2 fixation capacity to be dependent upon *Rhizobium* strain and efficiency of the gene that affect Nod-factor structure. Gicharu *et al.* (2013) also reported differences in nodulation and effectiveness between three climbing bean cultivars under the controlled conditions of the greenhouse. Generally results in the effect of nodulation and nitrogen fixation suggested that different landraces of beans had preference for certain rhizobia and native rhizobia were as good as some of the inoculants used Gicharu *et al.* (2013). This supports observations by Graham (1981), Kremer and Peterson (1983), Pacovsky *et al.* (1984) and Mostasso *et al.* (2002), who noted that though high nitrogen fixing strains of *Rhizobium* have been identified, they often do not always provide agronomic benefits in the field because such inoculants strains become excluded from the nodules of the host plant by the soil indigenous strains which are often more competitive for nodulation than introduced inoculant strains.

3.6. Percent nitrogen in the plant biomass

The % nitrogen in the plant biomass were generally significantly ($p \leq 0.05$) different among the landraces (Table 3). The % nitrogen was highest in Kabungu landrace inoculated with Biostacked inoculant (2.2%) and lowest in Kachumbaa landrace Native strains (1.20%). These results reveal probably that Kabungu landrace has high compatibility with the Biostacked inoculant than the other inoculants, thus fixing the highest quantities of nitrogen than the rest of the landraces. Nyayo, Msafiri, Nyamungu and Karanga landraces fixed high quantities of nitrogen with Biostacked inoculant (respectively 1.96, 1.67, 1.74 and 2.0 % nitrogen) than Nyayo, Msafiri and Nyamungu inoculated by Native strains (respectively 1.93,1.64 and 1.67%). These results suggests probably observed trend was due to genetic variability of symbiotic partners and partly due to the relatively broad host range symbiotic association exhibited by common bean. Similar findings reported by Oguctu *et al.* (2008) who noted significance variation of amount of plant nitrogen after selecting 7 efficient strain in cowpeain, the mean plant nitrogen were generally significantly ($p \leq 0.05$) different among the landraces, also supported by Samavat *et al.* (2012) who reported significance different in all inoculums strains in which some has highest effect in increasing effect of plant nitrogen in which commercial strain had highest effect in increasing plant nitrogen compared to native strains.

3.7. Effects of soil sterilization on plant growth variables under pots experiments

Soil sterilization did not result did not result in much ($p \leq 0.05$) significant differences as compared to unsterilized soils in most growth variables, with exception few differences in plant biomass (Table 4). This implies that soil sterilization did not alter the dynamics of nutrient availability as compared to those in the unsterilized soils.

Table 4 Means of variables for effect of landraces and sterilization on plant growth

Landraces/ Inoculant	Days 50% flowering			Number of pods per plant			Plant biomass (g)			100 seed weight (g)		
	N	BS	NS	N	BS	NS	N	BS	NS	N	BS	NS
Nyayo	34	34	34	10.0	10.0	10.0	3.90	3.64	2.24	51	51	49
Nyamungu	35	34	34	8.0	8.0	8.0	1.87	2.50	3.20	50	50	52

Landraces/ Inoculant	Days 50% flowering			Number of pods per plant			Plant biomass (g)			100 seed weight (g)		
	N	BS	NS	N	BS	NS	N	BS	NS	N	BS	NS
Soya	36	36	35	9.0	9.0	8.0	2.64	2.65	2.09	47	46	46
Kachumbaa	34	34	33	9.0	8.0	9.0	2.85	2.69	2.32	33	35	33
Msafiri	35	35	36	9.0	9.0	10.0	2.38	2.15	1.90	51	50	50
Karanga	35	35	35	9.0	10.0	10.0	1.46	1.84	2.75	37	40	37
Kasuka Nywele	34	34	34	9.0	10.0	9.0	3.19	3.17	2.98	50	50	50
Njano	34	34	34	10.0	9.0	10.0	3.52	3.48	3.07	38	38	38
Kabungu	35	35	33	9.0	9.0	10.0	2.37	2.35	1.58	48	48	47
P-value	<0.001			0.17			0.015			<0.001		
LSD (P≤0.05)	1.0			1.55			0.87			1.87		
Sterilization												
Unsterilized	35	35	34	9.00	9.00	9.00	2.99	2.53	2.65	45	45	45
Sterilized	34	34	34	9.00	9.00	9.00	2.38	2.91	2.72	46	45	46
P-value	0.983			0.833			<0.001			0.108		
LSD (P≤0.05)	1.17			2.39			1.50			3.25		
%CV	2.3			15.4			35.4			4.5		

Key: N = Native rhizobia; BS= Seeds inoculated with Biostacked**;** NS = Seeds inoculated with Nitro SUA

3.8. Effects of inoculation on plant growth variables

The effects of rhizobial inoculation on variables of plant growth presented in (Table 4). There were no significant differences between some inoculated bean landraces in the variables of plant growth.

3.9. Days to 50% flowering

The number of days to 50% flowering for the effect were generally significantly different (p≤0.05) among the landraces (Table 4). Effect of inoculants on number of days to 50% flowering were generally not significantly different among the inoculants, which implies that inoculation cannot alter the genetically fixed traits of the host plant. De Datta (1981) and Yoshida (1981) also reported genetic variations among genotypes of beans for days to 50% flowering as observed presently.

3.10. Number of pods per plant

The number of pods per plant were not affected by rhizobial inoculation but were generally not significantly different among some of the landraces (Table 4). These results are similar with the findings of De Datta (1981) who reported genetic variation of *Phaseolus vulgaris* in pods production per plant.

3.11. Plant biomass

The weight of plant biomass per plant were generally significantly (p≤0.05) different among the landraces (Table 4). The biomass was highest in Nyayo landrace inoculated with Natives strains (3.9 g) and lowest in Karanga landrace inoculated with Native strains (1.46 g). These results reveal Nyayo landrace has high compatibility with the Natives inoculant than other inoculants, thus resulting highest weight of plant biomass than the rest of landraces.

Nyayo, Kasuka Nywele and Njano landraces resulted in high plant biomass weight when inoculated with Biostacked inoculant (respectively 3.64, 3.17, and 3.48 grams per plant) than when inoculated with Nitro SUA (respectively 2.24, 2.98, 3.07 grams per plants). These results reveal that probably there is an important genetic variability associated with symbiotic nitrogen fixation among studied landraces that were manifested in plant biomass. These findings are consistent with Rodino *et al.* (2001) who reported genotypic variability associated with biological nitrogen fixation among 64 common bean landraces in which significant variability was observed in plant biomass. However, Westgate *et al.* (2008) reported little impact of inoculation on plant biomass when testing the impact of Biostacked inoculant on nodulation and nitrogen accumulation by a set of recombinant lines in *Phaseolus vulgaris.*

3.12. Weight of 100 hundred seeds

The means for the one hundred seed weight were generally significantly ($p \leq 0.05$) different among the landraces (Table 4). Means of landraces for one hundred seed weight were highest in landrace Nyayo inoculated with Native strains and Biostacked inoculant (51) lowest means were shown in Njano inoculated with all inoculants (38).

Effect of inoculation in one hundred seed weight on plant growth were also generally significantly different ($p \leq 0.05$) among some the inoculants and bean landraces. The only difference shown by Biostacked and Native inoculant which were more superior than Nitro SUA inoculant in Nyayo landrace, and Nitro SUA which was superior than Biostacked and Native inoculants in Karanga landrace. Otherwise there were no significant differences observed in the rest of inoculants. These results agree with Mehdi (2011) who reported significant differences in 100 weight of beans inoculated with of *Rhizobium leguminosarum.*

These results suggest that probably native rhizobia were quite competitive and that there was no need to introduce new inoculant strains. This supported work done by Kremer and Peterson (1983) who observed that though high nitrogen fixing strains of *Rhizobium* have been identified, they often do not provide much agronomic benefit in the field because they are often excluded from the nodules of the host plant by the soil indigenous strains which are often more competitive.

3.13. Landrace x inoculant interactions in unsterilized soils

3.13.1. Number of nodules per plant

The means of landrace and inoculants interactions of the number of nodules per plant in unsterilized soils conditions generally are statistically significantly ($p \leq 0.05$) different among landraces at (Table 5). However, the only means of inoculants number of nodules per plant of landraces Nyayo none-inoculated and Msafiri landrace none- inoculated have shown to be statistically significantly different from the means of the inoculant number of nodules per plant of the other landraces with and without inoculants interactions. The mean of Nyayo none inoculated is high (359) followed by the means of Msafiri landrace (256). It is an indication that these interactions between Nyayo landrace-none inoculated and Msafiri landrace none-inoculated have high effects on the production of nodules per plant than the rest of landraces with and without inoculants interactions. The means of other number of nodules per plant in the landraces with and without inoculants interactions which are statistically similar are presented in (Table 5).

Table 5 Effect of landrace x inoculant interaction on N_2 fixation on number and weight of nodules grown in unsterilized soils

Landraces/ Inoculant	Number of Nodules			Weight of Nodules (g)		
	N	BS	NS	N	BS	NS
Nyayo	359	134	114	266	238	203
Nyamungu	133	115	95	216	184	165
Soya	105	78	67	139	125	82
Kichumbaa	73	45	43	26	13	12
Msafiri	256	136.	123	414	250	213
Karanga	118	88	85	414	208	247
Kasuka Nywele	133	88	82	77	55	51
Manjano	63	41	72	69	30	57
Kabungu	150	124	101	249	198	156
%CV	17.0			23.6		

Landraces/ Inoculant	Number of Nodules			Weight of Nodules (g)		
	N	BS	NS	N	BS	NS
LSD (P≤ 0.005)	31.0			62.6		

Key: N = Native rhizobia; BS = Seeds inoculated with Biostacked; NS = Seeds inoculated with Nitro SUA

3.14. Dry weight of nodules per plant (mg)

The means of landrace and inoculants interactions of the dry weight nodules per plant (mg) in unsterilized soils were not significantly different among the landraces (Table 5). These results show that the interaction between landraces with and without inoculants has almost the same effects on the inoculant dry weight nodules per plant (mg). However, the means of inoculant dry weight of nodules per plant (mg) of Msafiri landraces none inoculated and Karanga landrace none-inoculated both have the highest mean of (414). The means of other inoculant dry weight of nodules per plant (mg) of the landraces with and without inoculants interactions are presented in (Table 5).

3.15. Proportion of effective nodulation

The means of landraces-inoculants interactions of the number of the percent effective nodules were statistically not significantly different among the landraces at (Table 6). These results indicate that the interactions between landraces with and without inoculants have almost the same effects on the percent effective nodulation. However, the Nyayo landraces inoculated with Nitrosua has high means of 91 % effective nodulation followed by Nyamungu landrace none-inoculated and Soya landrace inoculated with Nitrosua both with mean of 89%. The means of other percent effective of nodulation in the landraces with and without inoculants interactions are presented in (Table 6).

3.16. Percent plant total nitrogen

The means of landrace and inoculants interactions of the percent plant total nitrogen in unsterilized soils are statistically not significantly different among the landraces (Table 6). These results indicate that the interactions between landraces with and without inoculants have almost the same effects on the percent plant total Nitrogen. However, the Kabungu landraces non- inoculated has high means of (2.4) percent plant total Nitrogen followed by Kasuka Nywele landrace none inoculated with mean of (2.3). The means of other inoculant percent plant total Nitrogen in the landraces with and without inoculants interactions are presented in (Table 6). In overall there was no significant interaction between landraces and inoculant in all of the above nodulation phenotypic parameters probably N_2 fixation capacity are dependent upon *Rhizobium* strain and affectivity of gene that effect on Nod-factor structure and also on availability of nutrients to the plant Agha *et al.* (2004).

Table 6 Effect of landrace x inoculant interaction on N2 fixation on % effective nodules and plant total N2 grown in unsterilized soils

Landraces/ Inoculant	% Effective Nodules			Plant total N		
	N	BS	NS	N	BS	NS
Nyayo	85	88	91	2.2	1.7	1.9
Nyamungu	89	86	87	1.3	1.0	1.1
Soya	86	82	89	2.2	1.6	1.4
Kichumbaa	58	57	54	1.6	1.7	1.7
Msafiri	87	87	86	1.7	1.5	1.5
Karanga	84	84	85	2.0	1.9	1.8
Kasuka Nywele	82	86	86	2.3	2.0	1.9
Manjano	81	79	83	1.9	1.7	1.6
Kabungu	84	81	85	2.4	1.9	2.0
%CV	2.7			12.6		
LSD (P≤ 0.005)	3.6			0.37		

The results are in close conformity with findings of Zarrin *et al*. (2007) in the study interactive effect of *Rhizobium* strains and P on soybean yield, Nitrogen fixation and soil fertility who reported no significance interaction between inoculants and soy bean genotypes.

3.17. Plant growth

The means of landrace and inoculants (strain) interactions of the plant biomass (gms) in unsterilized soils were statistically not significantly different among the landraces (Table 7). These results indicate that the landraces with and without inoculants have almost the same effects on the plant biomass. However, Nyayo landrace without inoculation had the highest means of (5.8) plant biomass. The mean of plant biomass in the landraces with and without inoculants interactions are presented in (Table 7).

Table 7 Effect of landrace x inoculant interaction on plant growth of plant biomass and days 50% flowering grown in unsterilized soils

Landraces/ Inoculant	**Plant Biomass (g)**			**Days 50% Flowering**		
	N	BS	NS	N	BS	NS
Nyayo	6.0	2.0	2.0	34	34	34
Nyamungu	2.0	2.9	4.7	34	34	35
Soya	2.3	2.9	2.0	36	36	35
Kichumbaa	3.2	2.2	2.5	34	34	33
Msafiri	3.2	1.0	2.2	35	35	35
Karanga	1.3	2.4	2.7	35	34	35
Kasuka Nywele	2.7	3.6	3.0	35	34	34
Manjano	4.1	3.2	2.3	34	34	34
Kabungu	2.3	2.4	1.6	35	35	34
%CV	37.6			2.1		
LSD ($P \leq 0.005$)	1.67			1.21		

Key:Native rhizobia; BS = Seeds inoculated with Biostacked; NS = Seeds inoculated with Nitro SUA

The means of landrace and inoculants (strain) interactions of the days fifty percent up to flowering in unsterilized soils are statistically not significantly different among the landraces (Table 7). These results indicate that the interactions between landraces with and without inoculants have almost the same effects on the inoculum fifty percent flowering. However, the Soya landrace none inoculated has the highest means of (36) days fifty percent flowering. The means of other days up to fifty percent flowering in the landraces with and without inoculants interactions are presented in (Table 7).

The means of landrace and inoculants (strain) interactions for the number of the pods per plant in unsterilized soils were statistically not significantly different among the land races (Table 8). These results indicate that the interactions between landraces with and without inoculants have almost the same effects on the production of pods per plant. However, the Njano landraces inoculated with Nitro SUA has the highest mean of (11) pods per plant. The means of other inoculum pods per plant of the landraces with and without inoculants interactions are presented in (Table 8). The means of landrace and inoculants (strain) interactions of the days fifty percent up to flowering in unsterilized soils are statistically not significantly different among the landraces (Table 7). These results indicate that the interactions between landraces with and without inoculants have almost the same effects on the inoculum fifty percent flowering. However, the Soya landrace none inoculated has the highest means of (36) days fifty percent flowering. The means of other days up to fifty percent flowering in the landraces with and without inoculants interactions are presented in (Table 7). The means of landrace and inoculants (strain) interactions of the number of the one hundred seed weight (g) in unsterilized soils are statistically not significantly different among the landraces at (Table 8). These results reveal the interactions between landraces with and without inoculants have almost the same effects on the production of inoculants pods per plant. However, the Nyamungu landrace inoculated with Nitro SUA has high mean of (54.5) inoculant hundreds seed weight. The means of other inoculant hundreds seed weight (g) of the landraces with and without inoculants interactions are presented in (Table 8).

Table 8 Effect of landrace x inoculant interaction on plant growth on number pods per plant and 100 seed weight grown in unsterilized soils

Landraces/ Inoculant	Number of pods per plant			100 seed weight (g)		
	Native	BS	NS	N	BS	NS
Nyayo	10	10	10	52.4	49.5	49.6
Nyamungu	9	8	8	50.3	48.8	54.5
Soya	9	8	8	46.2	45.7	46.3
Kichumbaa	8	8	9	35.6	37.3	32.1
Msafiri	9	10	10	52.0	49.8	50.0
Karanga	10	9	10	37.9	41.3	37.1
Kasuka Nywele	9	10	9	50.6	49.6	46.6
Manjano	10	9	11	37.7	38.3	37.7
Kabungu	9	10	10	48.4	47.6	47.0
%CV	15.3			5.4		
LSD (P ≤0.005)	2.32			4.02		

Key:N=Native rhizobia; BS = Seeds inoculated with Biostacked; NS = Seeds inoculated with Nitro SUA

In overall these results reveals no significance different in all studied plant growth parameter, probably this suggests that *Rhizobium* strains x landraces genotypes specificity occur under certain soil conditions as also reported in chickpea by Somasegaran *et al.* (1988).

3.18. Landrace x Inoculant Interactions in sterilized soils

3.18.1. Number of nodules per plant

The means of landraces and inoculants interaction of the number of nodules per plant were generally significantly ($p \leq 0.05$) different among the landraces under sterilized soils (Table 9). The means of number of nodules per plant of landraces Nyayo inoculated with Biostacked and Msafiri inoculated with Biostacked significantly differ from the means of the rest of the landraces with and without inoculants interactions. The mean of Nyayo inoculated with Biostacked is higher (422) than the means of landraces with and without inoculants interactions under comparison. It is an indication that this interaction between landrace Nyayo inoculated with Biostacked inoculant has high effects on the production of nodules per plant than the rest of landraces with and without inoculants interactions. The means of other number of nodules per plant in the landraces with and without inoculants interactions which are statistically similar are presented in (Table 9).

3.18.2. Dry weight of nodules per plant (mg)

The means of landrace and inoculants interaction of the dry weight nodules per plant are not significantly different among the landraces under sterilized soils (Table 9). The means of dry weight of nodules per plant of Karanga landraces inoculated with Biostacked had high mean of (411.3) followed by Msafiri landrace inoculated with Biostacked with dry weight of nodules per plant in the landraces with and without inoculants interactions are presented in (Table 9).

Table 9 Effect of landrace x inoculant interactions on N fixation or number and weight of nodules grown in sterilized soils

Landraces/ Inoculant	Number of nodules				Weight of nodules (g)		
	N	BS	NS		N	BS	NS
Nyayo	137	422	126		252	297	220
Nyamungu	117	89	102		189	217	168
Soya	84	107	70		133	142	87
Kichumbaa	46	78	47		16	32	15
Msafiri	130	264	139		186	401	216
Karanga	83	117	83		288	411	245
Kasuka Nywele	90	133	80		60	76	56
Manjano	29	35	98		28	38	161
Kabungu	126	155	93		233	264	140
%CV	18				20.9		
LSD (P≤0.005)	33.95				57.89		

Key: N = Native rhizobia; BS = Seeds inoculated with Biostacked; NS = Seeds inoculated with Nitro SUA

However, the Nyayo landraces inoculated with Nitro SUA has high means of (91.1) percent effective nodes followed by Nyamungu landrace inoculated with Biostacked and Soya landrace inoculated with Nitro SUA both with mean of (88.67). The means of other percent effective of nodules in the landraces with and without inoculants interactions are presented in (Table 10).

3.18.3. Percent plant total nitrogen

The means of landrace and inoculants interaction of the percent plant total nitrogen are not significantly different among the landraces under the sterilized soils (Table 10). These results indicate that the interactions between landraces with and without inoculants have almost the same effects on the percent plant total nitrogen see (Table 10). However, the Kabungu landraces inoculated with Biostacked has high means of (2.7) percent plant total Nitrogen followed by Kasuka Nywele landrace inoculated with Biostacked with mean of (2.3). The means of other percent plant total Nitrogen in the landraces with and without inoculants interactions are presented in (Table 10).

Table 10 Effect of landrace x inoculant interactions on N fixation for % effective nodules and plant N grown in sterilized soils

Landraces/ Inoculant	%Effective nodulation			Plant total N		
	N	BS	NS	N	BS	NS
Nyayo	88	85	91	1.7	2.2	2.0
Nyamungu	87	88	86	1.0	1.3	1.4
Soya	82	86	89	1.8	2.1	1.9
Kichumbaa	57	58	55	1.8	1.7	1.7
Msafiri	87	87	87	1.6	1.9	1.5
Karanga	84	84	85	2.0	2.0	2.0
Kasuka Nywele	86	82	86	2.0	2.0	2.0
Manjano	80	80	83	1.5	2.0	1.6
Kabungu	81	84	88	2.0	2.6	2.0

Landraces/ Inoculant	%Effective nodulation			Plant total N		
	N	BS	NS	N	BS	NS
%CV	3.2			13.3		
LSD (P≤ 0.005)	4.3			0.2		

Key: N = Native rhizobia; BS = Seeds inoculated with Biostacked; NS = Seeds inoculated with Nitro SUA

4. Conclusion

The findings of this study have shown that variability among landraces in symbiotic nitrogen fixation exists. The landrace Nyayo, Nyamungu, Msafiri and Kabungu performed the best in most of the variables tested and in both experiments hence emphasizing the need to explore the potential of their rhizobia strains for improving the symbiotic performance of *Phaseolus vulgaris.* In case of inoculants native rhizobia had comparable symbiotic effectiveness to commercial inoculants in unsterilized soils and vice versa in sterilized soils conditions though soil sterilization was not significant. Based on the results in these studies it has also shown that, there is no significance interactions between strains and collected landraces.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that there is no conflict of interest.

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