Volume 1, Issue 2, pp. 25-31; March, 2013
Online International Journal of Microbiology Research

Full Length Research

Available Online at http://www.onlineresearchjournals.org/OIJMR

Effect of inoculating selected climbing bean cultivars with different rhizobia strains on nitrogen fixation

Gicharu GK[1], Gitonga NM[2], Boga H[3], Cheruiyot RC[4], and *Maingi JM[4]

[1]Department of Pure and Applied Sciences, Technical University of Mombasa, P.O. Box 90804 Mombasa, Kenya.
[2]Meru University of Science and Technology, Kenya.
[3]Department of Botany, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, Nairobi, Kenya.
[4]Department of Plant and Microbial Sciences, Kenyatta University, P.O. Box 43844, Nairobi, Kenya.

Downloaded, 11 March 2013 Accepted 24 March, 2013

Experiments were designed to investigate biological nitrogen fixation in indeterminate (climbing) bean cultivars namely *Cargamanto*, NG224-4 and G59/1-2. Greenhouse and field studies were carried out to determine the most efficient bean cultivars and rhizobia strains in nitrogen fixation. Each cultivar was inoculated with the rhizobia strains CIAT 899, USDA 2674, USDA 2676, the three strains combined and one was not inoculated. There were no statistical differences in nodulation between the different inoculant treatments but in the field, beans inoculated with rhizobia strain USDA 2676 formed the highest nodule number. The bean cultivars inoculated with a mixture of the three *rhizobia* strains gave the highest total plant dry weight. Treatments within each of the cultivars showed differences in total plant dry weight yield in response to rhizobia strain used. Field experiments showed that plant dry weight differed significantly between cultivars but there were no significant differences in yields.

Key words: Climbing beans, *rhizobium*, nitrogen fixation, inoculation, Central Kenya.

INTRODUCTION

In Kenya, the common bean is the most commonly grown grain legume with production of over 414,000 metric tons per year [1-3]. It comes second to maize as a subsistence crop and is a major source of protein in human diets. It is mainly grown in intercrop with maize (*Zea mays* L.) by the small-scale farmers.

A distinction is made between two growth habits of the common bean each having several forms. These habits and growth forms depend on genetic factors and ecological factors like temperature and photoperiod. The growth habits are determinate (bush beans) and indeterminate (climbing beans) [4]. The aim of development of the indeterminate bean genotypes was to increase yield potential and improve yield stability of the determinate beans [5,6]. It also aimed at facilitating the transfer of plant architecture traits and disease resistance. Indeterminate beans therefore produce greater seed yields than determinate genotypes in low plant populations, which should therefore benefit subsistence farmers who often plant low plant populations in order to reduce seed costs [6].

In Kenya, like other East African countries, a major problem with common bean production is low yield. Yield average is between 400-600 kg per hectare [7]. In contrast, South America has higher production efficiency with yields ranging from 800 -1,500 kg per hectare [7]. This indicates a two-fold difference in yields. Estimates of soil nutrient losses in sub-Saharan Africa [7,8], Asia and Latin America [9] suggested that there was a net removal of between 20 and 70 kg ha-1 of N from agricultural land each year. It was observed that these losses were likely to increase. To balance these losses of N, with the increasing demand for food production and fertiliser use,

*Corresponding Author's E-mail: jmanguske@yahoo.com.

it was suggested that inputs from Biological Nitrogen Fixation (BNF) would have to increase on average by more than 30 kg N ha^{-1} [10-13]

In the light of the aforementioned challenges, this research was designed to investigate BNF in three climbing bean cultivars grown in the East African region. The study was aimed at assessing how seed inoculation with rhizobia would contribute to more nitrogen fixation. The following were the objectives of the study:

1. To determine yield in climbing bean cultivars in response to inoculation with different rhizobia strains.
2. To identify the most effective Rhizobium strain in nitrogen fixation.
3. To determine whether climbing bean cultivars are nodulated by the same indigenous rhizobia strains.

MATERIALS AND METHODS

Study Area

Field experiments were carried out at the research farm of Jomo Kenyatta University of Agriculture and Technology (JKUAT) at Juja in Central Kenya. The university is located at elevation of 1,525 m above sea level, longitude 37° 00 ° E and latitude 10 ° 05° S. The site receives an average annual rainfall of 850 mm in a bimodal pattern with over 55% of the total falling in the long rain season (March-June) and 45% in the short rain season (October-January). Temperatures are moderate ranging from 13 °C – 26 °C [14].

Sourcing for Seeds and *Rhizobium* Inoculum

Seeds of the three climbing (indeterminate) bean cultivars were obtained from the College of Agriculture and Veterinary Science, University of Nairobi. The rhizobia strains used were obtained from Microbiological Resource Centre (MIRCEN) of the University of Nairobi.

Greenhouse Experiment

Greenhouse work was carried out to assess nodulation ability of the three bean cultivars when inoculated with different rhizobia strains under controlled conditions. Sterile polythene bags were filled with sterile vermiculite and covered with aluminium foil awaiting planting. Nitrogen free plant nutrient solution was prepared as described by [15]. Bean seeds were aseptically pre-germinated and those whose radicals had attained a length of 1-2 cm after incubation were considered ready for planting in the polythene bags.

Inoculation of the seedlings was carried out by pipetting 2 ml of inoculum onto the radical base. Each treatment was replicated four times and the bags were arranged on greenhouse benches in a complete randomised block design. After every 4 days, 50 ml of nitrogen free plant growth media was added.

The plants were harvested at flowering stage by uprooting (fifty days after planting). All the nodules were carefully detached from the roots and their number recorded. The plants were dried at 70° C to a constant dry weight. The total plant dry weights were determined using Sartorius weighing balance type H 160.

Field Experiments

The beans were planted in 60 plots each measuring 2.5 m by 2 m using Complete Randomised Block Design (CRBD). The treatments for each bean cultivar were as follows:

i. Not inoculated
ii. Inoculated with CIAT 899
iii. Inoculated with USDA 2674
iv. Inoculated with USDA 2676
v. Inoculated with CIAT 899, USDA 2674 and USDA 2676.

The crop was dry planted just before the beginning of the short rain season October-December 2005. Inoculation with the respective rhizobia was carried out by addition of 1 gram of the peat based inoculant to the moistened seeds followed by thorough mixing of the seeds with the inoculant. Weeding was done manually using hand hoes two weeks after emergence.

Plant Sampling and Harvesting

The plants were randomly sampled at flowering and at physiological maturity stages. At each stage, two plants were sampled per sub-plot. The plants were uprooted carefully to avoid root breakages. The nodules were carefully detached from the roots and their number per plant recorded. The plants were then oven dried in brown bags at 70 °C to a constant dry weight. The dry weights were then determined and recorded.

Laboratory Experiments

Laboratory experiments were carried out to establish characteristics of rhizobia isolated from the bean cultivars. Yeast Extract Mannitol Agar (YEMA) was used to grow and culture root nodule bacteria. It was prepared according to [16].

Ten nodules from the field were collected from each treatment at random and surface sterilised. They were then crushed with a pair of blunt-tipped forceps in a large drop of sterile water in a Petri-dish. A sterile inoculation loop full of the resulting suspension was streaked on Yeast Extract Mannitol Agar (YEMA) plates. The inoculated Petri-dishes were incubated at 28 °C for colonies to appear. The plates were observed daily for

Table 1. Effect of inoculation on nodulation of the three bean cultivars.

	Nodule number in different beans (Mean ±SD)		
Inoculum	**Cargamanto**	**NG-224-4**	**G59/1-2**
Control	6.0 ±2.0a	8.0 ±3.0a	9.0 ±3.0a
CIAT 899	81.0 ±31.0b	94.0 ±7.0bc	155.0 ±51.0e
USDA 2674	84.0 ±14.0b	99.0 ±19.0bcd	132.0 ±41.0de
USDA 2676	128.0 ±16.0cde	83.0 ±25.0b	123.0 ±33.0cde
Combined	84.0 ±18.0b	87.0 ±16.0b	131.0 ±32.0de
$LSD_{(0.05)}$ 34.3			

Note: First column indicates rhizobia inocula used while columns two three and four indicate nodule numbers on the three bean cultivars Cargamanto, NG-224-4 and G59/1-2 under the different treatments. Values followed by the same letters did not show statistical difference (p<0.05).

the appearance of colonies typical of rhizobia.

Characterization of Isolates

A number of morphological and biochemical tests were carried out to establish the characteristics of the rhizobia isolated from beans. These included Gram staining, use of Congo red dye, bromothymol blue dye, litmus milk, and peptone agar.

Data Analysis

Data collected was subjected to two way analysis of variance (ANOVA) to test significance between the treatments. Means were separated using Least Significance Difference (LSD). Genstat version 6.8 statistical package was used [17].

RESULTS

Results on nodulation in the greenhouse showed cultivar G59/1-2 had the highest ($P \leq 0.05$) nodule number at an average of 110.0 nodules per plant. It differed significantly from the other two bean varieties at $P \leq 0.05$, which had averages of 76.0 and 74.0 nodules per plant. No significant differences were noted between the three climbing bean cultivars in response to either of the three rhizobia strains at $P \leq 0.05$. All the inoculated plants produced more than 100 nodules on average (Table 1). However, different treatments (rhizobia strains) within a cultivar differed significantly in nodule number at $P \leq 0.05$. In cultivar Cargamanto inoculation with USDA 2676 produced the highest nodule number (128.0 ± 16.0) (Table 1). The highest nodule number among cultivar NG-224-4 treatments was produced by plants inoculated with USDA 2674 (99.0 ± 19.0) while among cultivar G59/1-2 treatments, beans inoculated with CIAT 899 had the highest ($P \leq 0.05$) nodule number (155.0 ± 51.0 on average). Cultivar G59/1-2 responded better to inoculation than the rest.

Total Plant Dry Weight

Cargamanto had the highest total dry weight and was significantly different from the other two cultivars at $P \leq 0.05$. Multistrain inoculated beans produced the highest dry weight and were statistically different from the others at $P \leq 0.05$. Between the different treatments within a cultivar, significant differences were noted in dry weight yield at $P \leq 0.05$. Multistrain inoculated Cargamanto had the highest average dry weight of 2.3 mg ± 0.2 while cultivars NG-224-4 and G59/1-2 inoculated with CIAT 899 gave the highest dry weight (1.9 mg ± 0.1 and 1.9 mg ± 0.1 respectively) (Table 2). Biochemical results on the isolated bacteria confirmed that they were *Rhizobium* (Table 3).

Field Studies

Results on nodulation at flowering showed that Cargamanto had the lowest average nodule number of 43.0 and was significantly different from the other two bean cultivars at $P \leq 0.05$. Cultivars inoculated with USDA 2676 had the highest average nodule number of 103.0 while those not inoculated had the lowest number at 60.0 nodules. Cultivars Cargamanto and G59/1-2 inoculated with USDA 2676 produced the highest average nodule number of 72.0 ± 24.0 and 167.0 ± 58.0 respectively as opposed to cultivar NG-224-4 whose highest nodule number was produced by beans inoculated with USDA 2674 at 147.0 ± 53.0 (Table 4) and were significantly different at $P \leq 0.05$). Among the inoculants used, USDA 2674 (in Cargamanto), CIAT 899 and USDA 2676 (in NG 224-4) and USDA 2674 (in G59/1-2) produced less nodules than the controls.

Total plant dry weight at flowering

Results on dry weight yield showed Cargamanto had the

Table 2. Effect of inoculation on dry weight of the three bean cultivars in the greenhouse.

Inoculum	Dry weight in climbing beans (Mean (mg) ± SD)		
	Cargamanto	**NG-224-4**	**G59/1-2**
Control	1.4±0.2bc	1.1 ±0.3a	1.0±0.1a
CIAT 899	1.7±0.2cde	1.9 ±0.2e	1.9±0.1e
USDA 2674	1.8 ±0.2de	1.8 ±0.1de	1.3 ±0.1ab
USDA 2676	1.9 ±0.2e	1.6 ±0.3cde	1.5 ±0.4bcd
Combined	2.3 ±0.2f	1.7 ±0.3cde	1.8 ±0.3de
LSD $_{(0.05)}$ 0.3			

Note: First column indicates rhizobia inocula used while columns two three and four indicate dry weight yield of the three bean cultivars Cargamanto, NG-224-4 and G59/1-2 under the different treatments. Values followed by the same letters in the same column or row did not show statistical difference (p<0.05).

Table 3. c1 reffers to cultivar Cargamanto, c2 NG224-4 and c3 G59/1-2.

Isolate from treatment	Congo red	Bromothymol blue	Milk litmus	Gram stain	Peptone agar
c1 Control	X	Y	P	-ve	-
c1 CIAT 899	X	Y	P	-ve	-
c1 USDA 2674	X	Y	P	-ve	-
c1 USDA 2676	X	Y	P	-ve	-
c1 Combined	X	Y	P	-ve	-
c2 Control	X	Y	P	-ve	-
c2 CIAT 899	X	Y	P	-ve	-
c2 USDA 2674	X	B	B	-ve	-
c2 USDA 2676	X	Y	P	-ve	-
c2 Combined	X	Y	P	-ve	-
c3 Control	X	B	B	-ve	-
c3 CIAT 899	X	Y	P	-ve	-
c3 USDA 2674	X	Y	P	-ve	-
c3 USDA 2676	X	Y	P	-ve	-
c3 Combined	X	Y	P	-ve	-

Note: Each bean received five treatments uninoculated, inoculated with CIAT 899, USDA 2674, USDA 2676 and the three inoculants combined. Columns 2,3,4,5 and 6 represents biochemical results when the isolates were grown on congo red, bromothymol blue, milk litmus, grams stain and peptone agar repectively.

Key:

a). In Congo red; x is pale/Translucent .
In Bromothymol blue; y is yellow colour, b is blue colour.
c).In Milk litmus; p is pink colour, b is blue colour.
d).In Peptone agar; (-) is lack of growth.
e).In Gram stain; -ve is Gram negative.

highest average total plant dry weight of 20.7 mg and was statistically different from the other two cultivars at P ≤ 0.05. Response to inoculation was statistically different at P ≤ 0.05 and showed that uninoculated plants had the highest average dry weight of 20.7 mg and the lowest was in plants inoculated with USDA 2676 whose average was 15.6 mg per plant (Table 5). Within treatments in a cultivar, significant differences at P ≤ 0.05 were noted with uninoculated Cargamanto having the highest dry weight of 27.4 mg ± 5.4; NG-224-4 inoculated with CIAT 899 with 20.6 mg ± 1.3 and G59/1-2 inoculated with USDA 2674 having the highest total dry weight of 20.3 mg ± 5.5 respectively (Table 5). From these results, it was notable that inoculating the climbing beans did not

Table 4. Effect of inoculation on nodulation of the three bean cultivars grown in the field.

	Nodule number in climbing beans (Mean ± SD)		
Inoculum	**Cargamanto**	**NG-224-4**	**G59/1-2**
Control	29.0 ±15.0ab	84.0 ±17.0de	67.0 ±23.0bcde
CIAT 899	40.0 ±19.0abc	70.0 ±24.0cde	98.0 ±23.0e
USDA 2674	23.0 ±8.0a	147.0 ±53.0f	65.0 ±29.0bcde
USDA 2676	72.0 ±24.0cde	70.0 ±10.0cde	167.0 ±58.0f
Combined	52.0 ±12.0abcd	80.0 ±25.0de	75.0 ±14.0cde
$LSD_{(0.05)}$ 39.0			

Note: First column indicates rhizobia inocula used while columns two, three and four indicate nodule numbers on the three bean cultivars Cargamanto, NG 224-4 and G59/1-2 as influenced by the different treatments. Values followed by the same letters in the same column or row did not show statistical difference (p<0.05).

Table 5. Effect of inoculation on dry weight of the three bean cultivars grow in the field.

	Dry weight (mg) of climbing beans (Mean ± SD)		
Inoculum	**Cargamanto**	**NG-224-4**	**G59/1-2**
Control	27.4 ±5.4e	15.3 ±3.2ab	19.4±2.5bc
CIAT 899	19.4±2.8bc	20.6 ±1.3cd	12.8±2.8a
USDA 2674	24.3 ±1.8de	15.2±1.4ab	20.3±5.5cd
USDA 2676	15.1 ±2.3ab	18.8±3.6bc	13.0 ±1.8a
Combined	17.3±2.2bc	15.6 ±3.2ab	19.3 ±2.8bc
$LSD_{(0.05)}$ 4.4			

Note: First column indicates rhizobia inocula used while columns two three and four indicate dry weights of the three bean cultivars Cargamanto, NG224-4 and G59/1-2when inoculated with different rhizobia. Values followed by the same letters in the same column or row did not show statistical difference (p<0.05).

Table 6. Effect of inoculation on seed dry weight of the three bush bean cultivars grow in the field.

	Seed dry weight(mg) of climbing beans (Mean± SD)		
Inoculum	**Cargamanto**	**NG-224-4**	**G59/1-2**
Control	40.5±6.3f	13.0±3.0a	25.2±14.8 bcd
CIAT 899	30.1±6.3cde	20.9±5.3abc	18.6±3.6ab
USDA 2674	28.2±3.7bcde	12.8±2.2a	20.5±4.0abc
USDA 2676	22.8±7.1abc	18.2±6.4ab	24.1±3.9bcd
Combined	36.6±13.6ef	22.7±6.3abc	33.7±7.9def
$LSD_{(0.05)}$ 10.3			

Note: First column indicates rhizobia inocula used while columns two three and four indicate dry weights of the three bean cultivars Ayenew, GLP24 and Ecab 0807 when inoculated with different rhizobia. Values followed by the same letters in the same column or row did not show statistical difference (p<0.05).

lead to higher total plant dry weights.

Seed dry weight

Between the three climbing bean cultivars, there were significant differences in average seed dry weight at P ≤ 0.05. Cultivar Cargamanto had the highest average seed dry weight of 31.6 mg and NG224-4 the lowest of 17.5 mg (Table 6). Multi-strain inoculation gave the highest (P ≤ 0.05) average seed dry weight of 31.01 mg though statistically, they were not different (P ≤ 0.05) from other inoculants. Within different treatments in a cultivar, uninoculated Cargamanto beans gave the highest yield of 40.45 mg ± 6.3, while among cultivars NG224-4 and G59/1-2 beans, multistrain inoculation though statistically not different from the others gave the highest seed dry weight at P ≤ 0.05 (Table 6).

DISCUSSION

Results from the greenhouse showed cultivar G59/1-2 was superior in nodulation to NG224-4 and Cargamanto cultivars. Different rhizobia inoculations did not have significant differences (P ≤ 0.05) among cultivars NG224-4 and G59/1-2 treatments. However, among cargamanto treatments, those inoculated with USDA 2676 had the highest (P ≤ 0.05) nodule number. This indicated differences in nodulation between the three climbing bean cultivars under the controlled conditions of the greenhouse.

In the field, beans inoculated with USDA 2676 produced the highest number of nodules on average. Inoculation with USDA 2676 produced the highest number of nodules in Cargamanto and G59/1-2 cultivars but among NG224-4 it was those inoculated with USDA 2674. Uninoculated beans were able to form as many nodules as those that had been inoculated. These results suggested that different cultivars of beans had preference for certain rhizobia and indigenous rhizobia were as good as some of the inoculants used. This supports observations by Graham [18]; Kremer and Peterson [19]; Pacovsky *et al.* [20]; Mostasso *et al* [21], who noted that though high nitrogen fixing strains of *Rhizobium* have been identified, they often do not provide an agronomic benefit in the field because they are excluded from the nodules of the host plant by the soil indigenous strains which are often more competitive for nodulation than inoculant strains.

Results on dry matter yield showed multistrain inoculated beans produced the highest dry weight in the greenhouse but in the field, it was those inoculated with USDA 2674 and some of the uninoculated plants. This showed that the native soil rhizobia in the uninoculated treatments were able to form effective nodules and therefore the beans accumulated higher dry weights. Greenhouse results showed multistrain inoculation in Cargamanto cultivar produced the highest dry weight. The same was noted among G59/1-2 cultivar though it was not significantly different from those inoculated with CIAT 899. No significant differences were noted among the different treatments of NG224-4. In the field uninoculated Cargamanto beans produced the highest total plant dry weight but among treatments in NG-224-4 and G59/1-2, it was beans inoculated with CIAT 899 and USDA 2674 respectively. However, they were not significantly different from the uninoculated treatments suggesting that there was no need to inoculate. These results supported observations by Burgos *et al.* [22]; Aguilar *et al.* [23]; Saxena *et al.* [24]; Muthini *et al.* [25] - 26], who noted that native rhizobia bacteria that exist in the fields, often out-compete the inoculant strains that only occupy a small proportion of nodules as observed in some areas of Latin America but contradicted the observation made by Mostasso *et al.* [21]; Hungria *et al.* [26] who noted that bean inoculation with *R. tropici* in Brazil was successful and *Rhizobium* inoculated onto beans enhanced their yields.

In seed yields, all the treatments were not significantly different. Among all the bean cultivars, those inoculated with the three rhizobia strains though not significantly different from the other treatments yielded slightly more. This showed that the indigenous rhizobia were quite competitive and that there was no need to introduce new inoculant strains. This supported work done by Kremer and Peterson [19] who observed that though high nitrogen fixing strains of *Rhizobium* have been identified, they often do not provide an 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.

Conclusion

From this study, differences were noted in nodulation between the three climbing bean cultivars. Under the controlled conditions of greenhouse, multistrain inoculated beans produced the highest nodule number but in the field, it was those inoculated with USDA 2676. In yield, multistrain inoculated beans yielded more than the other treatments. This showed that a combination of rhizobia is required if high yields are to be realised. It was also noted that the native soil rhizobia were as competitive and efficient as the inoculants strains when used alone.

REFERENCES

[1] Abate T, Ampofo JKO. Insect pests of beans in Africa. Their Ecology and Management. Annual Rev Entomol, 1996; 41: 45-73.

[2] Karanja D. Kenya National Bean Programme. Annual report, 2006.

[3] Katungi E, Farrow A, Chianu J, Sperling L, Beebe S. Common bean in Eastern and Southern Africa: a situation and outlook analysis, International Centre for Tropical Agriculture, 2009.

[4] Baudoin P, Vanderborght T, Kimani PM, Mwangombe AW. Crop Production in Tropical Africa. In: Directorate general for international co-operation. Ministry of Foreign Affairs, External Trade and International Co-operation. Kluwer Academic Press, Dordrecht Brussels-Belgium, 2001.

[5] Oscar C, Ceballo H, Blair MW. Generation means analysis of climbing ability in common bean (*Phaseolus vulgaris* L.). J Heredity, 2006; 97(5):456-465..

[6] Kelly JD, Adams MW, Varner GV. Yield stability of determinate and indeterminate dry bean cultivars. Theory Appl Genetics, 1987; 74: 516-521.

[7] Stoorvogel JJ, Smalling EMA, Janssen BH. Calculating soil nutrient balances in Africa at different scales. In: An Agro-ecological framework for integrated nutrient management with special reference to Kenya. PhD Thesis (E.M.A. Smalling). Agricultural University, Wageningen, 1993; P. 250.

[8] Badgley C, Moghtader J, Quintero E, Zakem E, Chappell MJ, Avilés-Vàzquez, K, Samulon A, Perfecto I. Organic agriculture and the global food supply. Renewable Agric Food Syst, 2007; 22: 86-108.

[9] Tandon HLS. Soil fertility and fertiliser use – an overview of research for increasing and sustaining soil productivity. In: The Integration of Natural and Man-made Chemicals in Sustainable Agriculture. Edtion HLS. Tandon, 1993; pp 1-32. CASAFA-ISSS-TWAS, New Delhi.

[10] Smalling EMA. Soil nutrient depletion in Sub-Saharan Africa in : The role of plant nutrients for sustainable crops production in Sub-Saharan Africa. H. Van Reuter and W.H. Prins. Eds. Dutch Association of Fertiliser Producers, Leidschenddam, the Netherlands 1993.

[11] Crews TE, Peoples MB. Legume versus fertilizer sources of nitrogen: ecological tradeoffs and human needs. Agric, Ecosyst Environ, 2004; 102, 279-297.

[12] World Bank. World Development Report 2008. The World Bank, Washington DC, USA.

[13] Wasike VW, Lesueur D, Wachira FN, Mungai NW, Mumera LM, Sanginga HN, Mugadi D, Wango P, Vanlauwe B. Genetic diversity of indigenous Bradyrhizobium nodulating promiscuous soybean [Glycine max (L)Merr.] varieties in Kenya: Impact of phosphorus and lime fertilization in two contrasting sites. Plant Soil 2009; 322(1-2) 153-163.

[14] Jaetzold R, Schmidt H (Eds). Farm management handbook of Kenya, Vol. 11/C. Kenya. National conditions and farm management information. Ministry of Agriculture GAT Nairobi and GTZ Eschborn, Rossdorf. 1993.

[15] Somasegaran P, Hoben H, Halliday J. The NifTAL manual for methods in legume-Rhizobium technology. 1985.

[16] Beck DP, Materon LA, Afandi F. Practical Rhizobium legume technology manual. Manual No. 9. International Centre for Agricultural Research in the Dry Areas, Allepo. 1993.

[17] Genstat. Genstat Statistical Software. Lawes Agricultural Trust, Rothamstead Experimental Station. 1995.

[18] Graham PH. Some problems of nodulation and symbiotic N2 fixation in *Phaseolus vulgaris L.* Field Crops Res, 1981; 4: 93-112.

[19] Kremer RJ, Peterson HL. Field evaluation of selected Rhizobium in an improved legume inoculant. Agro J, 1983; 75: 139-143.

[20] Pacovsky RS, Bayne HG, Bethlenfalvay GJ. Symbiotic interactions between strains of *Rhizobium phaseoli* and cultivars of *Phaseolus vulgaris L*. Crop Sci, 1984; 24: 101-105.

[21] Mostasso L, Mostasso FL, Dias BG, Vargas MAT, Hungria M. Selection of bean (*Phaseolus vulgaris L*.) rhizobial strains for the Brazillian Cerrados. Field Crop Res, 2002; 73: 121-132.

[22] Burgos PA, Castellanos J, Mora Y, Mora J. Field inoculation of common bean (Phaseolus vulgaris L.) with high efficiency Rhizobium strains. In Highlights of Nitrogen Fixation Research. Eds E. Martinez and G. Hernandez. Kluwer Acandemic/Plenum publishers, New York. 1999; pp. 255-257

[23] Aguilar OM, Lopez MV, Riccillo PM. The diversity ofrhizobia nodulating beans in North West Argentina as a source of more efficient inoculant strains. J Biotechnol, 2001; 91: 181-188.

[24] Saxena AK, Kingstone JJ, Mondal S, Shende R. Microbial Diversity: Current Perspectives and Potential application. International Publishing House PVT ltd, Newdelhi. 2005; pp 427-456

[25] Muthini JM. Nitrogen fixation in promiscuous soybean [Glycine max L) merril varieties in Eastern Kenya, PhD Thesis, Kenyatta University. 2008.

[26] Hungria M, Vargas MAT, Campo RJ, Chueire LMO, Andrade D de S. The Brazilian experience with the soybean (Glycine max) and the common bean (*Phaseolus vulgaris*) symbioses. In Nitrogen Fixation: From Molecules to Crop Productivityp. 2002.