EFFECTIVENESS OF RHIZOBIA ISOLATES FROM NJORO SOILS (KENYA) AND COMMERCIAL INOCULANTS IN NODULATION OF COMMON BEANS (*PHASEOLUS VULGARIS*)

N. W. Mungai[1] and N. M. Karubiu[2]
[1]Department of Crops, Horticulture and Soils, Egerton University, Njoro, Kenya
[2]Department of Animal Sciences, Egerton University, Njoro, Kenya
E-mail: mungain03@yahoo.com

ABSTRACT
Bean production in Kenya barely meets half the demand because of low soil fertility among other factors. While use of rhizobia inoculants can substantially increase bean yields, less than 1% of the farming population is aware of inoculants. The objectives of this study were, one, to isolate and evaluate indigenous rhizobia populations in two agro ecological zones in Njoro, and two, to test the efficacy of the isolates against commercial rhizobia inoculants in relation to nodulation and shoot dry matter of Rose Coco bean cultivar. Rhizobia isolates were collected from field 8 of Egerton University and Kerma farm in Njoro. The isolates were tested against Biofix (market available inoculant) and USDA 9030 (pure culture of *Rhizobia tropici*) under greenhouse conditions. Rhizobia inoculation had no effect on shoot dry weight (SDW), nodule dry weight (NDW) and shoot N content. However, significant differences were observed among rhizobia strains used, where Biofix produced a higher NDW and Kerma isolate resulted in higher shoot N content than other strains. Symbiotic effectiveness of 111%, 107%, 97% and 92% was observed for Kerma isolate, Field 8 isolate, Biofix and USDA 9030 respectively. Our results indicate that rhizobia isolates from Njoro had comparable symbiotic effectiveness to commercial inoculants and should be tested further using other bean varieties to assess their potential.

Key words: Beans, rhizobia, Kenya, symbiotic effectiveness, inoculant

1.0 INTRODUCTION

Kenya's current annual bean production of approximately 215,000 MT, barely meets half the annual consumption of 450,000 MT. The deficit must be met from imports. The average production per hectare is 500 kg or less, compared to 1800 to 2000 kg ha^{-1} potential (Africa Agriculture, 2008). The major limitation to bean production in many smallholder farms is declining soil fertility as a result of continuous cropping with minimal inputs or rotation to replenish soil nutrients. Nitrogen, for example, is a limiting nutrient in crop production for 35 to 45 per cent of farmers in the highlands, one of the most productive areas of the country (Odame, 1997). Some of the options that are currently being pursued to address low soil fertility include integrated use of organic (e.g., crop residues, animal manures, agroforestry tree prunings) and inorganic (fertilizers, phosphate rocks) resources, and use of rhizobia inoculants (Okalebo *et al*., 2007). Uses of crop residues usually conflicts with their other uses as fuel and fodder, and while most farmers recognize the value of animal manures, most have only few animals so the manure produced is not enough. Manures are also bulky and usually of low and variable quality. Use of rhizobia inoculants in other countries has been successful, and is an option that has potential to increase legume production. The potential for inoculants use in Kenya is great since an estimated 800,000 ha are planted to common beans annually (Africa Agriculture, 2008).

A survey in East and Southern Africa showed that farmers' attitudes to the use of inoculants were varied. Whereas 95% of the farmers were familiar with root nodules, only 26% considered nodules to have beneficial effects and less than 1% of farmers use inoculants in Kenya (Karanja *et al.*, 2000). Beans often demonstrate reduced physiological potential for symbiotic nitrogen fixation, however, they are preferred for their quick maturity, tolerance to short-term drought, ease of harvesting, rapid cooking and favourable taste therefore many farmers are reluctant to consider other legumes (Woomer *et al*., 1999).

Biofix is a market-available peat-based inoculant produced in Kenya. Notwithstanding its obvious potential to replace the often unavailable and expensive chemical fertilizers, Biofix has not been widely adopted in Kenya. Some of the limitations to the widespread use of Biofix include lack of awareness among farmers, unstable supply mechanisms, low production levels and low efficacy (Odame, 1997). While all the limitations are vitally important, that of low efficacy needs to be urgently addressed if bean yields are to be increased at the farm level. Causes of low inoculant efficacy include high indigenous rhizobia populations that out-compete introduced rhizobia, and unfavourable soil conditions especially soil acidity ($pH < 5.0$); low clay content ($< 15\%$) and low cation exchange capacity (CEC) (< 10 cmol kg^{-1}) (Howieson and Ballard, 2004).

Use of legumes that fix nitrogen has been shown as one way of improving soil N content. Benefits from BNF can be accrued in several ways, including breeding legumes for nitrogen fixation, introducing new legumes into cropping systems or using improved rhizobia strains. The latter two approaches can be achieved within shorter time frames and may effectively reverse negative N balance. Improved rhizobia strains (inoculants) for common beans are available in the market, but very few farmers are

aware of their benefits and those that know are not confident of the quality of the inoculants. The objectives of this study were two-fold:

(i) To isolate and evaluate indigenous rhizobia populations in two agro ecological zones in Njoro-division of Nakuru district.

(ii) To test the efficacy of the isolates against commercial rhizobia inoculants on effective nodulation and shoot dry matter of Rose Coco bean cultivar.

2.0 MATERIALS AND METHODS

2.1 Symbiotic Potential of Indigenous Rhizobia Populations Using Whole Soil Inocula

Composite surface soil (0-20 cm) samples were collected from two farms each in Kikapu and Kerma in Njoro in 2006. The two farms from Kikapu are designated KIKHP and KIKLP (Kikapu high and low P, respectively) and in Kerma as KERHP and KERLP (Kerma high and low P, respectively). Initial soil characteristics for the four farms are shown in Table 1.

Selected bean seeds were rinsed in 95% ethanol for 10 sec to remove waxy material and trapped air. Thereafter, the seeds were soaked in a solution of 1% sodium hypochlorite for 10 min to surface sterilize them and thoroughly rinsed in sterile water to remove any residual disinfectant. The seeds were pre-germinated on water agar (agar, 10.0 g L^{-1}) for 2 to 3 d. Two seedlings that were free from contamination and with good root development were transferred into Leonard jars containing sterilised sand. The seedlings were planted at 2 cm depth.

Ten-fold serial dilutions of the four soils were prepared, and I mL of $10^{-1}$and 10^{-2} dilutions used to inoculated the root systems of two replicate seedlings. Biofix, a commercial inoculant, was similarly diluted. Leonard jars were placed in a green house and watered with minus-N nutrient solution (Broughton and Diworth, 1994). Seedlings were harvested after 35 d of growth.

2.2 Rhizobia Isolation from Nodules_

Isolation methods as described by Somasegaran and Hoben (1994) were used. In brief, bean plants (Rose Coco cultivar) were uprooted carefully from Kerma farm and Field 8 (Egerton University), placed in plastic bags and transported to the laboratory. In the laboratory, a sieve (1.0 mm opening) was placed under each root sample to trap nodules that may become detached from the root. The roots were washed under a gentle stream of water. Nodules were cut from the plant, leaving about 0.5 cm of root attached to the nodule for ease in handling. The nodules were thoroughly washed with distilled water to remove all traces of soil, and then immersed in 95% ethanol for 30 s to break the surface tension and to remove air bubbles before soaking in a 5.25 % sodium hypochlorite solution for 4 min to surface sterilize them. Thereafter, they were rinsed thoroughly with sterile water to remove all traces of the sodium hypochlorite, crushed between blunt-tipped forceps and the nodule contents mixed with a drop of sterile water. A loopful of this suspension was streaked onto yeast manitol agar (YMA) plates and incubated at 28 °C for between 3 to 5 d. Representative colonies were selected and subcultured into fresh plates of YMA. Isolated strains were designated

as Kerma and Field 8 isolates.

2.3 Screening Rhizobia Isolates in Potted Field Soil

Composite surface soil (0-20 cm) sample was collected from Kerma Farm in Njoro in 2007, air dried and sieved through a 5 mm sieve. Kerma farm was previously under maize-bean intercrop for three years and under wheat for five years earlier. The farm had been limed with 3000 kg ha^{-1} of agricultural lime in 2006. The soil was analysed for soil pH, cation exchange capacity (CEC), extractable P (Bray 1), Exchangeable K, Ca, and Mg, electrical conductivity and organic C (Table 1). Field capacity of the soil was determined using the cylinder method (Somasegaran and Hoben, 1994) and the volume of water needed to adjust 2.5 kg of soil to field capacity determined as 688 cm^3. Plastic pots with a capacity of about 3 kg were perforated at the bottom and 2.5 kg of soil was weighed into each pot. Fertilizer equivalent to 25 kg P ha^{-1} was added to each pot as Triple superphosphate (TSP) and thoroughly mixed. Soil in all pots was tapped down to occupy nearly the same volume to achieve similar bulk density (of approximately 1 g cm^{-3}). Six surface-sterilised seeds were planted into each pot at a depth of 2 cm. Biofix culture used in this study was that which had been cultured following serial dilution of Biofix, growth in YMA and later in yeast manitol broth (YMB). Kerma and Field 8 isolates were also inoculated into YMB for use in the potted soil. USDA 9030 were regenerated from freeze-dried culture, grown in YMA and then in YMB. Each seed was inoculated with 1 ml of rhizobia culture as described below:

1. Biofix
2. Kerma isolate
3. Field 8 isolate
4. USDA 9030
5. Control 1 (no inoculation but with TSP)
6. Control 2 (no inoculation and no fertilizer)
7. Control 3 (no inoculation but with DAP)

The treatments were replicated five times. Treatments 1 to 5 received the equivalent of 25 kg ha^{-1} P as TSP, treatment 6 is unfertilized non inoculated control and treatment 7 received DAP at equivalent of 67.5 kg ha^{-1} to simulate farmers' practice. Phosphorus was applied across treatments to eliminate its deficiency effects on N-fixation. Sterilised coarse sand was added on top to control contamination and pots randomized on the bench (complete randomized design). Thinning was done after 5 days leaving 4 plants per pot. The plants were kept watered and harvested 35 days after emergence to determine dry weight of shoots and nodules for all treatments. The plants were cut at the sand interface to determine shoot dry weight. Roots were carefully removed from the soil, rinsed with a gentle stream of water and the nodules removed. The nodules and shoots were oven dried at 60 °C and then weighed. The shoots were then ground for total N determination.

Analysis of variance was done to assess treatments effects and Fisher's LSD for separation of means was used in SAS (SAS, 2001) at $\alpha = 0.05$. Contrast analysis was done to compare rhizobia isolates and commercial inoculants. Pearson correlation analysis was performed to reflect the degree of linear relationship of different parameters used to assess nitrogen fixation.

3.0 RESULTS

3.1 Use of Whole-Soil Inocula

Characteristics of the four soils (KIKHP, KIKLP, KERHP, and KERLP) used as source of inocula are shown in Table 1. All four soils were of moderate to high soil fertility based on organic C, total N and exchangeable K. However, Kikapu soils had higher organic C and total N and correspondingly lower C: N ratios than Kerma soils. The four soils differed in extractable P content, where two farms (KIKHP and KERHP) had high P content and the other two (KIKLP and KERLP) had very low P. All four soils were acidic.

Table 1: Characteristics of soils used as whole-soil inocula of bean rhizobia and for green house studies

Soil property	KIKHP†	KIKLP	KERHP	KERLP	G-house
Org. C (%)	3.2	3.5	2.7	2.6	2.3
Total N (%)	0.25	0.25	0.15	0.16	0.14
C: N ratio	13	14	19	16	16
Extractable P (mg kg^{-1})	47	2	43	4	29
Exchangeable K (mg kg^{-1})	1067	490	724	668	1985
pH ($CaCl_2$; 1:1 soil: H_2O ratio)	5.1	4.5	4.3	4.3	7.1
Calcium (mg kg^{-1})	-	-	-	-	3476
Magnesium (mg kg^{-1})	-	-	-	-	356
Ca: Mg Ratio	-	-	-	-	5.9
Sodium (mg kg^{-1})	-	-	-	-	38
Sodium % (ESP)	-	-	-	-	0.6
CEC (cmol kg^{-1})	-	-	-	-	26.8
Electrical conductivity (uS cm^{-1})	-	-	-	-	196

† KIKHP is Kikapu high P, KIKLP is Kikapu low P, KERHP is Kerma high P, KERLP is Kerma low P and G-house is soil used for greenhouse study.

Soil serial dilutions at 10^{-1} and 10^{-2} were used as a source of rhizobia inocula to assess symbiotic potential of indigenous rhizobia for beans. Table 2 shows results of bean shoot dry weight (SDW) grown under greenhouse conditions in Leonard Jars. No significant

differences were observed in SDW. Further, no nodules were found on these plants suggesting a lack of infective rhizobia in the soil extracts used. Results of SDW were comparable across the two dilution levels used as whole-soil inocula.

Table 2: Shoot dry weight (SDW) of Rose Coco bean as affected by inoculation with indigenous rhizobia of four Njoro soils

Treatment	Soil dilution	SDW(mg pl^{-1})
KIKHP†	10^{-1}	460
KIKHP	10^{-2}	328
KIKLP	10^{-1}	400
KIKLP	10^{-2}	430
KERHP	10^{-1}	330
KERHP	10^{-2}	330
KERLP	10^{-1}	335
KERLP	10^{-2}	310
Non-inoculated		410
BIOFIX		450
LSD_{005}		215

† KIKHP is Kikapu high P, KIKLP is Kikapu low P, KERHP is Kerma high P, and KERLP is Kerma low P.

3.2 Screening Rhizobia Isolates in Potted Soil

Inoculation with Biofix, Kevma and Field 8 isolates produced comparable SDW which was higher than with USDA 9030 (Table 3). Nodule dry weight (NDW) following inoculation with Biofix was higher than with Kerma and Field 8 isolates and USDA 9030, while shoot N content was higher following Kerma isolate and lowest with USDA 9030 (Table 3). Variations in fertilizer application did not result in significant differences in SDW, NDW and shoot N content among the control treatments. Overall, rhizobia inoculation had no effect on SDW, NDW and shoot N content when compared to control treatments.

Table 3: Shoot (SDW) and nodule dry weight (NDW) and percent shoot N of Rose Coco beans as affected by inoculation with different rhizobia isolates in potted soil

Treatment	SDW	NDW	Nitrogen
	——— mg plant^{-1} ———		—-- % ----
BIOFIX	613 ba	33 a	2.57 bc
KERMA isolate	601 ba	20 b	2.94 a
FIELD 8 isolate	510 bc	24 b	2.84 ba
USDA 9030	494 c	28 b	2.42 c
CONTROL 1	623 a	28 ba	2.86 ba
CONTROL 2	619 a	29 ba	2.65 bac
CONTROL 3	600 ba	24 b	2.62 bac
LSD_{005}	97	7	0.37

Control 1 – no inoculation with 25 kg ha^{-1} P,
control 2 - no inoculation no fertilizer,
control 3 – no inoculation with 67.5 kg ha^{-1} DAP (farmers' practice).
Different letters within a column denote significant differences at $P < 0.05$.

Contrast analysis was done to test the hypothesis that commercial inoculants perform the same as isolates in Rose Coco bean. Results show this hypothesis to be true for SDW and NDW primarily because of the significant differences between Biofix and USDA 9030 for these parameters. However, in terms of shoot N content the contrast for commercial vs. isolates was highly significant ($F= 9.74$, $P = 0.0042$) indicating that inoculation with the isolates resulted in higher shoot N than with commercial inoculants.

3.3 Symbiotic Effectiveness of Rhizobia Isolates and Inoculants for Beans

Table 4 shows the symbiotic effectiveness (inoculated shoot N/unfertilized control shoot N *100) of different inoculation treatments, with Kerma isolate showing the highest at 111% and USDA 9030 the lowest at 92%. Under our experimental conditions, Biofix and USDA 9030 performed poorer than the unfertilized control since their symbiotic effectiveness was < 100% (Table 4). The computed symbiotic effectiveness is in agreement with the other parameters used to assess rhizobia isolates (Table 2 and 3)

and generally confirms low inoculation effectiveness.

Table 4: Symbiotic effectiveness of rhizobia isolates and commercial inoculants on common beans

Treatment	*Symbiotic Effectiveness (%)*
BIOFIX	97 ba
KERMA isolate	111 a
FIELD 8 isolate	107 ba
USDA 9030	92 b
$LSD_{0.05}$	16

Different letters denote significant differences at P< 0.05

There was a positive linear relationship between NDW and SDW (Figure 1, Pearson correlation coefficient (r) = 0.5 p = 0.004). Relationship between shoot N content and SDW was less apparent (r= 0.3, p=0.06). These results suggest that other factors, such as nodule efficiency may be more important than nodule weight in estimating the amount of N fixed (Ogutcu, 2008).

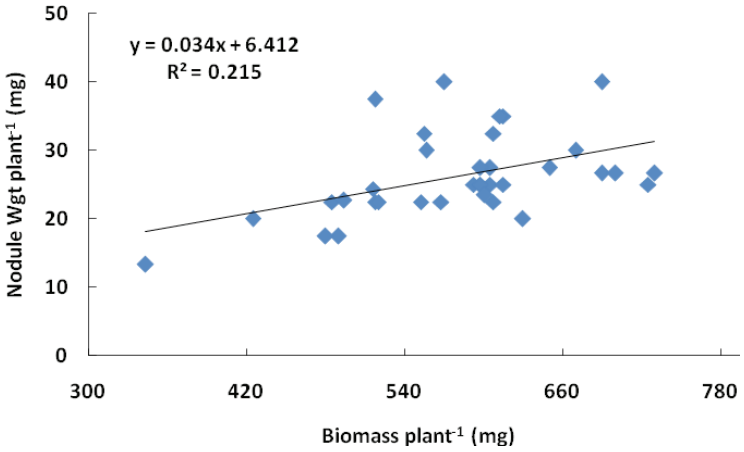

Figure 1: Relationship between nodule and shoot dry weight in beans inoculated with various rhizobia isolates

4.0 DISCUSSIONS AND CONCLUSIONS

4.1 Use of Whole-soil Inocula

Use of soil extracts at 10^{-1} and 10^{-2} dilution of four soils resulted in no nodulation and consequently no differences in SDW. Observed results could be attributed to use of N fertilizers or prolonged periods without bean cultivation in the selected soils resulting in low or no effective rhizobia (Diouf *et al*., 2008). High soil N can result in a reduction in the number of nodules that form (Diouf *et al*., 2008). Studies have shown that reinoculation and continuous cultivation may be necessary to help the build-up of rhizobia in soil, resulting in increases in nodulation and yield (Vlassak *et al.*, 1996; Raposeiras *et al*., 2006).

`Rhizobia are also affected by soil characteristics such as acidity and mineral composition. It was hypothesized that rhizobia population in the four soils would be different based on available P, hence leading to differences in shoot dry weight of inoculated beans. Soils with an organic C of 1.5 to 3.0 % and total N of 0.12 to 0.25 % would be considered to have moderate fertility, while extractable P and K contents of greater than 15 and 175 mg kg^{-1} are considered high (Okalebo, 2002). Raposeiras et al. (2006) reported response to inoculation with three highly effective strains alone or in pairs in the site with low fertility and not in the site with high fertility. It is possible that rhizobia populations were low in the four soils as a result of the high fertility. In addition, all four soils had low pH, which can limit rhizobia population directly, or indirectly cause toxic levels of aluminium and manganese, or deficiencies of calcium and molybdenum.

Bala *et al*. (2001) showed that soil dilutions may select certain rhizobia types/ genotypes depending on the dilution level and differences between strains in their abundance, competitiveness for nodule formation and the potential influence of the soil environment on competitive success. It may therefore be prudent to use trap hosts with varying rhizobia affinities, as well as the native species, use potted whole soil and also explore nodulation in situ where possible, to evaluate the diversity of indigenous or naturalized symbiotic populations. Rhizobia isolated from host plants rather than soil inocula were used for the second part of this study.

4.2 Inoculation with Rhizobia in Potted Soils

Rhizobia inoculation did not improved SDW and NDW over non-inoculated treatments in our study. Beans respond variably to inoculation in greenhouse and field studies. In a combination of 10 rhizobia strains and four bean varieties grown under greenhouse conditions, Diouf *et al.*, (2008) reported that only one elite combination outperformed non-inoculated bean treatment in SDW and N_2 fixation assessed by acetylene reduction activity. Field trials in SE Kenya showed that out of five *Rhizobium* strains tested only one (R3254) significantly increased pod and seed dry weight and seed yield of Tepary beans (Shisanya, 2002). R3254 also enhanced total nitrogen concentration in plant tissue. Rhizobia inoculation had no effect on nodule numbers, shoot biomass and grain yields of six grain legumes (including common beans) in two seasons in central Kenya (Chemining'wa *et al*., 2007).

Further, the lack of response to inoculation in our study can be attributed to several reasons: first, high numbers of indigenous rhizobia may have limited nodule

formation by introduced strains. Since, most indigenous strains often fix less nitrogen than inoculants strains; the benefit of inoculation may not be realized. Secondly, the number of rhizobia introduced during this study may not have been sufficiently large to out-compete indigenous rhizobia. Usually, 10^8 -10^9 of highly effective rhizobia per gram of inoculant should be introduced (Graham, 1999). Thirdly, culturing of Biofix (a multi-strain peat based inoculant) was done following serial dilution so that all treatments received rhizobia inoculation in liquid form. However, such preparations may disadvantage some of the strains in Biofix minimising its benefit as a multi-strain inoculant. Alberton *et al*. (2005) reported that dilution of the soil (used as a source of rhizobia inoculums) resulted in a decreased proportion of the most competitive strains, enabling the less competitive strains to nodulate the common bean. On the other hand, it may be that none of the strains in Biofix was competitive enough against indigenous rhizobia. Additionally, rhizobia strain competitiveness may be influenced by the presence of other strains in the same inoculant (Raposeiras *et al*., 2006).

Total nitrogen in legume plants is one of the parameters used to measure N fixation under experimental conditions. In our study, shoot N content for inoculated plants was the same with control treatments. Beck and Duc (1991) reported that in one line of chickpea (FLIP 83-98), neither total N nor yield were increased by inoculation but the proportion of crop N derived from fixation was increased from 32 to 80%. In crop rotation sequence, this would represent a nitrogen input into soil of more than 40 kg ha^{-1} from inoculation. Though chickpea yield was unaffected, an effect on the following cereal crop would be possible. This phenomenon, usually due to "soil N-sparing" and rhizodepositions is common with many legumes including common beans (Maingi *et al*., 2001).

Oguctu *et al*., (2008) selected seven strains with symbiotic effectiveness ranging from 225% to 394% as those with high potential for use as inoculants in chickpea because of their high shoot dry matter yields, N content and N fixed. Isolates selected for further screening should have high symbiotic effectiveness and none of the strains tested in this study met this criterion.

5.0 CONCLUSIONS

Inoculation with Biofix, USDA 9030 and rhizobia isolates from Njoro did not improve bean SDW, NDW and shoot N content over control treatments. However, Kerma isolates resulted in higher symbiotic effectiveness than USDA 9030. The higher symbiotic effectiveness of Kerma isolates from Njoro is an indication that such rhizobia may be better adapted and that they may have potential as sources of rhizobia inoculant. We suggest that further studies be carried out to better characterise indigenous or naturalised populations of rhizobia for common beans in Njoro. Such studies should include:

(i) Enumeration of native rhizobia in different soils using more than one bean variety.
(ii) Characterisation of rhizobia on the basis of growth rate and morphology, and acid reaction.
(iii) Characterisation using molecular markers such as Rep-PCR for strain and population level characterisation

ACKNOWLEDGEMENTS

Research funds from the Research and Extension Division of Egerton University are gratefully acknowledged. We also thank Rahab Wangechi, Samuel Gachara and Andrew Kere for their participation in processing samples for greenhouse work and data collection. Farmers from Kikapu and Kerma in Njoro are acknowledged for allowing soil collection from their farms.

REFERENCES

Africa Agriculture (2008). Kenya Seeks Increased Production of Beans – September 7, 2008. http://africanagriculture.blogspot.com/2008/09/kenya-seeks-increased-

production-of.html

Alberton O., Kaschuk G. and Hungria M. (2005). Sampling Effects on the Assessment of Genetic Diversity of Rhizobia Associated with Soybean and Common Bean. *Soil Biology and Biochemistry,* **38**, pp 1298-1307.

Bala A., Murphy P. and Giller K.E. (2001). Genetic Diversity of Rhizobia from Natural Populations Varies with the Soil Dilution Sampled. *Soil Biology and Biochemistry,* **33**, pp 841-843.

Beck D. and Duc G. (1991). Improving N_2-fixation in Faba Bean: *Rhizobium* Inoculation and N Nutrition. *Options Mediterraneennes,* **10**, pp 97-103.

Broughton W. J. and Dilworth M. J. (1994) Plant nutrient solutions: *In*: *Handbook for Rhizobia; Methods in Legume-Rhizobium Technology*. Somasegaran P., Hoben H. J. (Eds) Niftal Project, University of Hawaii, Hawaii, pp 245-249.

Chemining'wa G. N., Muthomi J. W. and Theuri S. W. M. (2007). Effect of Rhizobia Inoculation and Starter-N on Nodulation, Shoot Biomass and Yield of Grain Legumes. *Asian Journal of Plant Sciences,* **6**, pp 1113-1118.

Diouf A., Diop T.A. and Gueye M. (2008). Nodulation *in situ* of Common Bean (*Phaseolus vulgaris* L.) and Field Outcome of an Elite Symbiotic Association in Senegal. *Research Journal of Agriculture and Biological Sciences,* **4**, pp810-818.

Graham P. H. (1999). Biological Zinitrogen Fixation: Symbiotic. **In:** *Principles and Applications of Soil Microbiology*. Sylvia et al. (Eds), pp 322-345. Prentice Hall, Upper Saddle River, NJ.

Howieson J. and Ballard R. (2004) Optimizing the Legume Symbiosis in Stressful and Competitive Environments within Southern Australia—some Contemporary Thoughts. *Soil Biology and Biochemistry,* **36**, pp 1261-1273.

Karanja N., Freire J., Gueye M. and DaSilva E. (2000). MIRCEN Networking: Capacity Building and BNF Technology Transfer in Africa and Latin America. *AgBiotechNet,* Vol. **2** March, ABN 043.

Maingi J.M., Shisanya C. A., Gitonga N. M. and Hornetz B. (2001). Nitrogen Fixation by Common Bean (*Phaseolus vulgaris* L.) in Pure and Mixed Stands in Semi-arid South-east Kenya. *European Journal of Agronomy*, **14**, pp 1-12.

Ogutcu H., Algur O. F., Elkoca E. and Kantar F. (2008). The Determination of Symbiotic Effectiveness of Rhizobium Strains Isolated from Wild Chickpeas Collected from High Altitudes in Erzurum**.** *Turkey Journal of Agriculture and Forestry.* **32**, pp 241-248.

Okalebo J. R., Othieno C. O., Woomer P. L., Karanja N.K., Semoka J. R. M., Bekunda M.A., Mugendi D.N., Muasya R.M. Bationo A. and Mukhwana E. J. (2007). Available Technologies to Replenish Soil Fertility in Eastern Africa, p 45-62. **In**: Bationo *et al*. (Eds) Advances in integrated soil fertility management in sub-Saharan Africa: Challenges and opportunities. Springer, The Netherlands.

Raposeiras R., Marriel I. E., Muzzi M. R. S., Paiva E., Filho I. A. P., Carvalhais L. C., Passos R.V. M., Pinto P. P. and Horta de Sá N.M. (2006) *Rhizobium* strains Competitiveness on Bean Nodulation in Cerrado soils. *Brasília,* **41**, pp 439-447.

SAS Institute (2001). *SAS/STAT User's Guide, Version 8.0*. SAS Institute, Cary, NC.

Shisanya, C. A. (2002). Improvement of Drought Adapted Tepary Bean (*Phaseolus acutifolius* A. Gray var. latifolius) Yield Through Biological Nitrogen Fixation in Semi-arid SE-Kenya. *European Journal of Agronomy*, **16**, pp 13-24.

Somasegaran P. and Hoben H. J. (1994). Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. Springer, New York.

Vlassak K., Vanderleyden J. and Franco A. (1996) Competition and Persistence of *Rhizobium tropici* and *Rhizobium etli* in Tropical Soil During Successive Bean (*Phaseolus vulgaris* L.) Cultures. *Biology and Fertility of Soils,* **21**, pp 61-68.

Woomer P. L., Karanja N. K. and Okalebo J. R. (1999). Opportunities for Improving Integrated Nutrient Management by Small Hold Harmers in the Central Highlands of Kenya. *African Crop Science Journal,* **7**, pp 441-454.