Journal of Agriculture and Rural Development in the Tropics and Subtropics
Volume 107, No. 2, 2006, pages 149–159

Population Levels of Indigenous *Bradyrhizobia* Nodulating Promiscuous Soybean in two Kenyan Soils of the Semi-arid and Semi-humid Agroecological Zones

J. M. Maingi [*1], **N. M. Gitonga** [2], **C. A. Shisanya** [3], **B. Hornetz** [4] and **G. M. Muluvi** [5]

Abstract

Soybeans grown in Africa have been selected to nodulate effectively with indigenous *Bradyrhizobium* spp. populations since *Bradyrhizobium japonicum* populations are considered absent or in very low numbers in African soils. The major objective of this study was to estimate total population of *Bradyrhizobia* specific to soybean in two agro-ecologically different study sites, Kiboko in Makueni District, Southeast Kenya (semi-arid to arid conditions) and Kaguru in Meru District, East Kenya (semi-humid climate) . The population of the indigenous rhizobia specific to soybeans was determined using the Most Probable Number (MPN) plant infection technique. In these experiments, the total *Bradyrhizobia* populations, the population sizes of taxonomically defined slow-growing *Bradyrhizobia* specific to soybean and the population sizes of *Bradyrhizobia* spp. specific to tropical *Glycine* Cross (TGx) varieties were determined for the two study sites. Cowpea, *Vigna unguiculata*, cultivar Ken Kunde I was used to estimate the total *Bradyrhizobia* spp. population. Clark soybean, *Glycine max*, was used to estimate the population sizes of taxonomically defined slow-growing *Bradyrhizobia* spp. specific to soybean while a TGx genotype, SB12-TGx1869-31E was used to determine the population sizes of *Bradyrhizobia* spp. specific to TGx varieties. The results of the MPN counts indicated that the total *Bradyrhizobia* population in Kiboko was between 2.59×10^4 and 1.89×10^5. The population size of taxonomically defined slow-growing *Bradyrhizobia* in Kiboko was between 2.59×10^2 and 1.89×10^3 cells per gram of soil sample while the approximate *Bradyrhizobia* population specific to TGx genotype was between 7.81×10^2 and 5.67×10^3 cells per gram of soil. In Kaguru, the approximate total *Bradyrhizobia* population was between 1.04×10^2 and 7.56×10^3 cells per gram of soil. The population size of taxonomically defined slow-growing *Bradyrhizobia* was be-

* corresponding author

[1] John M. Maingi, Department of Plant and Microbial Sciences, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya, E-mail: jmanguske@yahoo.com

[2] Nkanata M. Gitonga, see [1]

[3] Chris A. Shisanya, Department of Geography, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya

[4] Berthold Hornetz, Department of Geosciences, University of Trier, D-54286, Trier, Germany

[5] Geoffrey M. Muluvi, Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya

tween $1.33x10^2$ and $9.72x10^2$ cells per gram of soil while the approximate *Bradyrhizobia* population specific toTGx genotype was between $2.37x10^2$ and $1.73x10^3$ per gram of soil. These populations were adequate to give satisfactory results on nodulation and nitrogen fixation in the two study sites.

Keywords: Bradyrhizobia, soybean, Glycine Cross, TGx, Glycine max, nodulation, Most Probable Number, soil, nitrogen fixation, Kenya

1 Introduction

Soybean has become famous as the plant that will help feed the world's present and future population and help to solve world protein deficiency. The soybean seed is the richest natural vegetable food. Soybean is an important aid to agriculture (Hill, 1978, pp. 340-341); it can help to enrich the soil with nitrogen through biological nitrogen fixation. Soybean as food is not as important in Africa as it is in developed countries or in Asia where it originated. The beans have a strong flavour, which is not liked by many local people. However, soybeans have a lot of uses in industry. Most soybeans grown in tropical Africa are for oil production and the protein-rich seed cake is fed to animals. Some soybean processors also produce soy flour, which has low carbohydrate content and is an excellent food for diabetics. The seeds can also be processed to give soymilk, which is an excellent source of protein for babies, especially those just weaned from breast-feeding. Cultivation of this crop in Sub-Saharan Africa has not been substantial but popularisation programmes by governments and non-governmental bodies have helped to create awareness to small scale farmers of the importance of soybean not only as a crop for improving their economic status but also as an important high protein food. Soybean is not an indigenous crop to Africa (Okogun and Sanginga, 2003) and it needs either inoculation with specific effective strains of *Bradyrhizobium japonicum* or addition of nitrogen fertilizer. Nitrogen is one of the major nutrients required by plants and is often the most limiting under cultivation. The small-scale farmers in Africa are resource poor and cannot afford the expensive nitrogen fertilizer inputs required to increase soybean yield. Many of the farmers in Africa cannot also afford the use of inoculants that provide an alternative and cheaper source of nitrogen for crop production than the N fertilizers (Kaleem, 2002). The technology for use of inoculants is also cumbersome and difficult to apply by the farmers (Hornetz *et al.*, 2000). They face problems in acquisition and storage of inoculants because cooling facilities are not readily available. Such requirements place constraints on the farmer's capacity to effectively use inoculants. As a result, soybean crops as are grown by farmers in Africa receive no inoculants and little or no commercial nitrogen fertilizer.

To avoid the need for inoculation, soybean breeders at the *International Institute of Tropical Agriculture* (IITA), Nigeria, developed new soybean genotypes for Africa. These genotypes, known as Tropical *Glycine* cross (TGx), nodulate with Bradyrhizobium sp. populations indigenous to African soils (Abaidoo *et al.*, 2000; Osunde *et al.*, 2003). These cultivars have been tested in a number of African countries (Mpepereki *et al.*, 2000; Javaheri, 1996). In Nigeria for example, the development of "promiscuous"

soybean cultivars has led to the spread of the crop to large parts of Semi-humid Guinea savanna where it was not being grown before (OSUNDE *et al.*, 2003).

Much still needs to be done to ensure optimum productivity by the TGxs. One of the main areas that need attention is the quantification of the nitrogen fixed by the rhizobia, and in this way assess the symbiotic performance of the association of the TGxs and the indigenous rhizobia in the soil (OSUNDE *et al.*, 2003). The numbers of indigenous Bradyrhizobia as well as the soil available nitrogen have been cited as some of the main factors that have a significant effect on symbiotic relationships (KEYSER and LI, 1992). The major objective of this study was to estimate the number of indigenous *Bradyrhizobia* in two ecologically different sites in Kenya, Kiboko in Makueni district, southeast Kenya and Kaguru in Meru district with the aim of assessing symbiotic relationship and performance of the indigenous Bradyrhizobia and promiscuous soybean varieties.

The experiments were carried out in the Greenhouse of the Department of Plant and Microbial Sciences, Kenyatta University Nairobi, using soil samples from the two sites in East and South East Kenya. The objectives of the study were: (1) to estimate the population sizes of taxonomically defined slow-growing soybean rhizobia (*Bradyrhizobium japonicum* and *Bradyrhizobium elkanii*, (2) to estimate the total *Bradyrhizobium* spp. populations and (3) to determine the population sizes of *Bradyrhizobium* spp. specific to TGx.

2 Materials and Methods

2.1 The Study Sites

The soil samples for this study were obtained from Kenya Agricultural Institute (KARI), Kiboko Sub-centre (AEZ: LM 5-6) in Makueni District, SE-Kenya and Kaguru Farmers Training Centre (FTC) (AEZ: UM 2-3) in Meru District, East Kenya respectively. KARI, Kiboko Sub-Centre (latitude 02° 12' S, longitude 3° 43' E), is located about 160 km south east of Nairobi, Kenya. The soils of the study area are well drained Fluvisols, Ferralsols and Luvisols. The soil pH is 6.9 (measured in $0.01 ml^{-1}$ $CaCl_2$). Analysis of soil nitrogen and phosphorous by the method described by FORSTER (1995) indicates a deficiency of both nutrients. Rainfall is bimodally distributed, with median monthly maximum in April (126 mm) and November (138 mm). The mean annual rainfall is about 582 mm. The short rains (October- January) generally have more rainfall and are more reliable than the long rains (March-June) (MUSEMBI and GRIFFITHS, 1986). Mean monthly temperatures are highest in February and October, prior to the onset of the rains in March and November, respectively (KMD, 1984). Kaguru Farmers Training Centre (FTC) is located at latitude, 0° 05' S and longitude 37° 40' E. The soils are well drained, extremely deep, dark reddish brown, friable clay, with acid humid top soil (humid nitosols). The soil pH measured at the time of the study was 4.83. The rainfall is bimodal; the long rains normally start at the end of March while the short rains start normally in October. The short rains are normally more reliable and give higher yields (JÄTZOLD and SCHMIDT, 1983).

2.2 Soil Sample Collection

Ten soil sub-samples were collected from each of the two study sites, Kiboko in Makueni district and Kaguru in Meru district. Surface debris were cleared from the area to be sampled. After clearance of debris from the surface, a soil core was removed from 15-20 cm depth with a soil auger (ABAIDOO *et al.*, 2002). Each soil sub-sample was cylindrical in shape, 3-5 cm in diameter. In the greenhouse, the soil sub-samples were thoroughly mixed together to obtain a homogenous composite sample.

2.3 Seed Procurement

The seeds which were used in this experiment were of *Vigna unguiculata*, cultivar Ken Kunde I (cowpea), *Glycine max*, cultivar Clark (Clark soybean) and a TGx soybean genotype, SB 12- TGx 1869-31 E. The cowpea seeds were purchased from Kenya Seed Company, Nairobi Kenya. Clark soybean seeds were obtained from United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Soybean Germplasm Collection, Department of Crop Sciences University of Illinois, USA. The SB12-TGx 1869-31E seeds were obtained from International Institute of Tropical Agriculture (IITA), Ibadan Nigeria.

2.4 Leonard Jar Assemblies

The Leonard jar assemblies used were a modification of that described by VINCENT (1970). The assembly was composed of a plastic cup, 8 cm mouth (brim) diameter which tapered to a bottom diameter of 4 cm. The cup containing the rooting medium (vermiculite) was inserted into a larger plastic vessel containing the nutrient solution. Eight hundred mls of plant nutrient solution was added into the lower container of each Leonard jar assembly. A sponge connecting the upper and the lower units of the jar irrigated vermiculite with the nutrient solution. The whole set up was insulated with a khaki paper bag.

2.5 Rooting Medium

The rooting medium, which was used in this study, was vermiculite. This material was washed thoroughly for three successive days by changing the water three times per day and stirring frequently. The final rinse was with distilled water and the pH of the medium was adjusted to about pH 6.8. After attaining the correct pH, water was drained off and the vermiculite packed into the small plastic cups of the Leonard jar assemblies. To reduce contamination and entry of water, the top cups were covered with lids and the assemblies were then steamed for one hour, twice in an autoclave to get rid of microorganisms.

2.6 Plant Growth Medium

Plant nutrient solution was prepared as described by BECK *et al.* (1993). Five stock solutions were prepared (Table 1). For each litre of full-strength plant growth solution, 0.5ml were added from each of the five stock solutions. The pH of the solution was adjusted to 6.8 using $NaOH$ (1.0 M) or HCL (1.0 M). All solutions were sterilized by autoclaving at 121°C for 15 minutes.

Table 1: Nitrogen free nutrient solution (Source: BECK *et al.* (1993))

Stock	*Compound*	*Amount* (g^{-1})	*Final Solution Concentration*
1	$CaCl.\,2H_2O$	294.1	1.00mM
2	KH_2PO_4	136.1	0.50 mM
3	$MgSO_4.7H_2O$	123.3	0.25mM
	K_2SO_4	87.0	0.25mM
	$MnSO_4.H_2O$	0.338	1.00μM
4	H_3BO_3	0.247	0.30μM
	$ZnSO_4\,H_2O$	0.288	0.50μM
	$CuSO_4\,5H_2O$	0.1	0.20μM
	$NaMoO_2\,:H_2O$	0.048	0.01μM
	$CoSO_4\,7H_2O$	0.056	0.01μM
5	Fe Citrate+	5.4	10.00μM

For each litre of full strength solution, 0.5 ml was added from each of the five stock solutions.

2.7 Sterilization and Pregermination of Seeds

Cowpeas and soybean seeds of good viability (more than 80 %), undamaged and of uniform colour and size were selected MAINGI *et al.* (1999). The seeds were surface-sterilized by immersing them into a 3 % solution of sodium hypochlorite for 5-10 minutes (3 % sodium hypochlorite solution was prepared by adding 10 parts of commercial bleach [5.25 % sodium hypochlorite] to 7.5 parts of water). The seeds were rinsed with 8 changes of sterile distilled water after surface sterilization. They were then soaked in clean sterile distilled water and allowed to imbibe it for one hour. They were transferred aseptically to 2 % water agar plates with a spoon-shaped spatula. Twenty seeds were placed in each plate. The plates with the seeds were incubated upside down at 28°C to enable the radicles to grow away from the water agar. The incubation period was four days. Seedlings whose radicles attained a length of 1-2 cm after the incubation period were considered ready for transferring to Leonard jar assemblies.

2.8 Planting in Leonard Jar Assemblies

A pair of flame sterilized forceps was used to prepare one hole in the rooting medium in each Leonard jar. Seeds with radicle length of 1-2 cm were picked up with the sterile pair of forceps and placed one per hole, with the radicle facing downwards. The holes were deep enough to accommodate pre-germinated seeds 0.5 cm below the surface.

The seedlings were maintained for eight days in the Leonard jar assemblies before inoculation with serial dilutions of soil samples. For the preparation of the serial dilutions, one hundred grams of the composite soil sample were diluted in 900 ml of sterile distilled water. A four-fold dilution series was made from 4^{-1} to 4^{-10}. The Leonard jar assemblies with the seedlings were set in quadruplicates in the greenhouse and inoculated with 2 ml of the dilutions following the procedure of SOMASEGARAN and HOBEN (1994). After inoculation with the soil samples the plants were maintained in the greenhouse for 4 weeks after which they were harvested.

2.9 Harvesting of Plants

At harvest, the stems of the plants were cut at the level of the growth medium. Root and adhering rooting medium were removed and put into a coarse sieve. The rooting medium was washed from the roots using a gentle stream of water. nodulation was observed (+, for nodulation or - for no nodulation) and the number of nodulated (+) plants (units) was recorded beside each dilution.

2.10 Estimation of Bradyrhizobia Population

At harvest, the total number of nodulated units was obtained by summing up the nodulated units at each dilution level (Tables 2 and 3). Uninoculated controls were used to check for sterile conditions. The numbers of Bradyrhizobia in the soil were determined using the Most Probable Number (MPN) plant infection technique. The MPN was calculated from the most likely number (m) obtained from the MPN tables according to the formula:

$$MPN = \frac{m \times d}{v} \tag{1}$$

where: m is the most likely number from MPN tables, d is the lowest dilution in the series and v is the aliquot used for inoculation (SOMASEGARAN and HOBEN, 1994). An estimated range for each of the population was obtained by multiplying and dividing the estimated population by a factor, 2.7 fiducial limit at 95% confidence level (BECK *et al.*, 1993).

3 Results and Discussion

The three soybean cultivars used in the study formed nodules when they were inoculated with serial dilutions of soils from the two study sites but there were great variations in the number of nodulated units per cultivar per study site. The estimated total *Bradyrhizobium* spp. population in Kiboko soil was between 2.59×10^4 and 1.89×10^5 cells per gram of soil sample while the population size of taxonomically defined slow-growing rhizobia (*Bradyrhizobium japonicum* and *Bradyrhizobium elkanii*) was between 2.59×10^2 and 1.89×10^3 cells per gram of soil sample. The population estimates from the TGx genotypes less the population estimates from soybean, cultivar Clark represented approximate population sizes of *Bradyrhizobium* spp. population specific to TGx genotype (ABAIDOO *et al.*, 2002). This was estimated to be between 7.81×10^2 and 5.67×10^3 cells per gram of Kiboko soil sample (Table 2). In Kaguru, the approximate total *Bradyrhizobium* spp. population was between 1.04×10^2 and 7.56×10^3 cells per gram

of soil sample while the population size of taxonomically defined slow-growing rhizobia (*Bradyrhizobium japonicum* and *Bradyrhizobium elkanii*) was between 1.33×10^2 and 9.72×10^2 cells per gram of soil. Approximate *Bradyrhizobium* spp. population specific to TGx genotype in Kaguru soil was between 2.37×10^2 and 1.73×10^3.cells per gram of soil sample (Table 3).

Table 2: Nodulated units planted with Cowpea, Clark and SB 12 TGx-1869-31E in Kiboko soil.

Dilution	*Total number of nodulated units*		
	Cowpea	*Clark*	*SB 12 TGx-1869-31E*
4^{-1}	4	4	4
4^{-2}	4	4	4
4^{-3}	4	3	3
4^{-4}	4	2	3
4^{-5}	4	1	2
4^{-6}	4	0	1
4^{-7}	2	0	1
4^{-8}	1	0	0
4^{-9}	0	0	0
4^{-10}	0	0	0
Control	0	0	0
Total	27	14	18

Number of replications (n)= 4

Generally, population sizes of the different types of rhizobia were higher in Kikoko soil than in Kaguru soil. The total *Bradyrhizobium* spp. population was about 2.58×10^4 to 1.81×10^5 cells per gram higher in Kiboko soil than in Kaguru soil while the population of slow growing rhizobia was approximately 1.26×10^2 to 9.18×10^2 cells per gram higher in Kiboko soil than in Kaguru soil. The population size of taxonomically defined slow-growing rhizobia was approximately 5.44×10^2 to 3.94×10^3 cells per gram of soil higher in Kiboko than in Kaguru. The total *Bradyrhizobium* spp. population was higher in the two sites as compared to the population sizes of slow-growing rhizobia and also the rhizobia specific to TGxs. The population size of the taxonomically defined slow-growing rhizobia was the lowest in the two soils.

The plant infection count is commonly used to estimate numbers of rhizobia in soil or to determine the quality of inoculants produced in sterile conditions (Beck *et al.*, 1993; Somasegaran and Hoben, 1994). Empirical models describing the response

Table 3: Nodulated units planted with Cowpea, Clark and SB 12 TGx-1869-31E in Kaguru soil.

Dilution	*Total number of nodulated units*		
	Cowpea	*Clark*	*SB 12 TGx-1869-31E*
4^{-1}	4	4	4
4^{-2}	4	3	3
4^{-3}	4	2	3
4^{-4}	3	1	2
4^{-5}	2	1	2
4^{-6}	1	0	1
4^{-7}	0	0	0
4^{-8}	0	0	0
4^{-9}	0	0	0
4^{-10}	0	0	0
Control	0	0	0
Total	18	11	15

Number of replications (n)= 4

to inoculation of legumes (THIES *et al.*, 1991) indicated that density as estimated by the MPN- plant infection assay is one of the primary factors determining the magnitude of response to inoculation. This is one of the main reasons why the *Bradyrhizobia* populations were determined in the two field sites before inoculation of soybeans with commercial inoculum. The size of field populations of rhizobia may vary within short distances in a field. For this reason much care should be taken in sampling so that a truly representative sample of the field soil is obtained. This was put into consideration when samples were being collected from the two field sites. Sampling was done diagonally in the fields to ensure uniformity of the process. Lack of compatible rhizobia in the soil has been cited as one of the potential barriers to the introduction of soybean in most African regions (MPEPEREKI *et al.*, 2000). However the *Bradyrhizobia* populations observed in the two study sites are adequate to give satisfactory results on nodulation and nitrogen fixation. The results are in line with findings by NAMBIAR *et al.* (1983) that most cultivated tropical soils have a rhizobial population of more than 100 rhizobia cells per gram of soil capable of nodulating the legumes grown on such soils.

The Kiboko site had higher *Bradyrhizobia* populations in all the categories than the Kaguru site. Soil analysis indicated that the Kaguru soil was acidic. *Bradyrhizobia* strains normally fail to multiply in acid conditions (COOPER *et al.*, 1985). Therefore most legumes which depend on symbiotic nitrogen fixation require a neutral or slightly

acidic soil for growth (Brockwell *et al.*, 1991). The low pH could be one of the possible reasons why the *Bradyrhizobia* population was low in Kaguru site compared to Kiboko. These variations in *Bradyrhizobia* populations have also been reported in other studies. Abaidoo *et al.* (2002) reported that the population sizes of indigenous promiscuous soybean *Bradyrhizobia* in soils from African countries were highly variable.

4 Conclusion

Strains of *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* were detected in the soils from the two study sites. However the numbers of these strains were far much lower than those of total *Bradyrhizobium* spp. (also referred to as cowpea miscellany) and *Bradyrhizobium* spp. populations that nodulate TGx soybean genotypes. Good infectivity was observed in *Bradyrhizobium* spp. (TGx) in this study. This implies that these rhizobia can be potential local source of strains for inoculum production and use in other soybean growing areas in Kenya where the *Bradyrhizobium* spp. populations are ineffective or inadequate. From this study, it can also be concluded that the TGx varieties are more restrictive in their *Bradyrhizobium* spp. requirements for effective nodulation than cowpea as evidenced by the fact that the population size of the cowpea miscellany was higher than that of *Bradyrhizobium* spp. (TGx) in the two study sites. This knowledge of the population characteristics of indigenous bradyrhizobia populations in East and South East Kenya will be very valuable for developing strategies to improve Biological Nitrogen Fixation (BNF) for increasing TGx soybean yields at low costs.

Acknowledgement
The work presented here is part of a larger project funded by the Volkswagen Stiftung, Hannover, Germany. The authors are grateful for the generous financial assistance.

References

Abaidoo, R. C., Keyser, H. H. and Singleton, P. W.; Population and symbiotic characteristics of indigenous *Bradyrhizobium* spp. that nodulate TGx soybean Genotypes in Africa; in: *Challenges and imperatives for biological nitrogen fixation research and application in Africa for the 21st century. Ninth congress of the AABNF*, edited by Karanja, N. and P., K. J. H.; 167–188; John Philips Africa Limited, Nairobi; 2002.

Abaidoo, R. C., Keyser, H. H., Singleton, P. W. and Bortharkur, D.; *Bradyrhizobium* spp. (TGx) isolates nodulating the new soybean cultivars in Africa are diverse and distinct from *Bradyrhizobia* that nodulate North American soybeans; *International Journal of Systematic and Evolutionary Microbiology*; 50:225–234; 2000.

Beck, D. P., Materon, L. A. and Afandi, F.; Practical *Rhizobium*-legume technology manua; Manual No. 9. International Centre for Agricultural Research in the Dry Areas, Aleppo, Syrian Arab Republic, pp. 1-245; 1993.

Brockwell, J., Pilka, A. and Holliday, R. A.; Soil pH is a major determinant of the numbers of naturally occurring *Rhizobium mililoti* in non-cultivated soils of New South Wales; *Aust. J. Exp. Agric.*; 31:211–219; 1991.

Cooper, J. F., Wood, M. and Bjourson, A. J.; Nodulation of *Lotus pedunculutus* in acid rooting solution by fast and slow-growing rhizobia; *Biol. Biochem.*; 17:487–492; 1985.

Forster, J.; Soil Nitrogen; in: *Methods in Applied Soil Microbiology and Biochemistry*, edited by Alef, K. and P. Nannipieri, P.; 79–87; Academic Press, London; 1995.

Hill, A. F.; *Economic Botany: A textbook of useful plants and plant products*; Tata McGraw-Hill Publishing Company Ltd. New Delhi; 1978.

Hornetz, B., Shisanya, C. A. and Gitonga, N. M.; *Studies on the ecophysiology of locally suitable cultivars of food crops and soil fertility monitoring in the semi-arid areas of SE-Kenya*; Materialien zur Ostafrika-Forschung, Heft 23; Geographische Gesellschaft Trier; 2000.

Javaheri, F.; *Nitrogen fixation by promiscuous soybean varieties in small scale cropping systems of Zambia and Zimbabwe*; Ph.D. thesis; University of Zimbabwe, Harare; 1996.

Jätzold, R. and Schmidt, H.; Farm management handbook of Kenya, Vol. 11/C: East Kenya: Natural conditions and farm management information; Ministry of Agriculture/GAT Nairobi and GTZ Eschborn; 1983.

Kaleem, F.; Biological nitrogen fixation in Soybean/Sorghum cropping system; in: *Challenges and imperatives for biological nitrogen fixation research and application in Africa for the 21st century. Ninth congress of the AABNF*, edited by Karanja, N. and Kahindi, J. H. P.; 50–62; John Philips Africa Limited, Nairobi; 2002.

Keyser, H. H. and Li, F.; Potential for increasing biological nitrogen fixation in soybean; *Plant and Soil*; 141:119–135; 1992.

KMD; Climatological statistics for Kenya; Kenya Meteorological Department (KMD), Nairobi; 1984.

Maingi, J., Shisanya, C. A., Gitonga, N. M. and Hornetz, B.; Biological nitrogen fixation in selected legumes of the semi-arid Makueni District of Southeast Kenya; *Der Tropenlandwirt. Journal of Agriculture in the Tropics and Subtropics*; 100(2):205–213; 1999.

Mpepereki, S., Javaheri, F., Davis, P. and Giller, K. E.; Soyabeans and sustainable agriculture. Promiscuous soyabeans in southern Africa; *Field Crops Research*; 65:137–149; 2000.

Musembi, D. K. and Griffiths, J. F.; The use of precipitation data to identify soil moisture patterns and the growing seasons in Eastern Kenya.; *Journal of Agricultural and Forest Meteorology*; 37:47–61; 1986.

Nambiar, P. T. C., Rao, M. R., Reddy, M. S., Flyod, C. N., Dart, P. J. and W., W. R.; Effect of intercropping on nodulation and nitrogen fixation by ground nut; *Experimental Agriculture*; 19:79–86; 1983.

Okogun, J. A. and Sanginga, N.; Can introduced and indigenous rhizobial strains compete for nodule formation by promiscuous soybean in the moist savanna agroecological zone of Nigeria?; *Biology and Fertility of Soils*; 38(1):26–31; 2003.

Osunde, A. O., Gwam, S.and Bala, A., Sanginga, N. and Okugun, J. A.; Responses to rhizobial inoculation by two promiscuous soybean cultivars in soils of the Southern Guinea savanna zone of Nigeria; *Biology and Fertility of Soils*; 37:274–

279; 2003.

Somasegaran, P. and Hoben, H.; Methods in Legume-rhizobium technology; NIFTAL project. University of Hawaii, Paia, Honolulu; 1994.

Thies, J. E., Singleton, P. W. and Bohlool, B. B.; Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes; *App. Environ. Microbiol.*; 57:19–28; 1991.

Vincent, J. M.; A manual for the practical study of root nodule bacteria; IBP Handbook No. 15. Blackwell Scientific Publications, Oxford; 1970.