World Journal of Agricultural Sciences 6 (4): 381-387, 2010
ISSN 1817-3047

Biological Nitrogen Fixation by Promiscuous Soybean (*Glycine max* L. Merril) in the Central Highlands of Kenya: Response to Inorganic Fertilizer Soil Amendments

[1]Mugendi Ezekiel, [2]Gitonga Nkanata, [1]Cheruiyot Richard and [1]Maingi John

[1]Department of Plant and Microbial Sciences,
Kenyatta University, P.O. BOX 43844-00100, Nairobi, Kenya
[2]School of Agriculture and Enterprise Development, P.O. Box 43844-00100, Nairobi, Kenya

Abstract: Biological nitrogen fixation (BNF) by promiscuous soybean cultivars offers a potential for minimizing the investment made by resource–limited farmers in central highlands of Kenya. Nitrogen fixation in this grain legume is influenced by factors such as availability of mineral elements and prevalent weather conditions. Nitrogen (N), Phosphorus (P) Potassium (K) and Sulphur (S) are intimately involved in plant metabolism, growth and N_2 fixation. In this study field, laboratory and greenhouse experiments were carried out to investigate the effects of PKS fertilizer application on nodulation and nitrogen fixation of two promiscuous soybean varieties. Early maturing TGx 1740-2F (SBI9) and late maturing TGx 1448-2E (SB20) were the main factors while fertilizer inputs were the sub-factors. Nodulation status, plant biomass production and yield components were used for data generation. The transformed data was subjected to analysis of variance- ANOVA using PROC GLM package to determine the main effects of the treatments and their interactions. Specific pair-wise comparisons of treatment levels were done using the least significant differences (LSD) test at P = 0.05 and correlations using 'PROC CORR'. In all sites the main effects of carbon level in the field, soybean varieties and treatments on nodulation, plant biomass production and yield components were significantly different at ($P \leq 0.05$). The effects due to the interaction of these factors were not significant. Laboratory and greenhouse results indicated that the isolates obtained were slow growing *Bradyrhizobium elkanii, Bradyrhizobium japonicum* and the fast growing *Sinorhizobium fredii.*

Key words: Promiscuous soybean · Nitrogen fixation · Soil amendments

INTRODUCTION

In Sub-Saharan Africa (SSA), a widening gap exists between food production and population growth [1]. In addition to declining grain production in the recent years, food legume yields have also decreased. According to the World Bank projection [2], the demand for food in SSA will double by 2015 from its level in the year 2000. Although Africa has the potential to feed itself, food production is getting inadequate and unsustainable. Many farmers face urgent problems of making sure there is enough food for their families for the whole year and earn sufficient income from the farm. Population growth [3] and the increasing size of towns mean that the amount of land available to grow food for each family is decreasing [4]. This has pushed cultivation into marginal areas leading to natural resource degradation.

Symbiosis between leguminous plants and soil bacteria commonly referred to as rhizobia is of considerable environmental and agricultural importance [5]. They are responsible for an estimated 180 × 106 tonnes per year of biological nitrogen fixation world-wide, which is equivalent to generation of resource equivalent to US $ 160-180 billion [6]. The symbiotic component alone contributes about 120 × 106 metric tonnes per year [7] to global nitrogen economy; this represents more than 65% of the nitrogen used in agriculture [8] and is several-fold larger than the input of nitrogen from N fertilizers, which is estimated at 65 × 106 tonnes per annum [6]. Therefore, the fixation of N_2 by legumes can play a key role in agricultural sustainability in SSA.

Crop yields in Central Highlands of Kenya are poor due to low soil fertility. Being resource poor, most

Corresponding Author: Ezekiel Mugendi Njeru, Department of Plant and Microbial Sciences, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya, E-mail: njeruezek@gmail.com

smallholders in this region like their counterparts in the rest of sub-Saharan Africa (SSA), typically apply negligible amounts of mineral fertilizers. Soybean farming is one of the most cost-effective ways in which smallholder farmers can maintain soil fertility and yet reap other benefits from the crop [9]. Although soybean is relatively a new crop for smallholders in the Central Highlands of Kenya, its cultivation is expected to gain popularity in the near future because of the increasing need for food and fodder. The crop is known for its high nitrogen fixing ability [10], thus improving soil nitrogen (N) content [11]. Nodulation and nitrogen fixation in soybean occurs effectively if other mineral elements such as Phosphorus, Potassium and Sulphur (PKS) are present in the soil. This necessitates constant addition of PKS fertilizers to boost the soil mineral nutrient level. The general objective of this study therefore was to determine the effects of soil amendments with PKS fertilizers on nodulation, nitrogen fixation and yield components of promiscuous soybeans in the Central Highlands of Kenya.

MATERIALS AND METHODS

Field experiments were conducted in Chuka and Muthambi Divisions, Meru South District, in the Central Highlands of Kenya. The area is in upper midlands 2 and 3 (UM2 and UM3) with an altitude of approximately 1500m above sea level. The average maximum temperature is 27°C; the minimum is 14°C, while the average temperature is 20.5°C. The area receives annual rainfall varying from 500 to 2200 mm bimodally. Long rains (March to June) and short rains (October to December). The soils are mainly humic nitisols [12] derived from basic volcanic rocks. Eight farms were identified in each division. In each farm, two sites were selected based on total organic carbon (one site with the lowest C and one site with the highest C). A mixture of sulphuric acid and aqueous potassium dichromate as described in section 3.3 was used to determine the soil organic carbon [13].

Experimental Design: The experiment was laid out as a randomized complete block design (RCBD) with each farm serving as a replicate. The promiscuous soybean varieties SB19 (TGX 1740-2F), an early maturity variety and SB20 (TGX 1448-2E), a late maturity variety were the main treatments while fertilizer inputs were the sub-treatments (Table 1). Plot size was 4 M by 2.70 M.

Land Preparation: The experimental fields were cleared of grasses and other prevalent weeds using mechanical methods, followed by demarcation. The fields were ridged at 45 cm intervals to a depth of 30 cm. Soybean seeds of high viability and quality were carefully sorted to increase chances uniform germination. All the fertilizer inputs were applied in the planting line on the trough and incorporated before planting. After application of the fertilizer, soybeans were planted in rows, 45 cm apart and drilled to be thinned to 5cm distance between plants. Weed control was done manually by periodically scouting the plots and uprooting the weeds wherever necessary.

Laboratory and Greenhouse Experiments: Laboratory experiments were carried out to isolate and characterize the *rhizobia* that naturally nodulate promiscuous soybeans cultivars SB19 and SB20 in Meru South. These included morphological and biochemical tests. Morphological tests included microscopic examination of cell morphology, gram staining and motility tests. Biochemical tests included growth on yeast extract manitol agar (YEMA) incorporating bromothymol blue dye and YEMA incorporating, Congo red dye [14]. Greenhouse experiments were carried out to authenticate the isolates as *rhizobia*. Sterile host plants were grown in sterilized nitrogen free medium in vermiculite tubes with or without inoculation with the rhizobial isolates.

Table 1: Treatment structure (Implemented on high carbon and low carbon sites)

Treatment	Variety	Sub-treatments	Fertilizer	Amount of fertilizer in kg/ha	Seed kg/ha
1	SB19	1	None	0P 0K 0S	140
	SB19	2	PKS	60P 60K 24S	140
	SB19	3	5P	5P 0K 0S	140
	SB19	4	10P	10P 0K 0S	140
	SB19	5	25P	25P 0K 0S	140
	SB19	6	50P	50P 0K 0S	140
2	SB20	1	None	0P 0K 0S	140
	SB20	2	PKS	60P 60K 24S	140
	SB20	3	5P	5P 0K 0S	140
	SB20	4	10P	10P 0K 0S	140
	SB20	5	25P	25P 0K 0S	140
	SB20	6	50P	50P 0K 0S	140

Data Analyses: Data collected on parameters; nodule number, nodule fresh weight, plant biomass production and yield components (pod fresh weight, a thousand grain weight and haulm weight) was analyzed and presented using both descriptive and quantitative statistical procedures. Statistical analyses were done using SAS [15]. The transformed data was subjected to analysis of variance- ANOVA using PROC GLM package to determine the main effects of the treatments and their interactions. Specific pair-wise comparisons of treatment levels were done using the least significant differences (LSD) test at P = 0.05 and correlations using 'PROC CORR'.

RESULTS AND DISCUSSION

The parameters put into consideration across the two experiments were phenological observations, nodulation status (nodule number and nodule fresh weight), plant biomass production, pods fresh weight, grain yields and haulms at final harvest. A phenological phase was considered to have occurred when 50% of the crops had attained the characteristic of that phase. Physiological maturity was considered to have taken place when 95% of the plants had turned golden yellow and 75% of the plants had their pods filled with seeds and hardened.

Phenological Observations: Application of different amounts of P fertilizers did not significantly ($P \le 0.05$) influenced percentage crop emergence (Table 2). However, application of 60 kg P ha^{-1} +60 kg K ha^{-1} +24 kg S ha^{-1} (PKS) fertilizers significantly ($P \le 0.05$) differed from other fertilizer amendments in crop emergence. This could be attributed to the moisture stress caused by the fertilizers immediately after planting which may have had a scorching effect on the germinating seeds. Some of the seedlings that germinated further dried up due to lack of rainfall for about two weeks after planting. Varietal differences and soil carbon level did not significantly influence percentage crop emergence.

Fertilizer application did not significantly influence the plant height. However, application of 50 kg P ha^{-1} resulted in the highest plant height (Table 2). Significant plant height differences were observed ($P \le 0.05$) between variety SB19 and SB20 (Table 3). No significant pest or disease incidences were observed on the crop at both sites.

The early maturing variety SB19 took, 62 days to attain 50% flowering, 81 days to form 50% of the pods and 124 days to mature. Variety SB20 took 70 days to attain 50% flowering and 90 days to form 50% of the pods and 146 days to mature (Table 3). Pods of both varieties did not shatter at maturity. Similar results on resistance of the varieties to pod shattering have been reported by Tukamuhabawa *et al.* [16].

Nodulation: Carbon level on the field had a significant effect on nodule number and nodule fresh weight at ($P \le 0.05$). Fields with high carbon level had a higher nodule fresh weight compared to low carbon level fields (Table 4). Nodulation was not significantly different within the two varieties at ($P \le 0.05$). However, variety SB20 had a greater nodule fresh weight mean of 1.4g compared to SB19, which had a mean of 1.2g (Table 5).

The nodule number was significantly influenced by application of PKS fertilizer and application of 25 kg P ha^{-1} (Table 6). However, the nodule fresh weight was not significantly influenced by application of different levels of P fertilizer and application of PKS fertilizer. Increased level of P fertilizer however resulted to concomitant increase in nodule fresh weight. These results are in agreement with Raychaudhuri [17] who reported increased nodule number and nodule weight with increased P levels. Averaged across fields and varieties, the nodule fresh weight increased by 112.5% when fertilizer application was increased from 10 kg P ha^{-1} to 25 kg P ha^{-1} (Table 6).

Application of PKS fertilizers had a significant positive effect over control ($P \le 0.05$) on nodule number of the soybean varieties. However, nodule fresh weight was not significantly different among PKS and control. Application of PKS fertilizer resulted in nodules with greater nodule fresh weight mean of 1.6g compared to the mean of 0.6g from the control (Table 6). The nodule fresh weight showed significant positive correlation with plant biomass production (r=0.50, n=80) and pod fresh weight per plant (r=0.37, n=80). These results suggest that nodulation in promiscuous soybeans is strongly related to plant biomass production and yields due to increased biological nitrogen fixation. Therefore, a tremendous potential exists to improve BNF and yield components from promiscuous soybean varieties.

Plant Biomass Production: Plant biomass production was significantly ($P \le 0.05$) influenced by level of carbon in the field (Table 4) and variety (Table 5). Variety SB20 produced higher plant biomass of mean 82.8g compared to the mean of 68.0g for variety SB19. Successive application of P fertilizer did not significantly influence plant biomass production (Table 6). However, application of PKS significantly ($P \le 0.05$) increased plant biomass compared to application of 10 kg P ha^{-1} and the control. Application of 25 kg P ha^{-1} increased plant biomass per plant by 10% while application of PKS resulted to 29% increase.

Table 2: Effect of fertilizer application on percentage crop emergence 4 weeks after planting

Fertilizer amendment (Kg ha^{-1})	% Crop emergence	Plant height (cm)
Control	97.3a	52.2a
PKS	89.4b	54.9a
10 P	97.5a	52.6a
25P	98.5a	54.6a
50P	97.7a	57.8a
LSD 5%	2.6	5.6

Means followed by the same lower case letter (s) and within the same column are not statistically different at ($LSD_{0.05}$)

Table 3: Days to onset of flowering, pod-set and maturity of promiscuous soybean varieties

Variety	Onset of flowering	50% flowering	Onset of pod formation	50% podding	Maturity	Plant height (cm)
SB19	55	62	62	81	124	43.4
SB20	64	70	68	90	146	65.2

Table 4: Performance of promiscuous soybean varieties in low and high carbon fields at 50% podding

FIELD	Nodule number	Nodule fresh weight (g)	Plant biomass (g)
High carbon	5	1.5	87.0
Low carbon	3	1.1	64.5
LSD (5%)	1.1	0.2	12.8

Table 5: Effect of maturity class on nodule number, nodule fresh weight and plant biomass production at 50% podding

Variety	Maturity class	Nodule number	Nodule fresh weight (g)	Plant biomass (g)
SB19	Early	4	1.2	68.0
SB20	Late	4	1.4	82.8
LSD (5%)		1.1	0.2	12.8

Table 6: Effect of fertilizer amendments on nodule number, nodule fresh weight and plant biomass production at 50% podding

Fertilizer amendment	Nodule number	Nodule fresh weight (g)	Plant biomass (g)
Control	2b	0.6a	68.3b
PKS	4a	1.6a	88.0a
10 kg P ha^{-1}	3ab	0.8a	67.1b
25kg P ha^{-1}	4a	1.7a	74.8ab

Means followed by the same lower case letter (s) and within the same column are not statistically different ($LSD_{0.05}$)

Table 7: Effect of field carbon on yield components on promiscuous soybeans

Field	Pod fresh weight (g)	1000 seed weight (g)	Haulm fresh weight (g)
High carbon	41.4	126.1	14.9
Low carbon	38.0	126.2	13.9
LSD (5%)	5.9	1.1	2.4

Table 8: Effects of fertilizer amendments on yield components of promiscuous soybeans

Fertilizer amendment	Pod fresh weight (g)	1000 seed weight (g)	Haulm fresh weight (g)
Control	3.2b	124.6c	1.2c
PKS	4.2ab	127.0ab	1.8a
10P	3.7ab	125.5bc	1.2bc
25P	3.9ab	126.4abc	1.4abc
50P	4.7a	128.0a	1.6ab
LSD (5%)	1.1	1.79	0.4

Means followed by the same lower case letter (s) and within the same column are not statistically different ($LSD_{0.05}$)

Table 9: Performance of the soybean varieties

Variety	Maturity class	Pod fresh weight (g)	1000 - seed weight (g)	Haulm fresh weight (g)
SB19	Early	38.3	123.0	9.9
SB20	Late	41.1	129.6	18.8
LSD (5%)		5.9	1.1	2.4

Table 10: Morphological and biochemical tests on *Bradyrhizobia* isolates from promiscuous soybean

Isolate	Cell morphology	Colony color	Bromothymol blue	Congo red	Gram stain	Mucocity
NH1	Bar	Cream	Y	X	-	+
NH2	Bar	Cream	Y	X	-	+
NL1	Bar	Cream	Y	X	-	+
NL2	Bar	White	B	X	-	-
SH1	Bar	Cream	Y	X	-	+
SH2	Bar	White	B	X	-	-
SL1	Bar	Cream	Y	X	-	+
SL2	Bar	Yellow	Y	X	-	-
NH1	Bar	Cream	Y	X	-	+
NH2	Bar	Cream	Y	X	-	+
NL1	Bar	White	B	X	-	-
NL2	Bar	Cream	Y	X	-	+
SH1	Bar	Orange	B	X	-	-
SH2	Bar	Cream	Y	X	-	+
SL1	Bar	Yellow	Y	X	-	+
SL2	Bar	Cream	Y	X	-	+

Key: B- Blue colour-characteristic of slow growing rhizobia
Y- Yellow colour-characteristic of fast growing rhizobia
X- No absorption of Congo red dye

Pod Fresh Weight: Pod fresh weight was not significantly influenced by field carbon level (Table 7). However, Pod fresh weight per plant was higher on high carbon level fields compared to the low carbon fields. Soybean variety did not significantly ($P \leq 0.05$) increase the pod fresh weight.

Application of varying amounts of P fertilizer did not significantly influence the pod fresh weight per plant. However, increasing the amount of P from 25 kg P ha^{-1} to 50 kg P ha^{-1} resulted to 25 % increase in pod fresh weight per plant (Table 8). There were strong relationships between pod fresh weight and nodule number (r=0.46), pod fresh weight and nodule fresh weight (r=0.35,) and between pod fresh weight and total plant weight (r=0.49).

1000 - Seed Weight: Field carbon did not significantly influence 1000 seed weight of both promiscuous soybean varieties (Table 7). However, soil amendments with different fertilizers significantly ($P \leq 0.05$) influenced 1000 seeds weight. Soil amendment with 50 kg P ha^{-1} resulted in the highest 1000 seed weight (Table 8). Variety SB20 differed significantly ($P \leq 0.05$) from variety SB19 in 1000 seeds weight (Table 9).

Haulm Fresh Weight: Differences in haulm fresh weight per plant were significant ($P \leq 0.05$) between varieties (Table 9). Application of 50 kg P ha^{-1} significantly increased haulm fresh weight compared to application of 10 kg P ha^{-1} fertilizer. Soil amendments with PKS fertilizer resulted in the highest haulm fresh weight per plant.

Laboratory and Greenhouse Experiments: The isolates obtained were Gram negative, rod shaped cells, which were water clear, white or cream colored with transparent or translucent areas. This is a characteristic, which is typical of all rhizobia [18]. On BTB medium, 75% of the isolates turned the growth medium from deep green to yellow, which indicated production of acidic substances, which diffused into the medium [14, 19]. The production of acid substances is a characteristic of fast growing rhizobia [20] and therefore these results probably indicated presence of fast growing *Sinorhizobium fredii.* About 25% of the isolates turned the medium from deep green to blue indicating production of alkaline substances, which diffused into the medium. This is a typical characteristic of slow growing *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* [21].

The isolates weakly absorbed Congo red dye. This biochemical characteristic helped in recognition of the relatively uncolored rhizobial colonies avoiding the chance of mistaking *Agrobacterium* for *Rhizobia,* which gives all the biochemical tests, designed for *Rhizobia*. The morphological and biochemical test results conformed to the standard methods used for identification of *Rhizobium* isolates earlier described by Somasegaran and Hoben [14] and Vincent [19]. Results from various biochemical tests indicated that 25% of the rhizobia isolated were slow growing while 75% were fast growing These results suggest the presence of both the fast growing *Sinorhizobium fredii* and the slow growing *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* in Meru South soils. Therefore, promiscuous soybean varieties can perform well in the area and can increase soil fertility through BNF without inoculation with *rhizobium* bacteria.

Greenhouse experiments were used to authenticate the isolates as pure cultures of rhizobia. Nodulation was noted in the inoculated plants while absence of nodules was noted from the controls. Visual observations indicated a pink coloration, suggesting that there was atmospheric N fixation. Vis-à-vis to these results the isolates were regarded to as fully authenticated cultures and preserved in YEMA slants.

CONCLUSIONS AND RECOMMENDATIONS

Biological nitrogen fixation offers an economically attractive and ecologically sound means of reducing external N inputs and improving the quality and quantity of internal sources. The results from this study suggest that soil amendments with PKS fertilizers are desirable to achieve maximum benefits from soybean production. Considering the economics, increase in yield and fertilizer use efficiency, application of 50 kg P ha^{-1} in high carbon level fields and 60 kg P ha^{-1}, 60 kg K ha^{-1} and 24 kg S ha^{-1}, in low carbon level fields could be an optimum dose for an optimum yield of soybean grown on these soils. In addition, it is recommended that farmers ameliorate the soils by incorporation of organic fertilizers, which will definitely augment the soil carbon level and provide the essential nutrients for plant growth and development.

To conclude, there is need to demonstrate the benefits from BNF technology in terms of maintenance and improvement of soil fertility through long term experiments. A holistic approach to improve crop production in Kenya is needed. Research and development of management methods to conserve nitrogen and carbon in cropping systems should rely heavily on the application of BNF.

REFERENCES

1. Food and Agriculture Organization (FAO), 2006. FAOSTAT Website, vol. 2006, Food and Agriculture Organization of the United Nations, Statistics Division.
2. World Bank, 2007. World Development Report 2008. The World Bank, Washington DC, USA.
3. Staatz, J.M. and N. Dembele, 2007. Agriculture for Development in Sub-Saharan Africa. Background paper for the World Development Report 2008, Michigan State University, USA, pp: 69.
4. Nekesa, C.O. and J.G. Mureithi, 2004. Green manure legume seed production: Efforts of the legume Research Network Project (LRNP) In: Legume research network project, Kenya Agricultural Research Institute (KARI), Nairobi, Kenya, pp: 21-23.
5. Ogutcu, H., A. Adiguzel, M. Gulluce, M. Karadayi and F. Sahin, 2009. Molecular characterization of *Rhizobium* strains isolated form wild chickpeas collected from high altitudes in Erzurum-Turkey, Romanian Biotech. Let., 14(2): 4294-4300.
6. Saghal, M. and B.N. Johri, 2003. The changing face of rhizobia systematics. Current Sci., 84(1): 43-47.
7. Postgate, J., 1998. Nitrogen Fixation, Cambridge University Press, Cambridge.
8. Grahm, P.H., 1988. Principles and applications of soil microbiology (eds D.M. Sylvia,), pp: 322-345.
9. Osunde, A.O., A. Bala, M.S. Gwam, P.A. Tsado, N. Sanginga and J.A. Okogun, 2003. Residual benefits of promiscuous soybean to maize (*Zea mays* L.) grown on farmers fields around Minna in the south Guinea savanna zone of Nigeria, In: IITA Agric. Ec. and Environ., 100: 209-220. Ibadan, Nigeria.
10. Kasasa, P., S. Mpepereki and K.E. Giller, 2000. Qualification of nitrogen fixed by promiscuous soybean varieties under Zimbabwean field condition. In: Forum for working document. 1. programme and extended abstracts. Fourth Regional Meeting of the Forum for Agricultural Resource Husbandry, 11-14 July 2000, Lolongwe, Malawi, pp: 64-66.
11. Tran, T.Y., 2004. Response to and benefits of rhizobial inoculation of soybean in the South Vietnam, Vietnam.
12. Food and Agriculture Organization (FAO), 1989. Forestry and Food Security. FAO Forestry Paper, FAO (UN), 90: 1-2.
13. Okalebo, R.J., K.W. Gathua and P.W. Woomer, 2002. Laboratory methods of soil and plant analysis: A Working Manual (second edition). Department of Soil Science, Moi University, Eldoret, Kenya.
14. Somasegaran, P. and H.S. Hoben, 1994. Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. New York, pp: 450.
15. Statistical Analytical Systems, 1996. SAS/STAT User's Guide, Version 6, Vol. 2, 4th ed. SAS Institute, Cary, NC, USA.

16. Tukamuhabawa, P., K.E. Dashiell, P. Rubaihawo and M. Nabasirye, 2002. Determination of field yield loss and effects of environment on pod shattering in soybean, African Crop Sci. J., 10(3): 203-209.
17. Raychaudhuri, M., K. Kumar and S. Raychaudhuri, 1997. Effect of *Rhizobium japonicum* and phosphorus on growth band yield of soybean (*Glycine max*). Indian J. Agric. Sci., 67: 459-462.
18. Zakhia, F. and P. Lajudie, 2001. Taxonomy of Rhizobia. Agronomie, 21: 569-576.
19. Vincent, J.M., 1970. (IBP Handbook no 15). A manual for the practical study of root-nodule bacteria Blackwell, Oxford.
20. Beck, D.P., L.A. Materon and F. Afandi, 1993. Practical *Rhizobium*- Legume technology manual. Manual No. 9 International Centre for Agricultural Research in the Dry Areas, Aleppo, pp: 1-245.
21. Xu, L.M., C. Ge, J. Li, and H. Fan, 1995. *Bradyrhizobium liaoningensis* sp. Nov isolated from root nodules of soybean. Inter. J. Syst. Bact., 45: 706-711.