Nodulation and nitrogen fixation in promiscuous and non promiscuous soybean (Glycine max (L.) Merrill) varieties in Eastern Kenya

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Nodulation and nitrogen fixation in promiscuous and non promiscuous soybean (*Glycine max* (L.) Merrill) varieties in Eastern Kenya

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Abstract

Soil nitrogen deficiency is a major factor limiting soybean production. This problem can be alleviated by the use of nitrogen fertilizers which on the other hand adversely affect the environment, are expensive and unaffordable to most peasant farmers. Alternatively, attention is being paid to improving soil nitrogen through the use of environmental friendly biological nitrogen fixation of soybeans in an attempt to develop sustainable cropping systems. There is however inadequate knowledge on estimates of nitrogen fixation by soybean varieties in Kenya. In light of this, growth experiments were designed to investigate biological nitrogen fixation in promiscuous and non promiscuous soybeans. Results from the field experiment showed that the two promiscuous soybean varieties (TGx 1869 and TGx 1893) nodulated better than the non promiscuous Gazelle. Uninoculated Gazelle did not produce any nodule while uninoculated TGx 1869 and TGx 1893 nodulated with indigenous soil rhizobia. Inoculation affected nodulation since there was a significantly higher mean nodule number in inoculated soybeans (highest in TGx 1869 IN – 76) as compared to uninoculated (highest in TGx 1869 – UNC - 21) and(0.0) in nitrogen treated soybeans at podding. Inoculated soybeans had higher shoot dry weight (highest in TGx 1893 IN - 19.30 g) than uninoculated soybeans (highest in TGx 1869 UNC - 13.50 g) .Stover biomass was higher in inoculated soybeans (13.07 g) than in uninoculated treatments (6.91 g). Although there was no significant difference in seed dry weight of inoculated (14.10 g) and uninoculated (8.11 g), inoculated soybeans had higher seed dry weight. The inoculated TGx varieties had higher seed dry weight (TGx 1869 at 12.94 g and TGx 1893 at 14.10 g) than Gazelle (10.12 g). There is need to adopt growing of promiscuous TGx varieties and to exploit biological nitrogen fixation with the view of increasing soybean yields and decreasing overdependence on nitrogen fertilizers for sustainable agriculture.

Key words: *Bradyrhizobia*, Soybean, *Glycine* cross, inoculation, nodulation, promiscuous, TGx, nitrogen fixation.

Introduction

Soybean (*Glycine max* (L.) Merrill) originated as a domesticate in the eastern half of northern China around the 11th century. Soybean production has increased since the early part of the 20th century to the extent that soybean is now being produced in larger quantities than any other legume crop in the world (Giller, 2001). Soybean yield 803 kg/ha on average in sub-Saharan Africa (SSA).This yield is substantially greater than average common bean yields (665 kg/ha) and double that of cowpea (392 kg/ha) (Maingi, 2009).

Soybean is one of the most popular pulses in the world due to its nutrient composition and yield. Soybeans have various uses, both domestically and industrially. Apart from providing income for the betterment of the socio-economic status of farmers, soybean is a main human food. In human food use, soybean is graded as one of the most important source of proteins, edible oils and vitamins in the world (Borget, 1992). Soybeans are in big demand in the margarine, cooking oils, beverages, soap, paint, vanish and pharmaceutical manufacturing firms in Kenya. It is projected that by the year 2010, demand for soybeans in Kenya will have risen to 150,000 metric tons (GTZ, 1996).

Biological nitrogen fixation (BNF) is the most important contributor to the world supply of nitrogen to plants (Bohlool *et al.,* 1992). It is the only process that can effectively alleviate adverse environmental impacts imposed by N fertilizer while at the same time benefiting both plants and the farmer (Whitehead and Wright, 1995). BNF offers an economically attractive and sound means of reducing external inputs and improving internal sources (Ladha *et al.,* 1996). Lack of BNF knowledge, information on availability and use of inoculants (Woomer *et al,*. 2003), cost of inoculants (Javaheri, 1996) and presumed special storage conditions for inoculants (Mabika and Mariga, 1996) have been identified as major factors hindering adoption of BNF technology.

The international Institute for Tropical Agriculture (IITA) has conducted research on cowpea and soybean and it has succeeded in developing new soybean cultivars for Africa, known as Tropical *Glycine* cross (TGx) which are nodulated by *Bradyrhizobia* indigenous to African soils (Abaidoo *et al.*, 2000). These cultivars have been tested in a number of African countries as naturally nodulating, that is, promiscuous soybeans (Kasasa *et al.*, 1998). Due to their ability to readily fix nitrogen in soils, soybeans help to improve productivity of other food and cash crops, particularly in mixed crop and rotational farming (Mpepereki *et al.*, 2000).

Studies have shown that soybean has potential for soil fertility improvement through biological nitrogen fixation. Soybeans grown by most Kenyan farmers receive no inoculants and little or no commercial nitrogen fertilizer. They largely depend on N fixation from natural nodulation by indigenous *Bradyrhizobia* populations. While the potential of promiscuous and non promiscuous soybeans to fix nitrogen and the subsequent contribution to sustainable cropping systems have been demonstrated in Nigeria (Sanginga *et al.*, 1997) and in Zambia (Javaheri, 1996), no research work on this subject has been conducted in Kenya. The objective of this research were to establish the effect of inoculation and N fertilizer on nodulation and nitrogen fixation in promiscuous and non promiscuous as well as to compare nodulation and nitrogen fixation in promiscuous and non promiscuous soybeans.

Materials and methods

Field site

Field experiments were carried out at Kaguru Farmers' Training Centre (FTC) (AEZ: UM 2-3) in Meru District, Eastern Province during the short rain season of 2004. Kaguru FTC is locate at latitude, 0^o $05^/$ S and longitude 37^o $40^/$ E, with an elevation of 1527 meters above sea level. The soils are well drained clay. Rainfall is bimodal; the long rains normally start at end of March while the short rains start normally in mid October. The short rains are normally more reliable and give higher yields (Jatzold and Schmidt, 1983). Mean annual rainfall is about 1765 mm.

Seeds and inoculant sources

The soybean seeds which were used in this experiment were TGx 1869, TGx 1893 and Gazelle. Seeds of soybean TGx 1869 and TGx 1893 were obtained from *International Institute of Tropical Agriculture* (IITA), Ibadan Nigeria. Gazelle seeds were locally obtained from Kenya Agriculture Research Institute (KARI) Njoro. *Bradyrhizobium* inoculum used was USDA 110, which was obtained from Microbiological Resources Centre (MIRCEN) at the University of Nairobi, Kabete Campus.

Planting and care of plants

The experimental design was a randomized complete block design with four replications. Each of the soybean variety TGx 1869, TGx 1893 and Gazelle were inoculated with USDA 110, treated with N fertilizer and others uninoculated. A total of nine (9) different treatments were represented in nine plots per block. Each plot size measured 3m x 3m. Land preparations were done by hand on all experimental plots. During planting, two soybean seeds were planted per hole at 0.75m inter-row and 0.30m intra-row spacing. For N treatment, 40 kg/ha of DAP was applied at planting. Uninoculated plots were planted before those with inoculated soybeans to avoid contamination during planting.
Plants were thinned to one plant per hole two weeks after germination. The experimental plots were hand-weeded three times during the experimental period. Hand-weeding was done to avoid cross-contamination.

Sampling of plants

Plants were sampled at 75% flowering, 75% podding and at full physiological maturity. Four plants were randomly sampled from each plot and various parameters determined. At flowering and podding, plants were cut at about 5 cm above soil level. Roots of the plants were dug up carefully and repeatedly washed with clean water to remove soil particles and any contaminants. Nodules were detached from the roots and counted to determine their numbers. Plant materials were then partitioned into nodules, roots and shoot. The samples were air dried, before oven drying at 72^oC to constant weight. During final harvest, yield data of seeds and stover was assessed. The dry weights of the samples were determined and the data obtained were analysed.

Results

Nodulation at flowering was poor and nodule number among the three soybean varieties had no significant difference (Table 1). At podding, nodulation was better (highest mean nodule number 76 in TGx 1869 IN) than at flowering (highest mean nodule number, 17 in Gazelle IN) and nodule number among the soybean varieties differed significantly. The two promiscuous TGxs had higher nodule number (TGx1869 at 76, TGx 1893 at 47) than non promiscuous Gazelle (29) at podding. Inoculation significantly increased nodulation in both promiscuous and non promiscuous soybeans. Uninoculated non promiscuous Gazelle never nodulated while uninoculated promiscuous TGxs nodulated with indigenous rhizobia at both stages. None of the N treated soybean nodulated at any stage; hence no nodule dry weight was recorded.

Table 1: Nodule number and nodule dry weight per plant of different soybean varieties as influenced by different treatments at flowering and podding at Kaguru Farmers' Training Centre during the short rain season of 2004.

	Nodule number		Nodule dry weight (mg)	
Treatment	Flowering	Podding	Flowering	Podding
TGx 1869 DAP	0±0[a]	0±0[a]	0.00±0.00[a]	0.00±0.00[a]
IN	16±1[c]	76±2[d]	60.00±3.88[a]	1170±60.98[a]
UNC	2±1[a]	21±1[ab]	20.00±1.22[a]	140±8.89[a]
TGx 1893 DAP	0±0[a]	0±0[a]	0.00±0.00[a]	0.00±0.00[a]
IN	12±2[b]	47±5[c]	70.00±4.00[a]	480±10.38[a]
UNC	2±1[a]	3±1[a]	20.00±1.56[a]	70±5.00[a]
Gazelle DAP	0±0[a]	0±0[a]	0.00±0.00[a]	0.00±0.00[a]
IN	17±3[c]	29±2[bc]	34.00±2.65[a]	320±9.74[a]
UNC	0±0[a]	0±0[a]	0.00±0.00[a]	0.00±0.00[a]

Means (n=4) down a column followed by the same letter are not significantly different according to Tukey's HSD at 5% level. Soybean varieties: TGx 1869 = SB12-TGx 1869-31E; TGx 1893 = SB16-TGx 1893-7F and Gazelle. Treatments: DAP – Diammonium Phosphate fertilizer; IN – Inoculated; UNC – Uninoculated.

Shoot biomass of soybean were not significantly affected by inoculation at both stages (Table 2). Among the soybean varieties, shoot biomass of non promiscuous Gazelle (3.89g) was significantly higher ($P<0.05$) than the two promiscuous TGx 1869 (2.21g) and TGx 1893 (2.42 g) at flowering. However, at podding the two TGxs had significantly higher ($P<0.05$) shoot biomass than Gazelle (Table 2). TGx 1893 had higher shoot biomass than TGx 1869 at both stages, but the difference was not statistically significant at $P<0.05$. Shoot biomass of soybean were significantly affected by N treatment at flowering and podding as compared to inoculated and uninoculated soybeans.

Stover dry weight among the soybean varieties differed significantly. TGx 1893 DAP had the highest average stover dry weight of 19.09 g. TGx 1869 DAP and Gazelle DAP had averages of 17.02 g and 13.82 g respectively (Table 3). Stover dry weights of the promiscuous TGx 1869 (DAP - 17.02g; IN - 8.78 g; and UNC - 6.91 g) and TGx 1893 (DAP - 19.09 g; IN - 13.07 g; and UNC g) respectively were significantly higher ($P<0.05$) in comparison with those of non promiscuous Gazelle (DAP - 13.82 g; IN – 4.40 g; and UNC – 4.29 g).

Table 2: Shoot dry weight per plant of different soybean varieties as influenced by different treatments at flowering and podding at Kaguru Farmers' Training Centre during the short rain season of 2004.

	Shoot dry weight (g)	
Treatment	Flowering	Podding
TGx 1869 DAP	2.21±0.24[b]	44.00±4.68[c]
IN	1.13±0.12[a]	19.00±2.23[ab]
UNC	1.32±0.12[a]	13.50±2.20[ab]
TGx 1893 DAP	2.42±0.20[b]	52.00±6.68[c]
IN	1.27±0.16[a]	19.30±3.26[ab]
UNC	1.30±0.15[a]	12.80±2.09[ab]
Gazelle DAP	3.89±0.23[c]	23.30±2.03[b]
IN	1.29±0.10[a]	6.80±1.09[a]
UNC	1.34±0.11[a]	7.30±2.00[a]

Means (n=4) down a column followed by the same letter are not significantly different according to Tukey's HSD at 5% level. Soybean varieties: TGx 1869 = SB12-TGx 1869-31E; TGx 1893 = SB16-TGx 1893-7F and Gazelle. Treatments: DAP – Diammonium Phosphate fertilizer; IN – Inoculated; UNC – Uninoculated.

Inoculation had no significant effect on soybean stover and seed dry weight but nitrogen treatment had significant effect on the two parameters. Although the stover and seed dry weight of inoculated soybeans was higher than in uninoculated soybean, the differences were statistically insignificant. Seed dry weight per plant among the soybean varieties had no significant difference. However, the two promiscuous TGxs had higher average seed dry weight than non promiscuous Gazelle (Table 3).

Table 3: Stover and seed dry weight per plant of different soybean varieties as influenced by different treatment at final harvest at Kaguru Farmers' Training Centre during the short rain season of 2004

Treatment		Stover dry weight (g)	Seed dry weight (g)
TGx 1869	DAP	$17.02\underline{+}1.59^{ef}$	$24.44\underline{+}2.23^{b}$
	IN	$8.78\underline{+}1.02^{bc}$	$12.94\underline{+}2.04^{a}$
	UNC	$6.91\underline{+}1.00^{ab}$	$9.25\underline{+}1.35^{a}$
TGx 1893	DAP	$19.09\underline{+}2.22^{f}$	$20.07\underline{+}3.30^{b}$
	IN	$13.07\underline{+}1.70^{de}$	$14.10\underline{+}1.29^{a}$
	UNC	$11.50\underline{+}1.13^{cd}$	$13.96\underline{+}1.35^{a}$
Gazelle	DAP	$13.82\underline{+}2.35^{de}$	$22.43\underline{+}3.22^{b}$
	IN	$4.40\underline{+}1.18^{a}$	$10.12\underline{+}2.07^{a}$
	UNC	$4.29\underline{+}1.04^{a}$	$8.11\underline{+}1.02^{a}$

Means (n=4) down a column followed by the same letter are not significantly different according to Tukey's HSD at 5% level. Soybean varieties: TGx 1869 = SB12-TGx 1869-31E; TGx 1893 = SB16-TGx 1893-7F and Gazelle. Treatments: DAP – Diammonium Phosphate fertilizer; IN – Inoculated; UNC – Uninoculated.

Discussion

Results obtained on nodulation indicated that some soybeans did not produce any nodule. Those that did had low averages of between 2 to 76 nodules per plant. Majority of the nodules were effective in nitrogen fixation as evidenced by the pink coloration of the nodules which is indicative of leghaemoglobin (Amara *et al*., 1995; Sprent and Sprent, 1990). A few nodules had an inner white coloration, an indication that they were ineffective or they had not developed enough to a stage where they could fix nitrogen. Nodule number is frequently used as a measure of infectiveness (Beck *et al*., 1993). The low number of nodules per soybean plant was an indication of low infectiveness of indigenous and introduced soil rhizobial strains.

Nodulation at flowering had no significant difference among the three soybean varieties, but it had a significant difference at podding, where the promiscuous TGxs nodulated better than non promiscuous Gazelle. This could be attributed to its short growth cycle which in this research was 50 days to 75% flowering compared to promiscuous TGxs which took 60 -65 days to 75% flowering. The results are in line with what other researchers have found, that is, the early to maturing varieties have lower nodulation compared to the late maturing varieties (Hornetz *et al*., 2006; Hardarson and Atkins, 2003). This observation agrees with that of Russel and Jones (1975), who noted that early to mature varieties, tend to be weak in nitrogen fixation. Although there was a significant effect of inoculation on soybean nodule number and nodule dry weight, inoculation did not have any significant effect on soybean shoot biomass. At early stage (flowering) uninoculated soybeans had higher shoot biomass than inoculated soybeans. However, at podding, inoculated soybeans had higher shoot biomass than uninoculated soybeans. These results showed that inoculation had influence on shoot biomass but this influence was noted at later stage of soybean growth as opposed to early stage.

Non promiscuous Gazelle turned out to be the best in shoot biomass at flowering which differed significantly with those of the two TGxs. At podding however, the two TGxs had significantly higher shoot biomass than Gazelle. The results showed that soybean varieties responded differently to rhizobia strains during their life cycle and non promiscuous Gazelle out-competed the promiscuous TGxs at early stage of growth while promiscuous TGxs did better than non promiscuous Gazelle at later stages of growth.

Promiscuous TGx soybeans performed better than non promiscuous Gazelle in terms of stover and seed dry weight. Significant difference was noted in stover dry weight among the soybean varieties, but there was no significant difference in seed dry weight. The results showed that late maturing varieties (TGx) build more biomass thus the possibility of a positive N balance in the soil unlike the early maturing variety (Gazelle) which has relatively high grain yield compared to its biomass. Lee *et al*., (1991) reported that early maturing varieties are thus more likely to have a negative N balance as they have a high N harvest index (NHI).

Increased nodulation due to inoculation had significant effect on nodule number and nodule dry weight but did not have any significant advantage in soybean biomass (shoot and stover) over uninoculated control. Seed yield from inoculated soybeans was not significantly higher than that from the uninoculated soybeans, showing a degree of competitiveness among the introduced rhizobia strain and the indigenous rhizobia population. Thus, to achieve the development of sustainable cropping systems through the improvement of N fixation in promiscuous soybean, as proposed by Sanginga *et al*., (1997) and Mpepereki *et al*., (2000) a search for more aggressive rhizobia colonizer with higher N fixation potential than the present strain is clearly needed. Except in nodule and nodule dry weight, N treated soybeans had significantly higher values in all other parameters than inoculated and uninoculated soybeans, an indication that N fertilizer has greater contribution to soybean production than inoculation.

Promiscuous TGx soybeans are better performers than non promiscuous Gazelle in terms of nodulation, plant biomass and yields. Uninoculated promiscuous varieties (TGxs) nodulated with indigenous soil rhizobia while non promiscuous Gazelle did not. Nodulation increased in TGxs after inoculation, which means contrary to popular believe that TGxs nodulate effectively with indigenous rhizobia, they too need inoculation. In this research, differences were noted between and within soybean varieties in nodulation and dry matter weight at different stages of their growth cycle. In this respect, there is need for further research in the evaluation of soybean germplasm for traits affecting nitrogen fixation.

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