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Nitrogen Fixation by *Vigna radiata* L. Wilczek in Pure and Mixed Stands in SE-Kenya

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Abstract

Greenhouse and field experiments were carried out to determine the number of rhizobia in soils of SE-Kenya, nodulation and nitrogen fixation by green grams in two cropping systems was also determined. The Most Probable Number (MPN) of rhizobia cells capable of nodulating green grams were between 519 and 3,780 per gram of soil in SE-Kenya. These results were confirmed by lack of response to inoculation and effective nodulation of the control plants under field conditions. Green grams intercropped with maize had significantly higher dry weights than the ones grown as pure stand, 21 days after emergence (DAE). The increase was transitory because it was not observed at podding (42 DAE) and at physiological maturity (100 DAE). Green gram yield was not affected by maize intercrop. However, maize yields were significantly reduced by intercropping with green grams. Soil analysis from treatment plots before and after the cropping season indicated that green grams increased soil nitrogen slightly or maintained it at pre-planting levels. This was unlike pure maize plots where there was a decline in soil nitrogen (N).

Keywords: Intercropping, nitrogen fixation, nodulation, SE-Kenya, semi-arid areas, *Vigna radiata*

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1. Introduction

Small scale farmers in arid and semi-arid lands (ASALs) are resource poor and therefore cannot afford irrigation and inorganic fertilizers (Jaetzold and Schmidt, 1983). Legume-*Rhizobium* technology has been exploited in other areas as a substitute for organic fertilizers (Sprent and Sprent, 1990; Vance, 1997). However, this technology has not been evaluated in maize-legume intercropping systems common in the marginal areas of Kenya.

Like other dry lands in sub-Sahara Africa, high evapotranspiration rates and unreliable rainfall determine the type of crops grown in Kenya's ASALs. With current climatological approaches like the ENSO model (Frenken et al., 1993), rainfall is predictable in time and space. It is therefore possible to increase food production in ASALs by improving agronomic practices on crops adapted to the rainfall regimes in these areas (Hornetz, 1997; Stewart and Hash, 1982). In view of this, a project was carried out to study nitrogen fixation by *Vigna radiata* (green gram) in pure stands and intercropped with maize (*Zea mays* cv Kinyanya) in the semi-arid areas of SE-Kenya during the long and short rains of 1997. The plant cropping systems were selected on the basis of what is grown by the local people (the Kamba ethnic group living in SE-Kenya). This paper reports the results of the study.

2. Materials and Methods

Study area

The global position of the experimental site is 02° 10' S, 37° 40' E (determined using a GPS 45 XL personal navigator). The soils at the site are classified as rhodic ferralsols to ferric luvisols (Touber, 1983). The soils are well drained and texturally are sandy loams overlying sandy clays (Pilbeam et al., 1995). The soil chemistry indicates a deficiency of nitrogenous plant nutrients such as ammonium nitrate (0.07 to 0.09 mg/100 g of soil, at a depth of 0–15 cm) (Hornetz, 1997). Rainfall is bimodally distributed with mean monthly maxima in April and November; mean annual rainfall is about 562 mm year^{-1}. The short rains (October to January) generally yield more water and are more reliable than the long rains (March to June) (Musembi and Griffths, 1986). The lengths of agrohumid periods for drought adapted crops as calculated by Watbal-Module 1 computer program (Kutsch and Schuh, 1980) are 50–55 days (long rains) and 65 days (short rains) (Hornetz, 1997). Average monthly temperatures are highest in February (24.3°C) and October (23.4°C), prior to the onset of rains in March and November, respectively (KMD, 1984).

Seeds and cultures

Green gram seeds were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) at Kiboko, SE-Kenya. Undamaged seeds of uniform colour and size were selected for the experiments. Seeds for greenhouse work were germinated in petri-dishes and watered with sterilized and demineralized water under greenhouse conditions. *Bradyrhizobium* spp. strain CB1015 for inoculation of seeds in the field was obtained from Microbiological Resource Centre (MIRCEN), University of Nairobi.

Estimation of rhizobial numbers

The number of rhizobia in the soil was determined using the Most Probable Number (MPN) plant infection technique described by Becker et al. (1993). Pre-germinated seeds (Somarsegaran and Hoben, 1985) were transferred into Leonard Jars, one seedling per Leonard Jar assembly. Uninoculated control plants were used as material control to detect contamination over the growth period in the greenhouse experiment.

Field experiments

Field experiments were performed during the long and short rainy season of 1997. The results were basically the same for the two seasons. Thus the results presented in this paper are the pooled data from the two seasons. Rainfall amount during the season was 223 mm. The experimental plots were additionally irrigated so that soil moisture was optimum. Soil moisture was monitored in the experimental field daily using calibrated gypsum electrodes, for the entire growing period.

The field experiments were made up of 8 treatments (green gram in pure stands inoculated, green gram in pure stands uninoculated, green gram in pure stands plus nitrogen fertilizer applied at the rate of 40 kg ha^{-1} of CAN powder (26% N), green gram inoculated and interplanted with maize, green gram uninoculated and interplanted with maize, green gram plus N fertilizer and intercropped with maize, pure stands of maize plus N fertilizer, and pure stands of maize without N-fertilizer). Each plot was replicated 4 times in a complete randomised block design (CRBD). A replicate constituted a 3×3 m plot.

At planting time in the field all seeds were dressed with Furadan chemical to control cut-worms (*Agrotis ipsilon*). Maize was sown in rows of 75 cm apart with a spacing of 30 cm between plants in the row (44,400 plants ha^{-1}). Green grams were sown 50 cm by 20 cm giving a plant density of 100,000 plants ha^{-1}.

These planting geometries were adopted in order to achieve the plant populations, recommended in the semi-arid areas of SE-Kenya (Lenga and Stewart, 1983).

The crops were dry planted before the onset of the rains in each season. Triple super phosphate (TSP) granules (50% $P_2 O_5$) were applied at the rate of 40 kg ha^{-1}, to alleviate phosphorus deficiency. Four seeds were planted per hole for each of the plants. During the first weeding (7 days after emergence) plants were thinned to two per hole. The second weeding was carried out 21 days after emergence (plants were about 20 cm above ground). Soon after second weeding, CAN powder (26% N) was topdressed on N treatment plots at the rate of 40 kg ha^{-1}.

Plant sampling was carried out after 21 days, 42 days and finally at physiological maturity. One plant was harvested at random from each treatment replicate. The plants were dug up for nodule counting (in case of legumes) and determination of below ground biomass. Plant samples and nodules were dried to constant weight in an oven. Dry weights were determined using a high precision Sartorius balance. Nitrogen and carbon contents from the third harvest were analysed using the high sensitivity nitrogen-carbon analyser (Sumigraph NC-90). Soil samples were taken as composite samples (5 samples per plot then mixed to give a composite sample) and nitrogen analysed before and after harvest using the Kjeldahl method (Bremner and Mulvaney, 1982). Soil phosphorus was determined using the Olsen method (Watanabe and Olsen, 1965).

Average plot yield per treatment was used to calculate yield per hectare. All data were subjected to statistical analyses using the statistical package Statgraphics (STSC Inc., 1986) and treatment means separated using the Duncan's Multiple range test (Steel and Torrie, 1981).

3. Results and Discussion

MPN counts

The most likely number of rhizobia specific to green grams was calculated from the MPN results (Table 1), according to the method of Vincent (1970). See also equation (1)

$$MPN = \frac{mxd}{v} \qquad (1)$$

where m is the most likely number, d is the lowest dilution, and v is the aliquot used for inoculation.

Table 1. Nodulation of green gram plants inoculated with varying dilutions of soil

Soil dilution	Nodulation (+ or –) in Leonard Jar units I	II	III	IV	Total units nodulated
10^{-1}	+	+	+	+	4
10^{-2}	+	+	+	+	4
10^{-3}	–	+	+	+	3
10^{-4}	+	–	+	+	3
10^{-5}	–	+	+	–	2
10^{-6}	+	+	–	–	2
10^{-7}	+	+	–	–	2
10^{-8}	–	–	–	–	0
10^{-9}	–	–	–	–	0
10^{-10}	–	–	–	–	0

The rhizobia capable of nodulating green grams in the soils ranged from 519 to 3,780 cells per gram of soil. These numbers were above the threshold (50 rhizobia cells per gram of soil) at which inoculation may be excluded (Thies et al., 1991). The soils were collected during the dry season and it is expected that during the rains, along with other soil microorganisms rhizobia numbers will increase.

Field experiments

Twenty one days after emergence (21 DAE), green grams intercropped with maize had higher total nodule and plant dry weights than the ones in pure stands regardless of the source of N (Table 2). The higher dry weights were possibly due to reduced soil water loss as a result of shading by maize. Nodule numbers were not significantly different between treatments, indicating a highly infective population of green gram rhizobia in the soil. Increase in nodule dry weights in green grams intercropped with maize is further evidence that interplanting green grams and maize is advantageous to the legume at least for the first 21 days. At this stage there were no significant differences between maize treatments (Table 3).

At second harvest (42 DAE) there was no significant difference in pod number, nodule number, and nodule dry weight. Plant dry weights were however significantly high in green grams treated with nitrogen fertilizer and lower in green grams intercropped with maize and not treated with nitrogen (Table 4). These data and results in Table 1 indicate an effective indigenous population of green gram rhizobia in Kiboko soils. Applied N did not affect

Table 2. Effects of maize intercrop on growth and nodulation of green grams 21 days after emergence (21 DAE)

Treatment	Plant dry weight (g)	Nodule number per plant	Nodule dry weight (mg) per plant
Green grams + N*	1.54 a	21 a	4 a
Green grams uninoculated	1.51 a	29 ab	6 a
Green grams inoculated**	1.49 a	33 ab	6 a
Green grams + maize + N*	1.94 ab	26 ab	8 ab
Green grams + maize	1.88 ab	28 ab	8 ab
Green grams inoculated** + maize	2.38 b	36 b	11 b

Means (n = 8) followed by the same letter down the column are not statistically different (P = 0.05) by Duncan's multiple range test. *Nitrogen fertilizer was topdressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder; **Green grams were inoculated with *Bradyrhizobium* strain CB1015.

Table 3. Effects of intercropping with green grams on the growth of maize 21 days after emergence (21 DAE)

Treatment	Plant dry weight (g)
Maize + N*	4.87
Maize	6.28
Maize + green grams uninoculated + N*	6.06
Maize + green grams uninoculated	5.23
Maize + green grams inoculated**	5.09

There were no significant differences (P = 0.05) between treatment means (n = 8) by Duncan's multiple range test. *Nitrogen fertilizer was topdressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder; **Green grams were inoculated with *Bradyrhizobium* strain CB1015.

nodulation. A preliminary inference is that there is poor N-fertilizer uptake by green grams. Dry weight gains observed at 21 DAE in green grams intercropped with maize levelled out at 42 DAE. This was possibly due to competition for water and nutrients between maize and green gram crop, a phenomenon absent in pure stands. Maize was more affected by intercropping as is evident from the lower plant dry weights compared to maize grown in pure stands (Table 5).

Table 4. Effects of maize intercrop on growth and nodulation of green grams 42 days after emergence (42 DAE)

Treatment	Plant dry weight (g)	Nodule number per plant	Nodule dry weight (g) per plant	Pod dry weight (g) per plant
Green grams uninoculated + N*	46.16 b	195 a	0.53 a	2.57 a
Green grams uninoculated	41.02 ab	142 a	0.36 a	1.72 a
Green grams inoculated	40.33 ab	163 a	0.58 a	2.01 a
Green grams uninoculated + maize+ N*	41.95 ab	147 a	0.46 a	1.94 a
Green grams uninoculated + maize	37.99 a	158 a	0.59 a	2.43 a
Green grams inoculated **+ maize	37.98 a	180 a	0.54 a	2.05 a

Means (n = 8) followed by the same letter down the column are not statistically different (P = 0.05) by Duncan's multiple range test. *Nitrogen fertilizer was topdressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder; **Green grams were inoculated with *Bradyrhizobium* strain CB1015.

Table 5. Effects of green gram intercrop on the growth of maize 42 days after emergence (42 DAE)

Treatment	Plant dry weight (g)
Maize + N*	81.73 c
Maize	9.08 c
Maize + green grams uninoculated + N*	76.27 b
Maize + green grams uninoculated	67.36 a
Maize + green grams inoculated**	74.08 ab

Means (n = 8) followed by the same letter down the column are not statistically different (P = 0.05) by Duncan's multiple range test. *Nitrogen fertilizer was topdressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder; **Green grams were inoculated with *Bradyrhizobium* strain CB1015.

Green grams were harvested 100 DAE. There were no significant differences (P = 0.05) in plant, pod, and seed dry weights between treatments (Table 6). Low dry weight and seed yield of maize intercropped with green grams relative to pure stands was evident at final harvest 110 DAE (Table 7).

Maize and green gram yields (Table 8) were higher than the quoted national averages of 2700 kg ha^{-1} and 1000 kg ha^{-1}, respectively (KARI, 1994;

Table 6. Effects of maize intercrop on the growth and nodulation of green grams 100 days after emergence (100 DAE)

Treatment	Plant dry weight (g)	Pod dry weight (g) per plant	Seed dry weight (g) per plant
Green grams uninoculated + N*	27.37	35.24	25.45
Green grams uninoculated	25.57	39.68	23.71
Green grams inoculated**	29.16	34.82	26.51
Green grams uninoculated + maize + N*	27.47	30.92	23.79
Green grams uninoculated + maize	27.18	33.15	25.96
Green grams inoculated** + maize	25.69	31.56	24.54

Means (n = 8). There were no significant differences (P = 0.05) between the means by Duncan's multiple range test. *Nitrogen fertilizer was topdressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder; **Green grams were inoculated with *Bradyrhizobium* strain CB1015.

Table 7. Effects of green gram intercop on maize 110 days after emergence (110 DAE)

Treatment	Plant dry weight (g)	Seed dry weight (g) per plant
Maize + N*	118.35 d	98.03 c
Maize	116.08 d	91.58 c
Maize + green grams uninoculated + N*	82.14 b	66.03 a
Green grams uninoculated + maize	65.22 a	56.23 a
Maize + green grams inoculated**	88.79 c	79.04 b

Means (n = 8) followed by the same letter down the column are not statistically different (P = 0.05) by Duncan's multiple range test. *Nitrogen fertilizer was topdressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder; **Green grams were inoculated with *Bradyrhizobium* strain CB1015.

Poehlman, 1991). This shows that good agronomic management can improve productivity of the two crops in semi-arid areas of SE-Kenya.

Soil parameters

The analysis of soil nitrogen before and after the cropping season showed plots with green grams as a sole crop and in intercrop had stabilized and partly enriched soil with N during the vegetation periods (Fig. 1). These results show

Table 8. Effects of intercropping maize and green grams on their yield

Treatment	Green gram yield (kg ha^{-1})	Maize yield (kg ha^{-1})
Green grams uninoculated + N*	2418 a	*
Green grams uninoculated	2379 a	*
Green grams inoculated**	2433 a	*
Green grams uninoculated + maize + N*	2549 a	2976 b
Green grams uninoculated + maize	2788 a	2521 a
Green grams inoculated** + maize	2698 a	2609 ab
Maize + N*	*	4120 c
Maize	*	3914 c

Means (n = 8) followed by the same letter down the column are not statistically different (P = 0.05) by Duncan's multiple range test. *Nitrogen fertilizer was topdressed 10 DAE at the rate of 40 kg ha^{-1} of CAN powder; **Green grams were inoculated with *Bradyrhizobium* strain CB1015.

that indigenous as well as inoculated rhizobia are able to fix nitrogen with green grams in both (sole and intercrop) cropping systems. Maize plots were however characterised by a general slight decrease in nitrogen, possibly caused by the high nutrient demand of the maize plants.

Phosphorus contents in the soil increased in all of the plots by 20–30% (average), being the highest in plots with inoculated plants (Fig. 2). This shows that amendment of the soil with triple super phosphate increased soil P. A general improvement of phosphorus in the Kiboko soils has also been reported by Hornetz (1997) in field experiments with sorghum and tepary beans after a cropping season. This was probably caused by increased soil microbial activity especially mycorrhiza which facilitates the release of larger quantities of insoluble nutrients like phosphorus for the plants (Torrey and Clarkson, 1975, cited in: Hornetz, 1997).

Analysis of different plant tissues did not reveal significant differences in nitrogen and carbon contents between treatments and cropping systems of green grams (data not shown). For maize, however, the yield in intercropped plants was lower than control plants in pure stands. This, apparently, can be attributed to lower nutrient uptake of the intercropped maize plants resulting in weaker yield performance.

4. Conclusions

Most probable number counts and field nodulation results put together

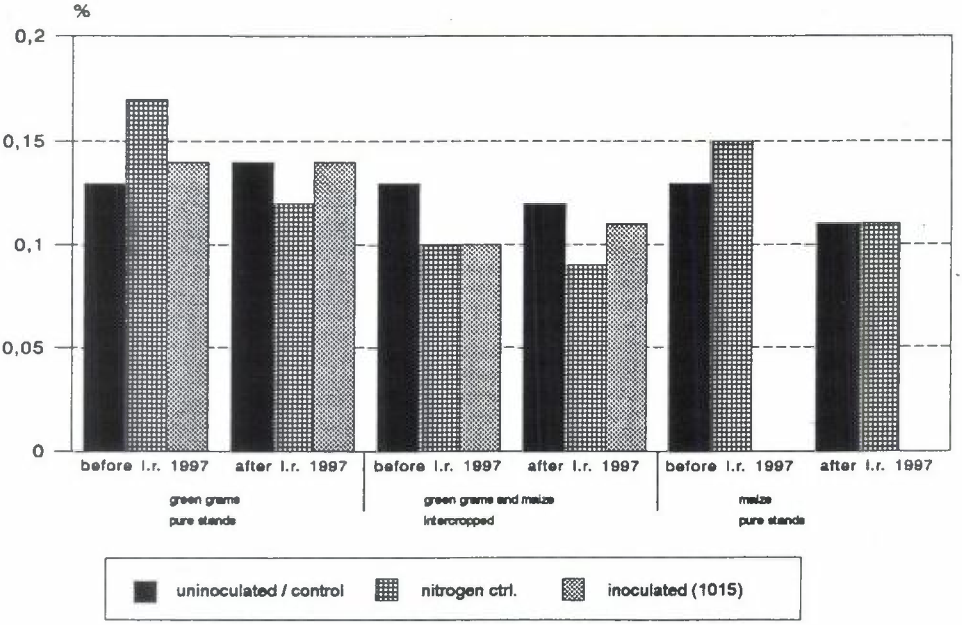

Figure 1. Nitrogen content in cropping systems with green grams and maize at Kiboko (topsoil, long rains 1997).

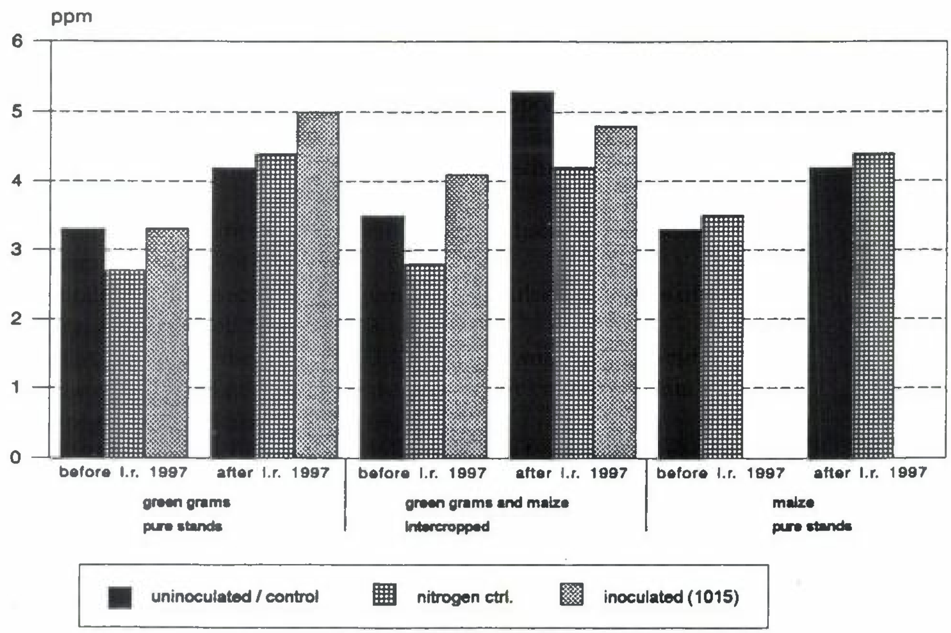

Figure 2. Phosphorus content in cropping systems with green grams and maize at Kiboko (topsoil, long rains 1997).

(Tables 2 and 3) suggests that there are adequate populations of infective and effective green gram rhizobia in the soils of semi-arid SE-Kenya. Therefore, inoculation may not be useful. Since the indigenous rhizobia are able to withstand adverse edaphic and environmental factors such as low soil pH, high soil temperatures and long dry spells they can be isolated, characterised and used in similar environments where need be. It is also necessary to quantify the amount of nitrogen fixed and formulate agronomic practices which enhance nitrogen fixation by the indigenous rhizobia.

Green grams are more competitive in intercropping systems with maize or other C4 plants, than other legumes such as beans or even the drought adapted tepary beans (*Phaseolus acutifolius*) (Hornetz, 1997). Elsewhere it has been shown that beans produced only 40% of their optimum yields when intercropped with sorghum (Katumani var. K369), whereas the cereals showed no significant yield reductions. Similar results have been reported in Africa by Steiner (1982: cited in Hornetz, 1997).

Poor fertilizer uptake by legumes, though surprising, was observed in the same soil in experiments involving cowpeas and common beans intercropped with maize (Pilbeam et al., 1995). More work needs to be carried out to establish the reason for the low fertilizer uptake. Since green grams maintain nitrogen and phosphorus status of the soil and are drought resistant (Hornetz et al., 1998) it is more advantageous to intercrop them with maize. This is especially applicable to resource poor farmers who farm purely for subsistence without applying any fertilizer into the soil. In case of unreliable rainfall which may lead to total maize crop failure, the farmer will be assured of getting some grain from the drought resistant legume.

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