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Yield and Nitrogen Fixation Response by Drought Tolerant Tepary Bean (*Phaseolus acutifolius* A. Gray Var. *Latifolius*) in Sole and Maize Intercrop Systems in Semi-arid Kenya

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Abstract: Tepary bean (TB), a drought tolerant bean variety has become popular among poor small-scale farmers in semi-arid Kenya, where it is predominantly intercropped with maize. Field experiments were conducted on effect of intercropping TB and maize on nitrogen fixation and crop yield in semi-arid Kenya over two cropping seasons. Experimental design was randomised complete block with eight treatments: TB sole crop not inoculated with *Rhizobium* (R3254) and without N fertilizer (N), TP sole crop not inoculated with R3254 with or without N, TB sole crop inoculated with R3254 without N, TB with maize intercrop not inoculated with R3254 with or without N and maize sole crop with or without N. Each treatment was replicated four times. Significant differences ($P \leq 0.05$) were observed in total plant dry weight in treatment R3254 at both 21 and 42 days after emergence (DAE). TB yields were significantly reduced in uninoculated intercrop. Inoculated TB treatments had significantly higher seed dry weights and yields ha^{-1} . Intercropping TB and maize suppresses the yield of the former under semi-arid conditions. Inoculating TB with *Rhizobium* strain R3254 was infective, effective and significantly improved TB yields in sole and intercrop. Soil analysis after the two cropping seasons indicated enhancement of soil N in sole TB plots above pre-planting levels. Maize plots exhibited a decline in soil N. Total N concentration in plant tissues was significantly enhanced in treatment R3254. There was a marked increase in soil P in all treatment plots following amendment.

Key words: Cropping system; crop yield; drought tolerant; *Phaseolus acutifolius*; *Rhizobium* inoculation; tepary bean

Introduction

The Government of Kenya has for a long time underscored the important role played by the arid and semi-arid lands (ASALs) of Kenya (Republic of Kenya, 1993) in food production. Currently, food production has been declining in these ASALs as population growth increases (Rao and Mathuva, 2000; Maingi *et al.*, 2001; Shisanya, 2002), long periods of fallowing no longer practiced and the land is cropped continuously after clearing. Fertilizer use is low because of socio-economic constraints and also its unavailability at the right time, high cost and risks from erratic rainfall. It is therefore a major challenge to sustain crop yields and economic returns in such low input agricultural systems, predominantly by small-scale farm. As a result, yields of cereals do not exceed 1 t ha^{-1} and legumes 0.5 t ha^{-1} per crop season (Tiffen *et al.*, 1994).

Various researchers have emphasized the importance of research on drought tolerant crop species of short cycle as a priority in addressing the food deficit problem in the ASALs (Hornetz *et al.*, 2000; Shisanya, 2002). Unfortunately, this has not received adequate attention (Shisanya, 1999). Most smallholder farmers in the ASALs cannot afford the required external inputs in the form of chemical N fertilizer to improve their food production. Researchers in Kenya have exploited the legume *Rhizobium* symbiosis as a substitute for the expensive N fertilizers in these ASALs (Gitonga *et al.*, 1999; Hornetz *et al.*, 2000; Maingi *et al.*, 2001; Shisanya, 2002). Nitrogen (N) contribution by legumes to other crops in the system depends on the species, biological N₂ fixation and growth of legumes as determined by climate and soil and management of residues.

In semi-arid Kenya, maize (*Zea mays* L.) is commonly intercropped or rotated with bean (*Phaseolus vulgaris* L.), pigeon pea (*Cajanus cajan* L. Millsp.) or cowpea (*Vigna unguiculata* L. Walp), although the relative proportion of the legume in these mixed systems is small. Tepary bean (*Phaseolus acutifolius* A. Gray var. *latifolius*), a drought tolerant legume (Hornetz, 1990) has recently assumed importance in the intercrop farming systems of semi-arid Kenya (Shisanya, 2002). The N removed by maize in this region is estimated to be as much as 25-40 kg ha⁻¹ per season, which means that a matching amount of N needs to be supplied for long term sustainability of production (Rao and Mathuva, 2000). Nitrogen fixation by bean is notoriously inconsistent, with or without inoculation (Maingi *et al.*, 2001), but cowpea nodulates well by the ubiquitous *Bradyrhizobia* sp. and fixes up to 20 kg N ha⁻¹ (Pilbeam *et al.*, 1995). Recently, Shisanya (2002) found that TB nodulates very well with *Rhizobium* sp. strain R3254 and fixes up to 260 kg N ha⁻¹. However, this study by Shisanya did not investigate the effect of intercropping maize and TB on nitrogen fixation by the latter under the semi-arid conditions. Earlier studies (Gitonga *et al.*, 1999; Maingi *et al.*, 2001) investigated the effect of intercropping on nitrogen fixation by common bean and green gram under semi-arid conditions, respectively. The main objective of this study was therefore to investigate the effect of intercropping maize and tepary bean on nitrogen fixation and yield under the semi-arid conditions of southeast Kenya.

Materials and Methods

Experimental site

The experiments were carried out at KARI Kiboko sub-centre (latitude 02° 12' S, longitude 37° 43' E, altitude 975 m a.s.l.), located at about 160 km southeast of Nairobi, the capital town of Kenya. The climate of the experimental site is described as hot and dry (Hornetz *et al.*, 2000). The soils of the study area are well drained Fluvisols, Ferralsols and Luvisols (Eichinger, 1999). The soil pH of the experimental field is 7.9 (measured in 0.01 M L⁻¹ CaCl₂). Rainfall is bimodally distributed, with median monthly maximum in April (126 mm) and November (138 mm). The medial annual rainfall is about 582 mm year⁻¹. The short rains (SR) (October-January) generally have more rainfall and are more reliable than the long rains (LR) (March-June) (Hornetz *et al.*, 2000). The lengths of the agrohumid periods for drought-adapted crops are 50-55 days (LR) and 65-70 days (SR) (Jaetzold and Schmidt, 1983). Average monthly temperatures are highest in February (24.3° C) and October (23.4° C) (KMD, 1984), prior to the onset of the rains in March and November, respectively.

TB seeds and Rhizobium culture

Seeds of maize and tepary bean were obtained from local farmers. Undamaged seeds were carefully selected to ensure uniformity in size. Commercial *Rhizobium leguminosarum* biov. *phaseoli* strain R3254 used to inoculate TB was obtained from the Microbiological Resource Centre (MIRCEN), University of Nairobi. The infectivity of this strain had been determined in an earlier study (Shisanya, 2002).

Soil and plant samples analyses

Soil samples were collected to a depth of 60 cm using a soil auger before planting. Five sub-samples were collected from each plot, mixed thoroughly in polythene bags and transported to the laboratory. Sub-sample was air-dried while the remainder was refrigerated at 4° C. The air-dried sub-samples were sieved (2 mm) and a further sub-sample was ground to pass through a 0.25 mm sieve for total C and N analyses. The colorimetric method described by Anderson and Ingram (1993) was used for soil organic C. Total N was measured calorimetrically following Kjeldahl digestion (Forster, 1995a; Bremner and Mulvaney, 1982). Soil P was determined following the method of Foster (1995b). Analyses of soil N and P were 0.7 mg N kg⁻¹ and 3.0 mg P kg⁻¹ soil in 0-60 cm soil depth, respectively. The C/N ratio and CEC are 11.7 and 7.8 ML⁻¹, respectively. Nitrogen concentration in plant tissues was done using the high sensitivity nitrogen - carbon analyser (sumigraph nc-90). Soil N and P changes in treatment plots over the growth period were calculated using the following equation:

$$\Delta (N/P) = (N/P)_h - (N/P)_{pp} / (N/P)_{pp} \times 100 \quad (1)$$

where:

Δ = % change in N/P in treatment plots

N = soil nitrogen in treatment plots

P = soil phosphorus in treatment plots

h = harvesting period

pp = pre-planting period

Field experiments

The experiments were conducted over two seasons, i.e. LR 2000 (March - June) and SR 2000/2001 (October - January). The field experiments were made up of eight treatments as follows:

1. Tepary bean as sole crop with N fertiliser and no inoculation (TB + N - INOC);
2. tepary bean as sole crop with no N fertiliser and no inoculation (control) (TB - N - INOC);
3. tepary bean as sole crop and inoculated with Rhizobium strain 3254 and no N fertiliser (TB - INOC - N);
4. tepary bean - maize intercrop with N fertiliser added and no inoculation (TB + M + N - INOC);

5. tepary bean - maize intercrop with no N fertilizer and no inoculation (TB + M - N - INOC);
6. tepary bean - maize intercrop inoculated with Rhizobium strain 3254 and no N fertiliser (TB + INOC + M - N);
7. maize as sole crop with N fertiliser (M + N) and
8. maize as sole crop with no N fertiliser (M - N)

Each plot was replicated four times in a complete randomised block design (CRBD). A replicate constituted a 3 × 3 m plot size. Land preparation was done by tractor ploughing followed by harrowing. A basal dose of triple superphosphate granules (TSP; 50% P₂O₅) fertiliser was applied at the rate of 40 kg ha⁻¹ to all the plots. Before planting, TB seeds for inoculation treatment were rinsed in 10% sucrose solution as described (Gitonga *et al.*, 1999). Inoculation with rhizobia was carried out by addition of 6.7 g of filtermud based inoculant for every kg of moistened sucrose coated TB. This was followed by thorough mixing of the seeds with the inoculant until the seeds were uniformly coated. The inoculated seeds were kept covered in a container to avoid exposure to direct sunlight. The crops were planted just before the onset of the rains in both seasons. For each of the treatments 3 seeds/hole were planted. During the first weeding, i.e. 7 days after emergence (DAE), plants were thinned to 2 seeds/hole. The resulting plant densities for tepary bean and maize were 10 plants m⁻² and 4.4 plants m⁻², respectively. At 10 DAE, when the plants were about 20 cm above ground, calcium ammonium nitrate (can) powder (26% N) was top-dressed on N treatment plots at the rate of 40 kg ha⁻¹. Tepary bean plants were sampled 21 DAE, 42 DAE and 70 DAE. These periods corresponded to first nodule formation, flowering and full maturity, respectively. The physiological maturity period for maize was 90 DAE. Four plants were randomly sampled from each treatment replicated and various parameters assessed. The plant material was partitioned into roots, stems and leaves. The TB plants were dug up for nodule counting and determination of below ground biomass. Plant samples and nodules were oven dried at 70°C to constant weight. Dry weights of the various parts of the plant samples were determined and recorded using a high precision Sartorius balance. From the final harvest, the following data were collected: pod number and pod dry weight of TB per plant, cob dry weight per plant in maize, seed (grain) dry weight per plot, 100 seed dry weight and yield per treatment plot. Data obtained were subjected to analysis of variance (ANOVA) using the statistical computer package statgraphics version 6.0 and treatment means separated using Duncan's multiple range test (DMRT) at P ≤ 0.05 level (Steel and Torrie, 1981).

Results

The LR 2000 season field results

There were significant differences (P ≤ 0.05) in nodule number, nodule dry weight and plant dry weights between the inoculated treatments and the other treatments, 21 DAE (Table 1). All the measured parameters, however, were higher in the sole cropped inoculated TB than in the inoculated TB - maize intercrop (Table 1). The effect of N fertiliser treatment on total dry matter of intercrop plants or sole crops was not significant at this stage of growth. Generally, the total

Table 1: Effect of different TB-maize cropping system on total per plant dry weight of TB during the LR 2000 and SR 2000/2001 season at 21 DAE. Means (n = 24) followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test

Treatment	Nodule number (plant ⁻¹)		Nodule dry weight (mg plant ⁻¹)		Total plant dry weight (g plant ⁻¹)	
	LR	SR	LR	SR	LR	SR
TB + N	16 ^b	18 ^b	7.8 ^b	8.0 ^b	2.9 ^b	3.2 ^b
Control	11 ^b	14 ^b	5.4 ^b	5.7 ^b	2.5 ^b	2.9 ^b
TB + INOC-N	30 ^a	38 ^a	14.7 ^a	15.2 ^a	3.8 ^a	4.5 ^a
TB+ M + N-INOC	15 ^b	16 ^b	7.4 ^b	7.6 ^b	2.6 ^b	2.8 ^b
TB + M-N-INOC	12 ^b	14 ^b	5.9 ^b	6.0 ^b	2.7 ^b	2.9 ^b
TB + INOC + M-N	26 ^a	34 ^a	12.7 ^a	14.8 ^a	3.6 ^a	4.1 ^a

Table 2: Effect of different TB-maize cropping system on total per plant dry weight of TB during the LR 2000 and SR 2000/2001 seasons at 42 DAE. Means (n=24) followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test

Treatment	Nodule number (plant ⁻¹)		Nodule dry weight (mg plant ⁻¹)		Pod number (plant ⁻¹)		Pod dry weight (g plant ⁻¹)		Total plant dry weight (g plant ⁻¹)	
	LR	SR	LR	SR	LR	SR	LR	SR	LR	SR
TB+N-INOC	44c	47c	21.6c	21.9c	39c	42c	41.1c	42.0c	26.8b	29.0b
Control	35d	38c	17.2c	17.6c	35c	38c	37.0c	39.9c	25.0b	28.5b
TB-INOC-N	78e	85a	39.2a	45.0a	60a	75a	63.3a	65.8a	32.8a	38.0a
TB+M+N-INOC	38a	42d	18.6c	19.2c	34c	39c	35.8c	37.0c	22.4b	25.4b
TB+M-N-INOC	32d	35b	15.7c	16.2c	30c	35c	31.6c	34.0c	19.6b	20.3b
TB+INOC+M-N	62b	70b	30.4b	34.2b	48d	55d	50.6b	53.5b	25.6a	25.6b

Table 3: Effect of different TB-maize cropping system on total TB seed dry weight, plant dry weight per plot and 100 seed weight at physiological maturity (70 DAE) during the LR 2000 and SR 2000/2001 seasons. Means (n= 24) followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test

Treatment	Total seed dry weight (g plot ⁻¹)		Total plant dry weight (g plot ⁻¹)		100 seed dry weight (g plot ⁻¹)	
	LR 2000	SR 2000/2001	LR 2000	SR 2000/2001	LR 2000	SR 2000/2001
TB+N-INOC	2467.5b	2497.0b	496.6c	500.6c	13.2b	13.3b
Control	2220.8b	2380.5b	420.5c	450.7c	12.9b	13.0b
TB+INOC-N	3210.6a	3500.5a	735.5a	750.0a	16.4a	16.6a
TB+M+N-INOC	2480.0b	2590.0b	570.4c	590.5c	13.0b	13.2b
TB+M-N-INOC	2160.0b	2160.0b	465.0c	480.6c	12.8b	12.9b
TB+INOC+M-N	2560.0b	2650.5b	600.8b	610.5b	13.6b	13.8b

Table 4: Effects of different TB-maize cropping system on yield during LR2000 and SR2000/2001 seasons. Means followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test

Treatment	TB yield (kg ha^{-1})		Maize yield (kg ha^{-1})	
	LR 2000	SR 2000/2001	LR2000	SR 2000/2001
TB+N-INOC	990c	1010c	-	-
Control	850c	996c	-	-
TB+INOC-N	1530a	1650a	-	-
TB+M+N-INOC	970c	980c	3850b	3900b
TB+M-N-INOC	950c	1000c	2560c	2670c
TB+INOC+M-N	1180b	1200b	3960b	3900b
M+N	-	-	4250a	4380a
M-N	-	-	2450c	2600c

Table 5: N concentration at harvest in TB plant tissues during the LR2000 and SR 2000/2001 seasons

Treatment	N concentration mg N g^{-1} (dry matter)					
	LR season 2000			SR season 2000/2001		
	Shoot	Root	Seed	Shoot	Root	Seed
TB+N	1.3b	0.6b	2.5b	1.7b	0.9b	2.8b
Control	1.1b	0.5b	2.2b	1.4b	0.8b	2.6b
TB+INOC-N	2.8a	2.0a	4.0a	2.9a	2.5a	4.4a
TB+M+N-INOC	1.9b	0.5b	3.0b	2.0b	0.8b	2.9b
TB+M-N-INOC	1.8b	0.4b	2.9b	1.9b	0.9b	3.0b
TB+INOC+M-N	1.5b	0.6b	2.6b	1.6b	0.8b	2.9b

dry weights of inoculated sole cropped TB plants at 21 DAE were slightly higher than the dry weights of the TB - maize intercrop.

At second harvest (42 DAE), inoculated treatments had significantly ($P \leq 0.05$) higher dry weights of all the parameters measured, whether in sole or intercrop, than the other treatments (Table 2). However, dry weights of the measured parameters were significantly lower in inoculated treatments in TB - maize intercrop. The effect of N fertiliser at this stage too was not significant.

At physiological maturity (70 DAE), sole cropped and inoculated TB had significantly ($P \leq 0.05$) higher dry weights of all parameters than the other treatments (Table 3). Though inoculated TB - maize intercrop had significantly ($P \leq 0.05$) higher total dry weight than the other treatments, it was significantly ($P \leq 0.05$) lower than inoculated sole TB crop (Table 3).

Inoculation had a significant effect on the yield of TB (Table 4). The yield of sole cropped TB was significantly ($P \leq 0.05$) higher than that in intercrop (Table 4). The yield response of maize to N fertiliser application was significant as compared to the same in TB (Table 4).

The SR 2000/2001 season field results

The results of the SR 2000/2001 season were very similar to those of the LR 2000 season in many aspects. Like in the previous season, significant differences ($P \leq 0.05$) were observed in nodule number, nodule dry weight and plant dry weight between inoculated and other treatments, 21 DAE (Table 1). At 42 DAE, treatment R3254 was significantly different from the rest in all parameters (Table 2). The effect of inoculation at this stage was significant though higher in sole cropped TB than in intercrop. At final harvest (70 DAE), significant effects of inoculation were observed in all parameters (Table 3). However, inoculation of TB with R3254 under sole crop had significantly ($P \leq 0.05$) higher dry weights of the parameters compared to intercrop (Table 3). Like in previous season, N fertiliser application effect was significant in sole cropped maize than in TB (Table 4).

Results of plant tissue N concentration, soil N and P

The data in Table 5 show the N concentration in various plant tissue parts at final harvest over the two seasons. Inoculated TB plants had consistently higher N concentration over N and control treatments in both seasons.

The analysis of soil N before and after the growing season (using equation 1) showed that TB legume plots had enriched their N content over the two seasons by between 15 - 70% (Figs. 1(a) and 1(b)). Soil P content in the soil was enhanced in all the plots by between 10 - 66% over the two seasons (Figs. 2(a) and 2(b)).

Discussion

Plant dry weight was used indirectly to estimate N_2 fixation in the present study. In field experiments, at 21 DAE, the effect of inoculating TB with R3254 had positive significant effects over the two seasons. The infectivity and effectivity of *Rhizobium* strain R3254 on TB was more pronounced at 42 DAE and at final harvest (70 DAE). For common bean in the study area, Pilbeam *et al.* (1995) reported that inoculation did not significantly improve yield over the control. N_2 fixation by common bean has been reported to be variable (Maingi *et al.*, 2001) due to many biotic and abiotic factors. High soil temperatures, as found in the study area and the incompatibility of some commercially available rhizobia strains are contributory factors in the failure of beans to nodulate and fix N_2 under semi-arid conditions of Kenya (Hornetz *et al.*, 2001; Shisanya, 2002). During all plant sampling stages, N fertilised plots had significantly lower dry weights of the parameters measured than inoculated treatments over the two seasons. Deficiencies in soil mineral N are a major constraint to crop growth and eventual productivity. Studies have shown that beans fertilised with moderate amounts of fertiliser N nodulate well during the early stages of growth and fix substantial amounts of N (Musandu and Ogendo, 2000). Apparently, this was not observed in the present study since N application had no significant

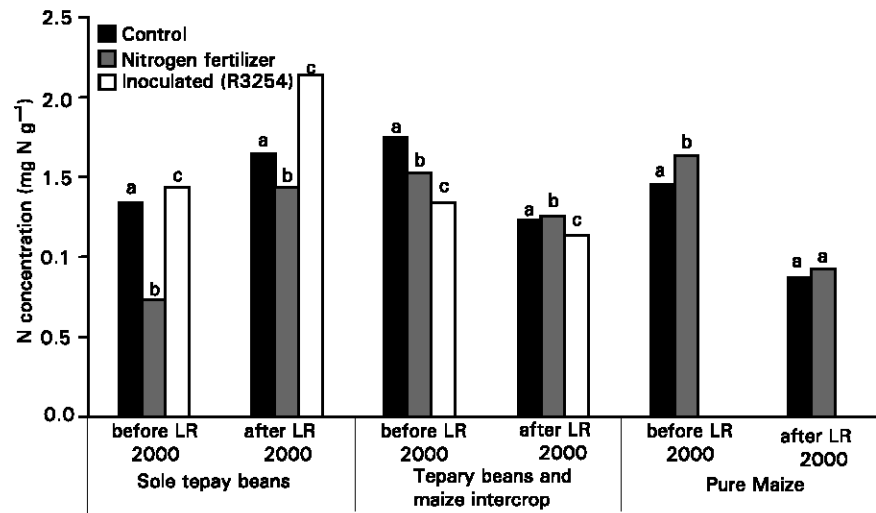


Fig. 1(a): Soil nitrogen (N) concentration in cropping systems with tepary beans and maize intercrop at Kiboko (0-60 cm soil depth, LR2000). Bars followed by the same letter are not significantly different by DMRT at $p \leq 0.05$

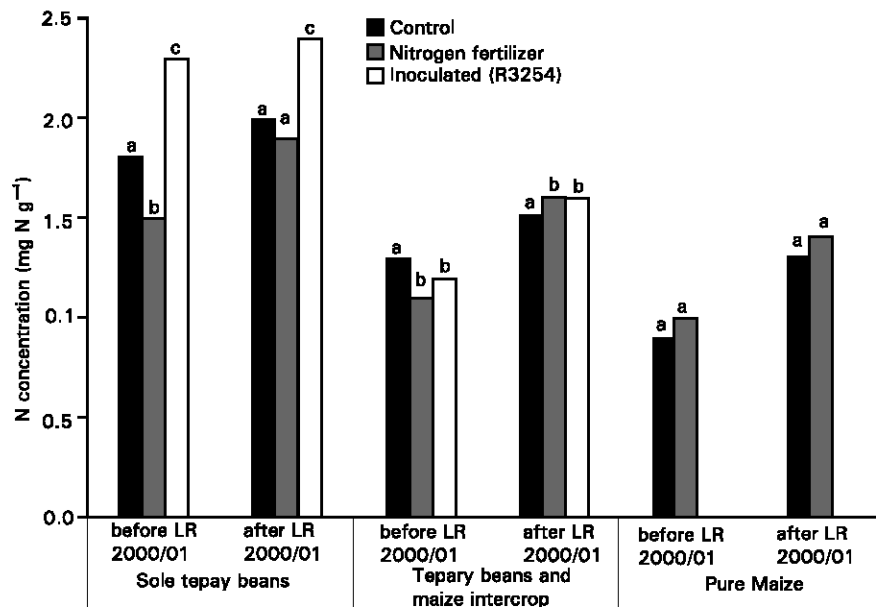


Fig. 1(b): Soil nitrogen (N) concentration in cropping systems with tepary beans and maize intercrop at Kiboko (0-60 cm soil depth, LR2000/01). Bars followed by the same letter are not significantly different by DMRT at $p \leq 0.05$

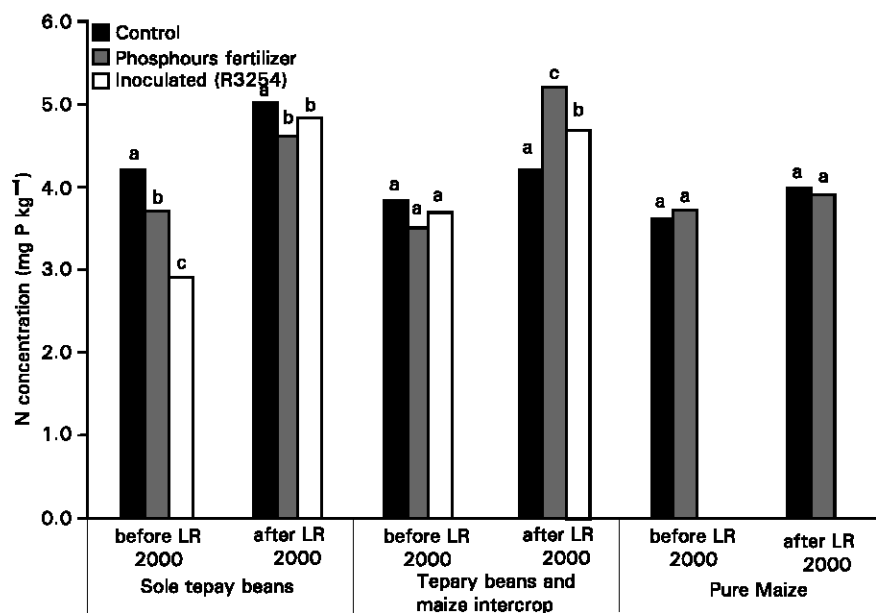


Fig. 2(a): Soil phosphorous (P) concentration in cropping systems with tepary beans and maize intercrop at Kiboko (0-60 cm soil depth. LR 2000). Bars followed by the same letter are not significantly different by DMRT at $p \leq 0.05$

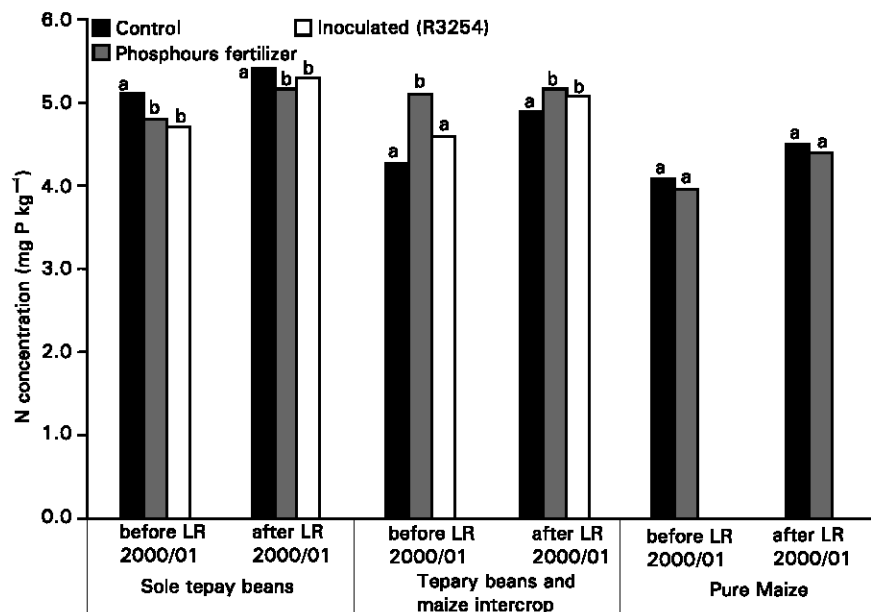


Fig. 2(b): Soil phosphorous (P) concentration in cropping systems with tepary beans and maize intercrop at Kiboko (0-60 cm soil depth. LR 2000/01). Bars followed by the same letter are not significantly different by DMRT at $p \leq 0.05$

effect on dry matter production or grain yield over the two seasons. A plausible explanation for this is that uptake of N may have been constrained by low rooting density of tepary bean (Shisanya, 1998) and inadequate soil moisture within the rooting zone that could have inhibited the mineral N moving to the plant through the plant roots (Shisanya, 2002). In addition, the aspect of rapid mineralisation of soil N cannot be ruled out under such semi-arid conditions (Hornetz *et al.*, 2000; Eichinger, 1999). Studies (Launauce, 1996; Danso *et al.*, 1988) have demonstrated that under arid, temperate and cold environments, very little N is transferred to the plants in the short-term period due to rapid mineralisation.

There were significant ($P \leq 0.05$) differences at final harvest between sole cropped inoculated TB and intercropped inoculated TB (Table 4) over the two cropping seasons. The second seasons yield parameters were higher than the previous. This could be attributed to the residual N benefits following the incorporation of TB stover from the first season into the soil. Results elsewhere (Kasasa *et al.*, 1999) have showed that soybean, for example, has significant residual fertility effects for maize and maize-based cropping systems. There is evidence that intercropping suppresses the legume growth. This has been confirmed elsewhere under limiting water conditions (Ayisi and Poswell, 1997; Hornetz, 1997). The legume yield suppression can further be explained in terms of competition between TB and maize for the limited N and P nutrients in the soil (Maingi *et al.*, 2001), sharing of fixed N_2 with the maize, which tend to reduce the photosynthetic activity (Hornetz *et al.*, 2000). Maize, belonging to the four-carbon dicarboxylic (C_4) group of plants, is usually a more competitive crop at the expense of a legume in maize-legume farming systems (Gitonga *et al.*, 1999).

Analysis of different plant tissues in different treatments showed that inoculated TB plants were significantly enriched with tissue N during the vegetative cycle over the two seasons (Table 5). The conclusion from this result is that *Rhizobium* strain R3254 is very infective and effective in N_2 fixation in TB under the semi-arid conditions of Kenya. Soil analysis of N indicated that inoculated treatment plots under sole crop and intercrop had enhanced soil N by between 15 - 70% (Figs. 1(a) and 1(b)). This is an indication that R3254 does fix nitrogen in both sole and intercrop. There was a general increase in P by between 10 - 66% in all the plots following the initial amendment (Figs. 2(a) and 2(b)). Soil P improvement has been reported in the study area in tepary bean - sorghum intercrop (Hornetz, 1997). The soil P improvement could also be attributed to microbial activity, especially mycorrhiza that facilitates the release of insoluble nutrients such as P for plants (Eichinger, 1999; Menge, 1983).

The results of this study show that intercropping tepary bean and maize is not advantageous to either crop under semi-arid conditions of Kenya. Maize tends to be aggressively competitive in terms of nutrient scavenging at the expense of the associated legume. Inoculation of tepary bean with R3254 was infective and effective under the semi-arid conditions irrespective of whether in sole or intercrop farming system. It is suggested that resource poor small-scale farmers, who may not have access to R3254, could practice rotation cropping of maize - tepary since the indigenous *Rhizobium* found in soils of the study area does fix some amount of nitrogen with tepary bean, which could be beneficial to the following maize crop.

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