

Quantification of Atmospheric N₂ Fixed by Cowpea, Pigeonpea and Greengram Grown on Ferralsols in Muheza District, Tanzania

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Abstract

Legume crops are usually intercropped with cereals in small-scale farming systems in Tanzania. This aims at taking advantage of legumes to replenish soil nitrogen (N due to legumes' N₂ fixation). Glasshouse pot experiments and field trials were carried out at Mlingano Agricultural Research Institute in Tanga, Tanzania, with the objective to find out how much N₂ can be fixed by cowpea, pigeonpea and greengram using native Rhizobia, thus substituting for the use of N-mineral fertilizers in maize production on Ferralsols. In the glasshouse pot experiment, the legumes were grown in 5L plastic pots for 40 days in soil sampled from 0 – 20 cm layer. The field experiment was maize – legumes intercropping whereby the N₂ fixation assessment was done 35 days after planting. In both cases, maize (Katumani variety) was the non- N₂- fixing reference crop. The legumes formed effective nodules with the native Rhizobia. Different quantities of N₂ gas were fixed by the different legumes, the differences being attributed to the legumes' differences in their genetic characteristics. In the field, monocropped cowpea, pigeonpea and greengram fixed 38, 21, and 49 kg N₂, respectively. In the intercrop system cowpea, pigeonpea and greengram fixed 16, 4, and 24 kg N₂, respectively. In both cases, the quantities of N₂ fixed were less than 50% of their total N accumulation. It was concluded that in Muheza, maize grown in association with the legumes would continue to need supplementation of mineral N fertilizer to maximize its yields.

Key words: Ferralsol, intercropping, legumes, nodules, N₂ fixation, N requirement

Introduction

Legumes depend both on soil mineral nitrogen (N) and biologically fixed atmospheric N₂ to meet their N requirements for growth and production (Buresh and De Datta, 1991). As a result, legumes thrive relatively better than cereals in N deficient soils. Field experiments have indicated that grain legumes grown in N deficient soils can fix substantial quantities of atmospheric N₂. The quantities of atmospheric N₂ fixed among and within legumes vary depending on their genetic characteristics, and factors such as soil and environmental conditions and presence or absence of inoculation. In West Africa, cowpea, for example, was reported to fix 122 kg N ha⁻¹year⁻¹ (Eaglesham *et al.*, 1981), and 201 kg N ha⁻¹year⁻¹ (Dakora *et al.*, 1987) in Nigeria and Ghana, respectively. In Kenya, cowpea fixed only 32 kg N

ha⁻¹year⁻¹ (Ssali and Keya, 1984). The length of the time span within which an annual grain legume crop can actively fix atmospheric N₂ depends on length of its life cycle within a season, while for perennial legumes the period is spread over several seasons. Research in West Africa with cowpea (Eaglesham *et al.*, 1982; Dakora *et al.*, 1987; Awonaike *et al.*, 1990) indicated that cowpea fixed between 47 and 201 kg N ha⁻¹, representing 54 - 70% of the accumulated N in a growth period of 60 days. In Brazil, however, cowpea fixed between 9 – 51 kg N ha⁻¹, which represented 32 – 74 % of the accumulated N within 110 days (Boddey *et al.*, 1990). Greengram in Thailand fixed 58 – 107 kg N ha⁻¹ (Firth *et al.*, 1973) and 64 – 66 kg N ha⁻¹ (Peoples *et al.*, 1991), both representing 89 – 90% of the total plant N in 57 to 64 days. In India, Kumar Rao *et al.* (1987) observed that pigeonpea fixed between 68 – 88 kg N ha⁻¹, representing 89 – 90% of the pigeonpea plant N

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accumulation.

These proportions of the legumes' N derived from atmospheric N₂ fixation indicate that the legumes could meet only part of their N requirements from N₂ fixation (Schroder, 1992; Brady and Weil, 2000). Other findings, however, showed that short duration pigeonpea could meet all their N requirements through N₂ fixation (Kumar Rao *et al.*, 1995).

Biological N₂ fixation is affected by several factors, including plant available soil N. High levels of soil N retard N₂ fixation by depressing nodule formation and/or inhibiting nitrogenase activity of mature nodules (Peoples and Herridge 1990; Giller and Wilson, 1991; Giller and Cadisch, 1995). Light energy is required by legumes for photosynthesis to provide the energy the required for the N₂ fixation process. Shading of legume plants under intercropping cropping systems reduces photosynthesis and hence supplies of photosynthates to nodules, which negatively affects N₂ fixation (Midmore 1993; Dakora and Keya, 1997).

Temperature affects the survival of Rhizobia, nodulation and N₂ fixation by legumes (Schroder, 1992). High soil temperatures retard nodulation and/or inhibit the activity of N₂ fixation. Low temperatures, on the other hand, retard the growth rate of plants, and delay nodule formation, leading to decreased rates of N₂ fixation (Day *et al.*, 1978). The optimum temperatures for legume growth and N₂ fixation vary widely between legume species, and this reflects their environmental adaptation (Giller and Wilson, 1991).

Availability of the major plant nutrients including phosphorus (Giller and Wilson, 1991; Giller *et al.*, 1997) and K, Ca and Mg (Giller and Wilson, 1991; Smithson *et al.*, 1993) is important for the N₂ fixation process. Micronutrients and beneficial elements are also important in legume nodulation and N₂ fixation (Giller and Wilson, 1991).

Quantification of the amount of N₂ fixed by grain legumes under maize-legumes cropping systems is essential, especially where N₂ fixation is the main N input to sustain plant growth and production. This information enables the farmer to determine the

amount of N that will have to be supplemented as mineral fertilizer for optimizing yields under such the cropping systems. The total N- difference method is the simplest method for such quantification (Giller and Wilson, 1991). It is least expensive, and can be used where only the facilities for determination of total N are available (McNeill *et al.*, 1996; Peoples and Giller, 1996). The technique estimates the N₂ fixed by a legume as the difference in total N uptake between a legume and a non fixing control plant, both grown on the same soil under identical conditions. Different non-legume crops have been used as reference crop using this technique. Ismail *et al.* (1997) in Egypt, for example, used maize variety hybrid 204 and barley variety hybrid 89 in the estimation of N₂ fixed by cowpea and faba bean, respectively. Kumar Rao and Dart (1987) in India used sorghum when estimating the quantity of N₂ fixed by pigeonpea.

In Muheza District, Tanzania, small scale farmers include different legumes in their maize cropping systems. The maize is grown as intercrop with the legumes, preferably cowpea, pigeonpea or greengram. The maize yields, however, are far below those produced under monocrop with use of mineral N fertilizers, which could be as high as 2.5 ton/ha. This necessitated investigations on the N₂ fixing potential of the legumes, to determine the extent to which the legumes could eventually supply N to a succeeding maize crop. This research was, therefore, carried out to find how much N is fixed by three legumes, cowpea, pigeonpea, and greengram, in a glasshouse and in the field under the existing environmental conditions and crop management practices in Muheza.

Materials and methods

This research was carried out at Mlingano Agricultural Research Institute in Muheza district, Tanzania located at 39° 52'E and 5° 10'S with altitude of 183 m.a.s.l. The area is characterized by a bimodal rainfall pattern with long and short rain seasons. The research included glasshouse followed by field experiment. Before starting the experiments, soil was sampled from the 0 – 20 cm depth, air dried, sieved through 2mm sieve, and analysed for site characterization.

Site characterization

The chemical and physical characteristics of the field experimental soil are presented in Table 1. This soil was a Rhodic Ferralsol (FAO, 1990). The textural class of the soil was clay. According to Landon (1991), the soils' reaction was medium acid, which is suitable for most annual crops. The total N was low, indicating the requisite soil N conditions for the legumes to amply fix N₂, also indicating a need for external N input to increase maize yields. The organic carbon was very low, whereas the C:N ratio indicated presence of a good quality soil organic matter, albeit in small quantities. The site had low available P, which necessitated application of recommended rates of TSP fertilizer for enhancing both the legumes' N₂ fixation and to meet the P

Table 1: Physical and chemical characteristics of soil from the experimental site

Parameter	Value
Clay (%)	52
Silt (%)	6
Sand (%)	42
pH : (H ₂ O)	6.0
pH : (KCl)	4.9
Organic carbon (%)	1.59
Total N (%)	0.12
C:N ratio	13
Bray 1 P (mg kg ⁻¹)	7
CEC (cmol(+)kg ⁻¹)	10.34
Exchangeable Ca (cmol(+)kg ⁻¹)	4.2
Exchangeable Mg (cmol(+)kg ⁻¹)	2.4
Exchangeable K (cmol(+)kg ⁻¹)	0.33
Exchangeable Na (cmol(+)kg ⁻¹)	0.09
Base saturation (%)	68

requirement for the maize crop. The exchangeable Ca and K were low, while exchangeable Mg was high.

Glasshouse pot experiment

The glasshouse pot experiment was carried out for 40 days to assess the potential of the cowpea, pigeonpea and the greengram to nodulate and fix N₂ with the native rhizobia in soils of the study area. Topsoil (0 – 20 cm) was sampled from the field

experimental site, and passed through a 6 mm sieve while fresh. Four kilograms of the sieved sample were placed into 5L capacity plastic pots, and thoroughly mixed with TSP fertilizer at 0.109g P pot⁻¹ (27.2 mg P kg⁻¹ soil). The soil moisture content was raised and maintained at 60% of its field capacity during the 40 days of the experiment. The experiment comprised five treatments, which included the three legumes, maize (Katumani variety) and an unplanted pot as control. The Katumani maize variety was the non-N₂-fixing reference crop. The treatments were replicated four times and arranged in a randomized complete block design (RCBD). Three seeds were planted per pot and thinned to two plants seven days after seedling emergence.

Forty days after planting the above ground plant portions were cut at 1 cm from the soil level using a razor blade. The roots were separated from the soil and the nodules were counted and assessed qualitatively for effectiveness as described by Alexander (1985). The roots and the above ground portions were washed using distilled water and oven dried to constant weight at 60°C. The dried legumes and maize plant materials were weighed and ground to pass through a 0.5 mm sieve, then total N determined following the procedure by Bremner (1965). The amounts of N₂ fixed by the legumes were estimated as the difference between N uptake of the legume crop and that of the Katumani maize plants, the maize being the non-N₂-fixing reference crop (Giller and Wilson, 1991). Uptake is the product of concentration of N in plant material and the dry matter weight of the plants. The difference between the N uptake of a N₂ fixing legume crop and a non-fixing reference crop is a measure of the N₂ fixed by the N₂ fixing legume crop. Total N of the soil from the pots was determined both before planting and after harvest.

Field experiment

A field trial was carried out to estimate quantities of N₂ fixed by the legumes under the maize-legumes intercropping systems. The treatments included the three legumes (cowpea, pigeonpea and greengram) intercropped with maize and as legume monocrops. Within the intercropped plots, rows of maize were alternated with rows of cowpea, pigeonpea, or greengram. The spacing of monocrop maize was 75

cm between rows and 30 cm within the rows, while it was 90 x 30 cm for the intercropped maize, with one maize plant per hill in each case. For the intercropped legumes, spacing was 20 cm within rows for cowpea and pigeonpea (two plants per hill each), and 10 cm within rows for greengram (two plants per hill). For the monocrop legumes, spacing was 50 cm between rows and 20 cm within rows for cowpea and pigeonpea (two plants per hill each), and 50 cm between rows and 10 cm within rows for greengram (two plants per hill). The N₂ fixation assessment was carried out for the legumes in the intercropped plots and those of the legume monocrops. The experimental design was the RCBD, with four replications in 5 m x 5 m plots. Adjacent to the intercropped and monocropped legume treatments, there were 5 m x 3 m plots on which the Katumani maize was grown. The spacing and number of plants per hill of the Katumani maize were the same as those of the adjacent monocropped legumes, whereas for the legume-maize intercropped treatments, plant to plant spacing and number of plants per hill were the same as those of the legumes in adjacent intercropped plots. All agronomic practices including planting time, TSP fertilizer rate and method of application, weeding, and pest control for the Katumani maize were the same as that for the legumes.

At 35 days after planting eight maize plants were sampled by cutting the above ground portions, and eight legume plants were uprooted and the above and below ground portions separated in the laboratory. The nodules on the legume roots were counted, and the N₂ fixed was determined as for the glasshouse pot experiment. All the data were subjected to analysis of variance using the MSTAT programme.

Results and discussion

Glasshouse pot experiment

The performance of the three legumes and the Katumani maize is presented in Table 2. The three legumes formed effective nodules with the native rhizobia of the soil. The number of nodules produced by the cowpea and pigeonpea were not significantly different ($P < 0.05$), but were significantly higher ($P < 0.001$) than those produced by the greengram. A cross section cut of the nodules of the three legumes showed a pinkish colour the intensity of which was

of the order cowpea > pigeonpea > greengram.

The %N contents of cowpea and pigeonpea were not statistically different ($p < 0.05$), but were significantly higher than that of the greengram. The dry matter yield of the cowpea was the highest and was significantly different ($P < 0.001$) from those of the pigeonpea and greengram. The N uptake of the cowpea and pigeonpea were not statistically different, but were significantly higher ($P < 0.001$) than that of the greengram. The quantities of N₂ fixed were statistically different ($P < 0.01$) among the three legumes. The proportions of the legumes' N derived from N₂ fixation by cowpea and pigeonpea were higher ($P < 0.01$) than that of greengram. There was no significant difference between the proportions of N₂ fixed by cowpea and pigeonpea, as was also reflected by nodule numbers. The legumes grown in the pots had no significant effect on the total soil N ($P < 0.05$). The quantities of N₂ fixed by the legumes (Table 3) were positively correlated with the number of nodules, dry matter yield, %N content and the N uptake.

The differences in the nodule numbers, the proportions of plant N derived from atmosphere and the quantities of N₂ fixed by the three legumes were due to the inherent variations among the legume species. Legumes vary in these parameters given the same environmental conditions (Giller *et al.*, 1994). The proportions of the total N uptake derived from N₂ fixation by the three legumes were lower compared to findings reported for cowpea in West Africa (Eaglesham *et al.*, 1982; Dakora *et al.*, 1987; Awonaike *et al.*, 1990), in Brazil (Boddey *et al.*, 1990), in Thailand (Firth *et al.*, 1973; Peoples *et al.*, 1991) and in India (Kumar Rao *et al.*, 1987). Although the differences could be attributed to differences in environmental conditions and varieties of the legume in question, the legumes on the Ferralsol in the current study obtained more N from the soil than what they fixed. This implies that the contribution of the legumes to the soil N will not be substantial taking into consideration that much of the legumes' N is removed through grain harvest and little is returned to the soil through residues.

The lack of increase in total N of the soils in the pots implies that there was no contribution of N to the soil

Table 2: Performance of legumes and Katumani maize in the pot experiment

Treatment	Total soil N (%)	Nodule number pot ⁻¹	Dry matter (g pot ⁻¹)	N tissue content, (%)	N uptake, ...g pot ⁻¹ ...	N ₂ fixed, (%)	Proportion fixed (Patm)
Cowpea	0.16	53a	6.900a	3.243a	0.22a	0.10a	45%a
Pigeonpea	0.17	50a	5.775b	3.285a	0.19a	0.07b	37%a
Greengram	0.16	23b	5.025b	2.960b	0.15b	0.03c	20%b
F-test	NS	***	***	*	***	**	**
CV %		14.2	5.5	4.1	7.95	21.5	17.7
Katumani maize	0.16	-	11.50	11.50	0.12	-	-
Control	0.17	-	-	-	-	-	-

Means within a column followed by the same letter are not statistically different ($P < 0.05$) according to Duncan's New Multiple Range Test *, ** and *** indicate statistical significance at $P < 0.05$, 0.01 and 0.001, respectively. NS indicates non significance at $p < 0.05$.

Table 3: Correlation between the quantities of N₂ fixed and nodule number, legume dry matter yield, %N and N uptake under the glasshouse experiment

	Nodule number pot ⁻¹	Dry matter, g pot ⁻¹	N content, %	N uptake, g pot ⁻¹
N ₂ fixed	0.790	0.627	0.644	0.931
Significance	**	*	*	***
R ₂	0.624	0.393	0.415	0.867

*, ** and *** indicate statistical significance at $P < 0.05$, 0.01 and 0.001, respectively.

by the potted plants, during the time they were growing. This indicates insignificant, or absence of, N release from the roots and/or nodules of the legumes to the soil during the growth period. The legumes were harvested when cowpea and greengram were at flowering stage. At this time, the nodules were at the peak of N₂ fixation, and nodules had not senesced to release the N they contain. Kumar Rao and Dart (1987) observed that nodule senescence in pigeonpea started 30 days after planting. It has further been reported by Chapman and Meyers (1987), Kumar Rao and Dart (1987) and Bergersen *et al.*, (1989) that much of the legumes' contribution to the soil N was through above ground residues, with little from roots and nodules. The lack of legumes' leaf fall, decaying of nodules and roots, during the growth period, accounts for the observed lack of change in total soil N in the pots in the present study.

Field Experiment

The performance of the three legumes in the field experiment is presented in Table 4. The three legumes, like was the case under the glasshouse pot experiment, formed effective nodules with the native rhizobia of the soil. The pigeonpea produced significantly lower numbers of nodules than those of the cowpea. The numbers of nodules of the cowpea and greengram, and similarly those of pigeonpea and greengram, were not significantly different ($p < 0.05$). The number of nodules of the same legume under intercropped and monocropped legumes were not significantly different ($p < 0.05$). A cross section cut of the nodules of the three legumes showed a pinkish colour the intensity of which was of the same trend as that under glasshouse pot experiment (i.e cowpea > pigeonpea > greengram) indicating differences in the effectiveness of N₂ fixation among the legumes, with cowpea fixing the highest amounts.

Within the intercropped plots, the quantity of N₂ fixed by greengram was significantly ($p < 0.05$) higher than that of the pigeonpea. Under monocropped legumes, the quantities of N₂ fixed by the cowpea and greengram were not significantly different, but were significantly ($p < 0.05$) higher than that of pigeonpea. More N₂ was fixed by the same legume under monocropping than under intercropping.

The proportion of legumes' N derived from N₂ fixation (Patm) of the same legume was not statistically ($p < 0.05$) affected by the cropping system. Within the intercropped legumes, the

proportion of pigeonpea N derived from fixation was significantly lower than that of the cowpea and greengram, while those of the cowpea and greengram were statistically the same. Within the monocropped legumes the proportions were not significantly different. The proportions were less than 50%, indicating that the legumes absorbed more N from the soil than the amounts of N₂ fixed, in both cropping systems.

Table 4: Quantities of N₂ fixed by the legumes and the proportion of legumes' N derived from N₂ fixation (*Patm*) under monocrop and intercrop systems

Treatment	Nodule number	N ₂ fixed,	Proportion fixed
	per plant	kg ha ⁻¹	(<i>Patm</i>)
Maize + cowpea	21a	15.70bc	37%a
Maize + pigeonpea	13b	3.60c	19%b
Maize + greengram	16ab	24.04b	38%a
Cowpea monocrop	22a	38.45a	40%a
Pigeon pea monocrop	12b	21.43b	25%ab
Greengram monocrop	18ab	49.13a	39%a
F- test	*	***	*
CV %	17.7	18.7	23.3

Means within the same column followed by the same letter are not statistically different ($P < 0.05$) according to Duncan's New Multiple Range Test

* and *** indicate statistical significance at $P < 0.05$ and 0.001 , respectively.

The differences in the nodule numbers, the proportions of legumes' N derived from atmosphere and the quantities of N₂ fixed by the three legumes, as also in the case of the glasshouse pot experiment, is attributed to the inherent variations between the legume species. The higher quantities of N₂ fixed by an individual legume when monocropped than when intercropped could partly be attributed to the differences in plant population density between the cropping systems. The population density of monocropped legumes was about twice that under intercropping. The higher population density influenced the quantities of the N₂ fixed when calculated on a per-hectare basis, but on a per plant basis the fixation rates would be similar.

The absence of statistical differences in the proportions of the legumes' N derived from N₂ fixation between monocropped and intercropped legume shows that the presence of the maize crop had no adverse shading effects on the N₂ fixation by the legumes. The legumes and the maize were sown at the same time. The legumes, being faster growers, attained a high enough height that was not affected by maize shading, and therefore maize did not adversely affect the process of photosynthesis, hence the N₂ fixation, by the legumes. These observations are contrary to those of Dakora and Keya (1997) and Van Kessel and Roskoski (1988), who reported the adverse effects of maize shading on intercropped cowpea. Differential cowpea varietal characteristics between the present and the cited studies, for example creeping versus upright varieties, could explain this difference.

Conclusion

The three legumes tested presently nodulated and fixed some N₂ with the native soil rhizobia, indicating the possibility of the legumes to grow well on the Ferralsol under the existing environment and management practices in Muheza. However, they were not efficient in N₂ fixation, as more than 50% of their N requirements were obtained from the soil. The quantities of N₂ fixed by the legumes in the field under the current management practices were low to cause a significant effect on the soil N replenishment. Based on the fact that much of the N₂ fixed is harvested in the grain, the contribution of the residues to the soil N is minimal. On those Ferralsols and management practices, use of mineral N fertilizer to the maize crop either grown as intercrop or in rotation (with the legume) should be adopted for maximizing maize yields.

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