

## Nitrogen management and biological nitrogen fixation in sorghum/pigeonpea intercropping on Alfisols of the semi-arid tropics

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### Abstract

Pigeonpea (*Cajanus cajan* L. Millsp) and sorghum (*Sorghum bicolor* L. Moench), are grown as intercrops on soils typified by low nitrogen (N), particularly Alfisols and Vertisols of the semi-arid tropical areas of India and East Africa. Proper management of fertilizer N in sorghum/pigeonpea intercropping systems is essential to enhancing biological nitrogen fixing ability of pigeonpea, and to increasing grain yields of sorghum.

In a 3-year study, we examined the effect of time and rates of fertilizer N application on biological N fixation (BNF) and fertilizer N recovery by pigeonpea and sorghum grown as a sole crop, and as an intercrop on an Alfisol in India. In 1993, N (50 kg N ha<sup>-1</sup>) was all applied at planting (basal) or at 40 days after sowing (delayed). During 1994-1995, four N rates 0, 25, 50, and 100 kg ha<sup>-1</sup> were applied (i) 100% at planting (basal) or (ii) 67% at 40 days, and 33% at 60 days after sowing (delayed).

Delaying N fertilization until 40 days after sowing (DAS), rather than applying at sowing significantly increased ( $p < 0.001$ ) total dry matter, grain yield (GY) and total N content (TNC) of the sorghum, but not of pigeonpea. Highest GY and TNC was recorded at an N rate of 50 kg ha<sup>-1</sup> with sole crop sorghum, and at 25 kg ha<sup>-1</sup> for intercrop sorghum. Delaying N fertilization to sorghum significantly ( $p < 0.001$ ) increased <sup>15</sup>N recovery in the shoot from 15 to 32% in sole crop, and 10 to 32% in intercrop. Similarly, there was a significant ( $p < 0.001$ ) increase in N recovery (calculated without <sup>15</sup>N, but by the difference between the nonfertilized N and fertilized plots) from 43-59% in sole crop and from 28-71% in intercrop sorghum. Pigeonpea fixed between 120-170 kg ha<sup>-1</sup> of atmospheric N throughout the cropping season. Nitrogen application to the sorghum row, but not to the pigeonpea, enhanced BNF by pigeonpea and maximized fertilizer recovery by sorghum in a sorghum/pigeonpea intercropping.

### Introduction

Pigeonpea and sorghum are important food grain crops in the semi-arid tropical areas in India, and Eastern Africa. Both crops are usually grown as intercrops on soils of low N content, and in dry environments where it is difficult to grow other food grains. Pigeonpea is able to meet a proportion of its own N requirement through biological N fixation (Kumar Rao et al., 1987). In addition, pigeonpea can increase the available phosphorus pool of cropping systems in which it is grown by accessing iron-bound P to a greater extent than other crop species in Alfisols (Ae et al., 1990). Thus, the inclusion of pigeonpea in intercropping systems helps to minimize competition for N and P with the cereal component.

Nitrogen management in cereal/legume intercropping has received little attention in the semi-arid tropics (SAT) probably because farmers

rarely apply N in intercropping systems. Reasons for this include unpredictable rainfall leading to low N use efficiencies, and the detrimental effect of fertilizer N on BNF by the legume. Naraian et al. (1980) and Tobita et al. (1994) reported that a moderate dose of 25-50 kg N ha<sup>-1</sup> was optimum for a sorghum/pigeonpea on an Alfisol in the SAT in India.

During a 3-year study, we investigated the rate and time of N fertilization on BNF by pigeonpea, and grain yield and fertilizer recovery by sorghum in sorghum/pigeonpea intercropping. The study also aimed to understand the extent to which pigeonpea grown in association with sorghum, instead of in pure stands, can help reduce the fertilizer N needs of sorghum on Alfisol typified by low N content.

### Materials and methods

Two field experiments were carried out on a

medium-deep Alfisol (Ferric Luvisols; Udic Rhodustalf) at ICRISAT Asia Center, Patancheru (17°38'N, 78°21'E) India, during the 1993-1995 rainy seasons.

### Experiment 1

#### Crop cultivation and management

The soil had an average available moisture storage capacity of about 100 mm, pH (H<sub>2</sub>O) 6.2, mineral N concentration of 40 mg kg<sup>-1</sup>, and available P concentration (Bray II) 10 mg kg<sup>-1</sup>. The experiment was a 4 × 3 factorial, set out in a split-plot design with three replicates. Main treatments were four cropping systems, namely (i) sole crop sorghum (ii) sole crop pigeonpea (iii) intercrop sorghum/pigeonpea and (iv) a bare fallow treatment (to serve as a control plot for monitoring N dynamics in the soil). The sub-plot treatments were (i) no N fertilizer applied (N<sub>0</sub>), (ii) a rate of 50 kg N ha<sup>-1</sup> all applied at planting (basal) and (iii) 50 kg N ha<sup>-1</sup> applied at 40 days after sowing (delayed). All of the N fertilizer was applied by opening the furrows and banding at 5-8 cm depth. The N source was urea. In the intercropping treatment, N was applied only to sorghum rows. Phosphorus was basally broadcast on all the experimental plots at the rate of 20 kg ha<sup>-1</sup> as single superphosphate, and mixed in the surface 5 cm of soil.

Medium-duration pigeonpea [*Cajanus cajan* (L) Moench] cultivar ICP 1-6, and grain sorghum [*Sorghum bicolor* (L) Moench] hybrid CSH 5, were sown either as a sole crop or as intercrop. The spacings were 60 × 30 cm for sole-cropped pigeonpea, 60 × 15 cm for sole-cropped sorghum, and 60 × 10 cm for both crops in the intercropping, which had a 2:1 (sorghum:pigeonpea) row proportion. This arrangement gave an identical number of plants on an area basis for each component in the sole and intercropping systems. Microplots measuring 90 × 90 cm for sole-cropped pigeonpea, 45 × 90 cm for sole-cropped sorghum, 50 × 90 cm for intercrop, and 45 × 60 cm for the bare fallow plots, were demarcated at the center of each plot with iron pegs. The <sup>15</sup>N-labelled urea (30 atom% excess, Shoko Co. Ltd., Japan) was applied by banding to crop rows in all microplots, at the same time and rate as for the surrounding non-labelled urea.

#### Crop harvest

Shoot samples were taken from a 0.8 m<sup>2</sup> area from each plot at 28, 40, and 78 days after sowing (DAS). At maturity (110 days for sorghum and 210 days for pigeonpea), an area of 4 rows by 4 m (9.6 m<sup>2</sup>) was

harvested to estimate total dry weight and grain yields. Plant samples were separated into leaves, stems and reproductive organs depending on the growth stage, dried at 70°C for 48 hr and weighed.

#### Chemical analysis

Oven-dried shoot samples were ground to pass a 0.4-mm screen for chemical analysis. Nitrogen in plant parts was estimated by the indophenol color formation method (Chaykin, 1969) after micro-Kjeldhal digestion.

#### <sup>15</sup>N analysis and <sup>15</sup>N recovery by crops

Four plants of each species were sampled at final harvest from the <sup>15</sup>N-treated microplots for the <sup>15</sup>N enrichment analysis. Another set of 4 plants were sampled outside the microplot for <sup>15</sup>N natural abundance (δ<sup>15</sup>N) analysis. The natural <sup>15</sup>N abundance in grains of pigeonpea and sorghum was estimated with a mass spectrometer (Finnigan Mat 251). The <sup>15</sup>N enrichment was estimated with an <sup>15</sup>N emission spectrometer (Jasco N-150).

The fractional contribution of N derived from fertilizer (%N<sub>dff</sub>) and the <sup>15</sup>N recovery by crops were calculated as:

$$\%N_{dff} = (1 - \text{atom } \% \text{ } ^{15}\text{N excess in pigeonpea or sorghum} / \text{atom } \% \text{ } ^{15}\text{N excess in fertilizer}) \times 100$$

The fertilizer N recovery (FRN) was estimated as:

$$\text{FRN} = N_{dff} / N_{\text{applied}} \times 100,$$

where the amount of N derived from fertilizer (N<sub>dff</sub>) was calculated as the N<sub>total</sub> (kg ha<sup>-1</sup>) × %N<sub>dff</sub>.

#### Estimation of nitrogen fixation by pigeonpea

The fractional contribution of fixed N derived from air (%N<sub>dfa</sub>) by pigeonpea in this study, was estimated using a delta <sup>15</sup>N<sub>a</sub> value of -2.1‰ for a medium-duration pigeonpea (ICP 1-6) grown solely on atmospheric N<sub>2</sub> (Tobita et al., 1994). The amount of N fixed by pigeonpea was calculated as:

$$N_{dfa} = N_{\text{total}} \times \%N_{dfa}$$

where N<sub>total</sub> is the total N accumulated by pigeonpea (kg ha<sup>-1</sup>).

### Experiment 2

The experimental site was 20 m from Experiment 1. The physical and chemical characteristics of this soils were similar except that N content of the soil was lower at this site. The sorghum and pigeonpea cultivars used were the same as in Experiment 1.

The experiment was a 4 × 4 × 2 factorial, set out in a split-split plot design with three replicates. Main treatments were four cropping systems as described in Experiment 1. The sub-treatments consisted of four N rates, 0, 25, 50, and 100 kg ha<sup>-1</sup>, applied (i) 100% at planting (basal) or (ii) 67% at 40 days (25 in 1995), and 33% at 60 days (50 in 1995) after

planting (delayed). The application method and source of N fertilizer, shoot sampling and estimation of total dry weight was done as described in Experiment 1.

#### Estimation of Biological nitrogen fixation

Xylem exudates were collected with a Pasteur syringe at 25, 37 and 65 DAS after cutting plants at the base and placing sleeves of black silicon rubber tubing over the exposed root stumps. The proportion of N derived from air (%Nd<sub>fa</sub>) by pigeonpea was estimated by the relative ureide abundance method (Peoples et al., 1989). The ureides (allantoin and allantoic acid) were measured together as phenylhydrazone derivative of glyoxylate (Young and Conway, 1942). Nitrate was measured by the salicylic acid technique (Cataldo et al., 1975). The  $\alpha$  amino-N contents of exudates were determined colorimetrically with ninhydrin (Yemm and Cocking, 1955).

#### Data analysis

All data were analysed using GENSTAT 5 Release 3.2 (Copyright 1995, Lawes Agricultural Trust Rothamsted Experimental Station), following standard analysis of variance procedures (Gomez and Gomez, 1984).

## Results

### Experiment 1

#### Fertilizer nitrogen recovery (FNR)

By including a nonfertilized control, we could compare the conventional difference method of determining fertilizer N uptake, and the direct labelling (<sup>15</sup>N) method (Fig. 1). The N recovery by the conventional difference method was higher than by the labelling method. Delaying N fertilization to sorghum for 40 days significantly ( $p < 0.001$ ) increased <sup>15</sup>N recovery in shoot from 15 to 32% in sole crop, and 10 to 32% in intercrop. Similarly,

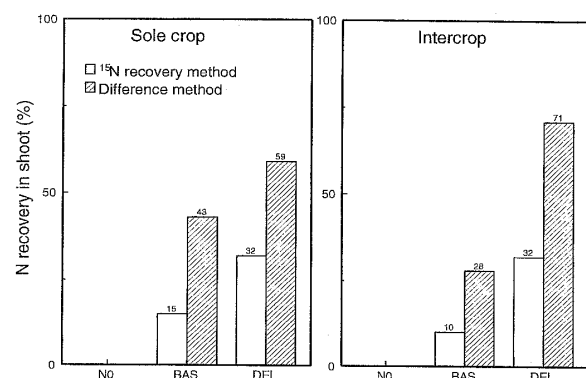


Fig. 1. Fertilizer N recovery in shoot of sorghum grown as a sole crop and as an intercrop with pigeonpea (Experiment 1).

there was a significant ( $p < 0.001$ ) increase in N recovery (by the difference method) from 43 to 59% in sole crop and from 28 to 71% in intercrop sorghum.

#### Nitrogen derived from fertilizer (Nd<sub>ff</sub>), soil (Nd<sub>fs</sub>) and air (Nd<sub>fa</sub>)

Delaying N fertilization to sorghum by 40 days resulted in a two-fold increase of %Nd<sub>ff</sub> (Table 1). Soil N accounted for 84-100% of the total N in sorghum, and 26-44% in pigeonpea. Contrary to the %Nd<sub>ff</sub> data, the %Nd<sub>fs</sub> in sorghum was significantly higher when N fertilizer was applied at planting than at 40 DAS.

Biological N<sub>2</sub> fixation by pigeonpea was estimated using the sorghum plants as a reference (Tobita et al., 1994). The proportion and amount of Nd<sub>fa</sub> by pigeonpea, calculated from  $\delta^{15}\text{N}$  values in grain of sorghum ranged from +2.3 to +4.1‰, and -0.5 to 1.6‰ in pigeonpea. The % Nd<sub>fa</sub> values ranged from 57-74%, but were not affected by timing of N fertilization (Table 1). Pigeonpea fixed 120-170 kg N ha<sup>-1</sup> of atmospheric N throughout the cropping season. The amount was slightly higher when pigeonpea was grown in association with sorghum than when grown as a sole crop.

### Experiment 2

#### Total Dry Matter Yield (TDMY) and N accumulation

A 25 kg N ha<sup>-1</sup> applied as "delayed N" gave the highest grain yield of intercropped sorghum compared to 50-100 kg N ha<sup>-1</sup> for sole-cropped sorghum (Fig. 2). Nitrogen accumulation by sorghum (sole and intercropped) was higher in the delayed than in the basal treatments.

#### Estimation of nitrogen derived from atmosphere (Nd<sub>fa</sub>)

In pigeonpea, fixed N is mainly translocated as ureide-N, hence the proportion of ureide-N to the other nitrogenous solutes (nitrate and  $\alpha$ -amino acids) in xylem exudates is a good indicator of % Nd<sub>fa</sub>. The %Nd<sub>fa</sub> by sole and intercropped pigeonpea before the first dose of delayed fertilization (25 DAS), second dose application (37 days) and 65 days were estimated.

There was a marked decrease in %Nd<sub>fa</sub> in the sole crop but not in intercropped pigeonpea as fertilizer rates increased (Fig. 3). Intercropped pigeonpea derived about 80% of the N in plant from air compared to 60% by sole crop at 65 DAS.

## Discussion

The 3 year study clearly demonstrates that there is a high potential for increasing grain yield of sorghum

Table 1. Fractional contribution of plant N derived from fertilizer (%Ndff), from soil (%Ndfs), and from atmosphere (%Ndfa) of sorghum and pigeonpea in sole crop and intercropping systems at harvest

Cropping System	Sorghum						Pigeonpea								
	% Ndff			% Ndfs			% Ndff			% Ndfs			% Ndfa		
	N0 <sup>a</sup>	Bas <sup>b</sup>	Del <sup>c</sup>	N0	Bas	Del	N0	Bas	Del	N0	Bas	Del	N0	Bas	Del
Sole crop	-	7.7	16.0	100	92.3	84.0	-	2.84	3.24	26.3	44.7	38.7	73.7	52.5	58.1
Intercrop	-	5.8	12.7	100	94.2	87.3	-	0.94	0.44	45.4	36.4	36.2	54.6	62.6	63.4
<b>Means</b>	-	<b>6.8</b>	<b>14.4</b>	<b>100</b>	<b>93.2</b>	<b>85.7</b>	-	<b>1.89</b>	<b>1.84</b>	<b>35.8</b>	<b>40.5</b>	<b>37.4</b>	<b>66.1</b>	<b>57.5</b>	<b>60.7</b>
SE (±)															
NT <sup>d</sup>		1.32			1.33			0.352			1.52			1.65	
CS <sup>e</sup>		0.27			0.28			0.106			4.96			4.93	
NT × CS		1.56			1.56			0.420			5.26			5.29	
P value															
NT		<0.001			<0.001			0.008			0.18			0.090	
CS		0.049			0.047			0.009			0.73			0.880	
NT × CS		0.060			0.090			0.051			0.03			0.003	

<sup>a</sup>N0 : No N fertilizer.

<sup>b</sup>Bas : N application of 50 kg N ha<sup>-1</sup> at planting (basal).

<sup>c</sup>Del : N application of 50 kg N ha<sup>-1</sup> at 40 days after sowing (delayed).

<sup>d</sup>NT : Timing of N application.

<sup>e</sup>CS : Cropping system.

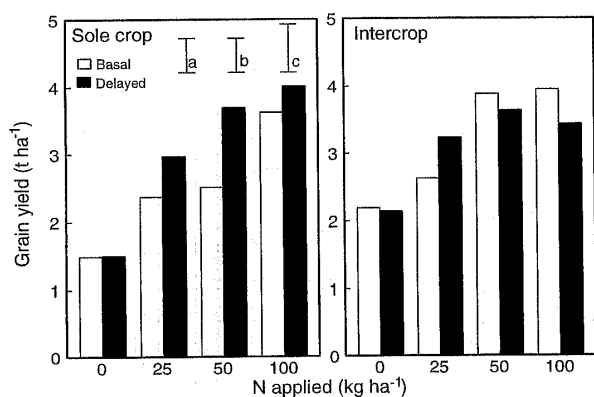


Fig. 2. Mean grain yields of sole crop and intercrop sorghum in response to timing and rates of N application during a 2-year period. Bars indicate LSD ( $p < 0.05$ ) values when comparing (a) cropping systems × time (b) cropping systems × fertilizer, and (c) cropping systems × time × fertilizer.

and enhancing BNF by pigeonpea through intercropping without necessarily increasing the quantity of fertilizer N use. In sorghum/pigeonpea intercropping, N application to the sorghum, but not to pigeonpea was effective in enhancing BNF by pigeonpea and maximizing fertilizer recovery by sorghum. Delaying fertilizer N application for 25 DAS resulted in a higher grain yield and total N amount than basal application. When sorghum is intercropped with pigeonpea, moderate N fertilization of 25 kg ha<sup>-1</sup> was optimum for grain yield and total N accumulation, compared to 50 kg N ha<sup>-1</sup> when grown as a sole crop (Fig. 2). Similar results were reported by Naraian et al. (1980), and Tobita et al. (1994) who recommended a moderate dose of 25-30 kg ha<sup>-1</sup> for sorghum/pigeonpea

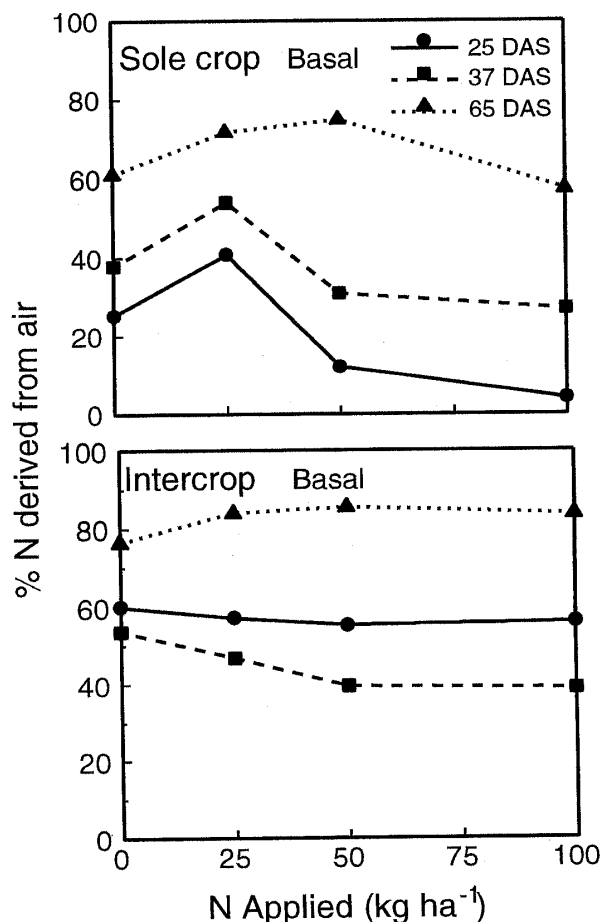


Fig. 3. Proportion of N derived from air (%Ndfa) by pigeonpea estimated by the relative ureide abundance method.

intercrops on an Alfisol in the SAT in India. The results indicate that pigeonpea grown in association with sorghum, instead of pure stands, can help

reduce the fertilizer N needs of sorghum in soils typified by low N.

In the SAT where intercropping is commonly practised, "basal N" is preferred to "delayed N" application by farmers because it is considered that the crops require N for their early growth. As reported by Adu-Gyamfi et al. (1996), however, the mineral N content in soil due to the long dry fallow period, in most cases, is adequate for early establishment of the component sorghum because of less competition for soil N by the associated pigeonpea.

#### *Fertilizer nitrogen recovery in plant*

Fertilizer N recovery (FNR) was calculated for crop uptake with or without the use of labelled  $^{15}\text{N}$  has been extensively reviewed (Jansson and Persson 1982; Jenkinson et al. 1985; Strong, 1995). Where the two FNR methods have been compared, recovery is usually higher for the difference method than for the  $^{15}\text{N}$  direct method. This is due to the "added N interaction" described by Jenkinson et al. (1985), which is also commented on in detail by Jansson and Persson (1982). However, few studies have compared the FNR of sorghum and pigeonpea in intercropping systems.

Using the difference method, FNR in the shoot by sole crop sorghum was higher for the delayed (59%) than for the basal treatment (43%). When sorghum was grown in association with pigeonpea, 71% of fertilizer N was recovered when fertilization was delayed compared to 28% for basal (Experiment 1). The plant  $^{15}\text{N}$  recovery method showed a similar trend to the difference method, although the values were lower (Fig. 1). Similar results were reported on an Alfisol by Adu-Gyamfi et al. (1996), Moraghan et al. (1984), and Tobita et al. (1994). Thus, FNR by sorghum was enhanced when (i) intercropped with pigeonpea compared to when grown as a sole crop and (ii) N application was split-delayed compared to 100% application at planting.

#### *Biological nitrogen fixation*

The amount of N fixed by pigeonpea estimated by the  $^{15}\text{N}$  natural abundance method, during the entire growth period of 210 days, was 120~170 kg N ha<sup>-1</sup>. The amount was higher by intercropped than by sole-cropped pigeonpea (Katayama et al., 1995; Tobita et al., 1994).

The  $^{15}\text{N}$  natural abundance and the  $^{15}\text{N}$  enrichment methods for estimating BNF by legumes are costly because they involve the use of mass and emission spectrophotometers, usually not available in laboratories of developing countries. The relative

abundance of ureides (the proportion of ureides,  $\alpha$ -amino acids and  $\text{NO}_3\text{-N}$  concentration in xylem sap) has been used to estimate %Ndfa by soybean and other ureide producing leguminous crops. Field application of this method to estimate %Ndfa by intercropped-pigeonpea does not exist in literature, although Peoples et al. (1989) estimated %Ndfa of pot-cultured young pigeonpea plants.

The %Ndfa estimated by the relative ureide abundance method supported the findings that BNF was enhanced by intercropping. The only drawback of using this method is that the amount of N derived from air at final sampling could not be estimated because xylem exudates could not be extracted from pigeonpea after flowering. The woody nature of stem of pigeonpea makes it difficult to extract xylem exudates irrespective of the soil moisture. Thus, the estimation of Ndfa by the natural abundance method is more desirable to quantify the total contribution of BNF by pigeonpea during the entire cropping period. Nevertheless, values of the %Ndfa by sole crop and intercrop pigeonpea at flowering compare favourably with reported values for sole-cropped and intercropped pigeonpea (Adu-Gyamfi et al., 1996; Katayama et al., 1995; Tobita et al., 1994).

#### **Conclusions**

The 3 year study clearly demonstrated that there is a high potential for increasing grain yield of sorghum and enhancing BNF by pigeonpea through intercropping and proper timing of N without necessarily increasing the quantity of fertilizer N use. In sorghum/pigeonpea intercropping, N application to the sorghum, but not to the pigeonpea, enhanced BNF by pigeonpea and maximum fertilizer recovery by sorghum. In semi-arid regions, heavy rains that occur at the onset of the rainy season may lead to a substantial leaching of accumulated soil mineral N during the long dry fallow period. Since the crop need for N, and the capacity for N uptake are small, delaying fertilizer application for 4-5 weeks can result in increased sorghum grain and dry matter yields, and enhanced BNF by pigeonpea.

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