# Comparison of the Requirements and Utilization of Nitrogen by Genotypes of Sorghum (*Sorghum bicolor* (L.) Moench), and Nodulating and Non-Nodulating Groundnut (*Arachis hypogaea* L.)

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# Comparison of the Requirements and Utilization of Nitrogen by Genotypes of Sorghum (*Sorghum bicolor* (L.) Moench), and Nodulating and Non-Nodulating Groundnut (*Arachis hypogaea* L.)

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ABSTRACT

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Nitrogen requirements and utilization of mineral nitrogen (N) by sorghum and groundnut were compared. At the maximum N use level, sorghum genotypes showed greater N use efficiency (120 kg biomass/kg N harvested) than groundnut genotypes (36 kg biomass/kg N harvested). Using a non-nodulating groundnut genotype (Non-nod) or sorghum as controls for soil N uptake, the amounts of $N_2$ fixed by the nodulated groundnut genotypes were estimated to be 183–190 kg N/ha. Nitrogen fertilization increased harvest index and percentage N translocated to seeds in sorghum genotypes, but decreased harvest index and had variable effects on percentage N translocated to seed in groundnut genotypes. Leaf nitrate reductase activity (NRA) and nitrate content in the leaves of two sorghum genotypes, one nodulating, and 'Non-nod' groundnut genotypes were also compared. The concentration of nitrate was lower in sorghum than in groundnut leaves, but NRA was higher in sorghum. It is suggested that either NRA in the groundnut leaves has relatively lower affinity for the substrate (higher *Km*, the Michaelis-Menton constant) or higher nitrate is required for the induction of nitrate reductase in groundnut than in sorghum. This implies that groundnut is a poor utilizer of fertilizer nitrogen.

## INTRODUCTION

Sorghum and nodulated groundnut differ in their methods of nitrogen (N) acquisition. Sorghum depends primarily on inorganic forms of N derived from mineralization of soil organic matter or fertilizers, and on N-deficient soils responds strongly to additions of fertilizer N (reviewed by Tandon and Kanwar, 1984). In contrast, nodulated groundnut depends primarily on symbioti-

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cally fixed N, and even on N-deficient soils responses to fertilizer N have been small and erratic (Acuna and Sanchez, 1968; Chesney, 1975; Gutstein, 1978; Balasubramanian et al., 1980; Rami Reddy et al., 1982). However, recent studies indicate that at very high yield levels, the N requirement of nodulated groundnut cannot be met from symbiotic $N_2$ fixation alone (Williams, 1979). Hence a nodulated groundnut should respond to fertilizer N and non-nodulating groundnut (Nambiar et al., 1982) would be expected to behave like sorghum and respond strongly to application of fertilizer N on N-deficient soils.

In order to understand the requirement and utilization of N by sorghum, nodulated and non-nodulated groundnut, an N fertilization study was undertaken using an improved hybrid and a traditional variety of sorghum, and two nodulating and one non-nodulating genotypes of groundnut. The biological fixation of $N_2$ by nodulated groundnut could be measured by the differential uptake method (Ham, 1978) using sorghum or non-nodulating groundnut as controls. The relationship between nitrate reductase (EC 1.6.6.1, NADH: nitrate oxidoreductase; NR) activity and leaf nitrate content was also examined.

## MATERIALS AND METHODS

Two experiments were conducted in Alfisol fields at the ICRISAT Center, near Hyderabad, India. A basal application of 17 kg P/ha was applied to all plots. The nitrate content (analysed by the method of Bremner, 1965) in these soils before starting the experiments was 1.7 ($\pm$0.07) ppm. This indicates very low available soil N. Both experiments were conducted during postrainy seasons and the crops were irrigated at intervals of 7–10 days to return the soils to field capacity. The plots were not inoculated since there were abundant native *Rhizobium* populations that nodulate groundnut ($10^2$–$10^4$ rhizobia/g dry soil).

### *Experiment 1: Comparative yield responses*

The first experiment was conducted during the 1983–84 postrainy season. The groundnut genotypes tested were Robut 33-1 and J 11 (nodulating) and a non-nodulating genotype (Non-nod). The sorghum genotypes were CSH 8R (an improved hybrid) and M 35-1 (a traditional variety). All crops were fertilized with five levels of nitrogen (0, 50, 100, 150, and 200 kg N/ha as urea). The crops were sown on raised beds, 1.5 m wide, with four rows of groundnut and two rows of sorghum per bed. Plant spacing within rows for groundnut was 10 cm (266 666 plants/ha), and for sorghum 20 cm (66 666 plants/ha). The crop was sown on 30 November. A factorial combination of treatments was laid out in a randomised block design with three replications. The urea was applied in six equal applications 11, 19, 28, 38, 50, and 60 days after sowing (DAS). Although nitrate is a better source for nitrate reductase induction,

urea was chosen as the nitrogen source (i) to avoid the effect of cations associated with nitrate and (ii) because urea is the most commonly used nitrogenous fertilizer and is hydrolysed and nitrified within 6–8 days under the above soil conditions (K.L. Sahrawat, ICRISAT, personal commun., 1983). The fertilizer required per plot was dissolved in 5 l of water and applied in small furrows (3–4 cm deep) opened on the sides of the ridges, which were then closed immediately. Sorghum genotypes were harvested 95 DAS, the groundnut genotype J 11 at 118 DAS, and the Non-nod and Robut 33-1 groundnut genotypes at 138 DAS. The final harvest area in each plot was 12 $m^2$.

*Experiment 2: Comparison of leaf nitrate reductase activity (NRA) and nitrate content*

This experiment was conducted during the 1984–85 postrainy season. The groundnut genotypes tested were Robut 33-1 and Non-nod. Sorghum genotypes were M 35-1 and CSH 8R. The crops were fertilized with 0, 67, 133, and 200 kg N/ha. The design of the experiment was the same as in experiment 1. The crop was sown on ridges 60 cm apart and the plot size was five rows of 5 m. Plant spacing within rows for groundnut was 10 cm (167 000 plants/ha), and for sorghum 15 cm (111 389 plants/ha). Urea was applied in four equal applications 14, 35, 60, and 80 DAS as described for the first experiment. Three randomly selected plants were sampled from each plot for leaf NRA and nitrate estimations. The total leaves from each branch were counted, separated, and divided into three equal portions representing top, middle, and lower leaves at each harvest. During the later stages of plant growth, senescent leaves from the lower parts of the plants were excluded from the estimations. Discs of 8 mm diameter were cut from the leaves and used for measurement of NRA. The rest of the leaves were dried at 60°C for 48 h and total weights were recorded. Leaf NRA and leaf nitrate of each portion, i.e. top, middle, and lower, were calculated and added up to estimate leaf NRA/plant and nitrate content/plant respectively.

*Leaf nitrate reductase activity*

NRA was measured by the method of Jaworski (1971). From each leaf one 8 mm diameter disc was cut and incubated in sodium phosphate buffer (0.1 *M* sodium phosphate, pH 7.5, 5% *n*-propanol, and 0.02 *M* $KNO_3$, approximately 2 ml buffer/leaf disc) in 250-ml beakers. The discs were subjected to vacuum infiltration for 2 min at $1 \times 10^3$ pascals, and incubated at 30°C for 30 min. The incubation mixture was filtered immediately through a nitrate-free Whatmann No. 1 filter paper and nitrite content was estimated using Szechrome NIT as described by Hunter et al. (1982).

*Leaf nitrate content*

The separated leaves were dried at 60°C for 48 h and finely ground to pass through a 1-mm sieve. Either 0.1 g of groundnut or 0.3 g of sorghum leaf powder were mixed with nitrate-free, activated charcoal in a ratio of 1:2, and the nitrate was extracted into 20 ml of distilled water and was estimated using Szechrome NAS (Hunter et al., 1982).

*Total nitrogen*

The nitrogen content in the plant tissue was determined as described by Technicon (Autoanalyser II, Industrial method NC 218-72 A, 11). Haulm and stalk were chopped in a knife-mill and 400–500 g of this was ground to pass through a 1-mm sieve. Eighty mg of the powder was digested with 4 ml of selenium:sulphuric acid mixture, diluted to 75 ml with distilled water, and 0.33 ml was used for estimation based on the Berthelot reaction (for details refer to Industrial method No. 218-72 A, 11, Technicon Industrial Systems, Tarrytown, NY 10591, U.S.A.).

## RESULTS

All results were tested for significance at the 5% level of probability and only significant results are discussed.

*Dry matter yield*

N application increased grain and shoot yield of sorghum and Non-nod groundnut genotypes (Tables 1 and 2). Haulm yields of the nodulated genotype Robut 33-1 were also increased at the highest N level. Higher grain yield was obtained in sorghum hybrid CSH 8R than in M 35-1 and the yield levels of both sorghum genotypes reached a plateau between 120 and 150 kg N/ha. Nitrogen fertilizer did not significantly influence the kernel yield of groundnut genotype Robut 33-1, but high N reduced the kernel yield of genotype J 11 (Table 2). Stalk yield increased in both sorghum genotypes, but genotype M 35-1 produced more dry matter than CSH 8R at higher N levels. Both kernel and haulm yields of the Non-nod groundnut genotype were lower than those of the nodulating groundnut genotypes, Robut 33-1 or J 11, and seed and stalk yields of sorghum. Increased N application increased harvest index in sorghum (from 41% to 53%), but decreased that of groundnut (from 47% to 39%, Table 3). N treatment × genotypes (within crop species) interactions were not significant; hence only genotype means are presented in Table 3.

TABLE 1

Effect of N fertilization of stalk/haulm yield (kg/ha)

| Nitrogen applied (kg/ha) | Sorghum | | Groundnut | | |
|---|---|---|---|---|---|
| | CSH 8R | M 35-1 | Non-nod | Robut 33-1 | J 11 |
| 0 | 1888 | 2192 | 1099 | 4793 | 3831 |
| 50 | 2941 | 3185 | 1732 | 4768 | 4423 |
| 100 | 3357 | 4301 | 2247 | 4816 | 4032 |
| 150 | 3449 | 4249 | 3407 | 4390 | 4810 |
| 200 | 3565 | 4651 | 2970 | 5656 | 5237 |
| SE | ± 193.0[a] | | | ± 291.3[b] | |

[a]Standard error for comparing the nitrogen levels of sorghum genotypes.
[b]Standard error for comparing the nitrogen levels of groundnut genotypes.

## *N uptake and measurement of $N_2$ fixation*

The total N uptake by Non-nod at nil nitrogen was very similar to that of the sorghum genotypes (Table 4), indicating that in this case sorghum also could be used as a 'non-fixing' crop to estimate the soil N uptake by nodulated groundnut using the differential N uptake method. Nitrogen fixed by nodulated groundnut genotypes, estimated by the differential N uptake method using Non-nod as a control for the soil N uptake, were 189 kg N/ha for genotype Robut 33-1 and 183 kg N/ha for genotype J 11.

An increased percentage of N was observed in the stalks of sorghum genotypes fertilized with 150 kg N/ha or more (Table 5). There was no change in the N content in the kernels of genotypes J 11 and Robut 33-1 (4.6–4.8%) but at high N levels, N content in the haulm of genotype Robut 33-1 was increased

TABLE 2

Effect of N fertilization on grain/kernel yield (kg/ha)

| Nitrogen applied (kg/ha) | Sorghum | | Groundnut | | |
|---|---|---|---|---|---|
| | CSH 8R | M 35-1 | Non-nod | Robut 33-1 | J 11 |
| 0 | 1251 | 1035 | 570 | 2586 | 2849 |
| 50 | 3051 | 1991 | 862 | 2494 | 2466 |
| 100 | 3758 | 2709 | 947 | 2939 | 2150 |
| 150 | 4140 | 2909 | 1300 | 2807 | 2090 |
| 200 | 4139 | 2922 | 1202 | 2590 | 2302 |
| SE | ± 266.0[a] | | | ± 222.1[b] | |

[a,b] See Table 1.

TABLE 3

Effect of nitrogen fertilization on harvest index

| Nitrogen applied (kg/ha) | Sorghum[a] | Groundnut[b] |
|---|---|---|
| 0 | 41 | 47 |
| 50 | 48 | 43 |
| 100 | 52 | 43 |
| 150 | 53 | 42 |
| 200 | 53 | 39 |
| SE | ±1.8 | ±1.5 |

[a]Mean of two genotypes (CSH 8R, M 35-1).
[b]Mean of three genotypes (Robut 33-1, J 11 and Non-nod).

(from 1.7% to 2.0%, Tables 5 and 6). Nitrogen application increased total N accumulation of groundnut genotype Robut 33-1, but did not significantly influence the total N accumulation of genotype J 11 (Table 4). However, both kernel and haulm N content of Non-nod increased linearly with increase in N. N contents in the shoot and seed and total N accumulation of both sorghum genotypes were much lower than those of groundnut genotypes and there was no increase in the N content in sorghum up to 100 kg N/ha (Tables 4, 5 and 6). Percentage of total N translocated to seed in sorghum genotypes increased with N fertilization responses (Table 7). N fertilization decreased percentage N translocated to kernels in genotype J 11 whereas there were no significant effects on genotypes Non-nod and Robut 33-1, up to 150 kg N/ha. However, at 200 kg N/ha, less nitrogen was translocated to kernels of genotype Robut 33-1.

TABLE 4

Effect of N fertilization on total nitrogen harvested (kg/ha)

| Nitrogen applied (kg/ha) | Sorghum | | Groundnut | | |
|---|---|---|---|---|---|
| | CSH 8R | M 35-1 | Non-nod | Robut 33-1 | J 11 |
| 0 | 30 | 25 | 29 | 218 | 212 |
| 50 | 58 | 40 | 43 | 208 | 203 |
| 100 | 64 | 56 | 60 | 231 | 188 |
| 150 | 82 | 66 | 97 | 222 | 195 |
| 200 | 104 | 87 | 90 | 246 | 206 |
| SE | | ±5.01[a] | | ±11.8[b] | |

[a,b] See Table 1.

TABLE 5

Effect of N fertilization on nitrogen content (%) in stalk/haulm

| Nitrogen applied (kg/ha) | Sorghum | | Groundnut | | |
|---|---|---|---|---|---|
| | CSH 8R | M 35-1 | Non-nod | Robut 33-1 | J 11 |
| 0 | 0.8 | 0.5 | 1.3 | 1.7 | 1.6 |
| 50 | 0.7 | 0.5 | 1.0 | 1.8 | 1.5 |
| 100 | 0.6 | 0.5 | 1.2 | 1.7 | 1.5 |
| 150 | 0.7 | 0.6 | 1.3 | 1.7 | 1.7 |
| 200 | 0.9 | 0.9 | 1.3 | 2.0 | 1.5 |
| SE | | ±0.04[a] | | ±0.08[b] | |

[a,b]See Table 1.

## *N utilization*

Sorghum and groundnut vary largely in protein content and N% in plant parts and hence differ in their N requirement to produce an equivalent biomass. The relationships between biomass production and total nitrogen harvested in the plant parts of the sorghum and groundnut genotypes are presented in Fig. 1. At maximum N use level, sorghum genotypes showed greater N use efficiency (120 kg biomass/kg N harvested) that groundnut genotypes (36 kg biomass/kg N harvested).

The reduction of nitrate to nitrite is believed to be the rate-limiting step in plants growing on mineral nitrogen. Hence to understand the N utilization pattern of groundnut and sorghum, ontogenic changes of nitrate reductase activity of these genotypes were studied during the 1984-85 postrainy season. In these experiments observations were limited to leaf nitrate reductase as

TABLE 6

Effect of N fertilization on nitrogen content (%) in seed

| Nitrogen applied (kg/ha) | Sorghum | | Groundnut | | |
|---|---|---|---|---|---|
| | CSH 8R | M 35-1 | Non-nod | Robut 33-1 | J 11 |
| 0 | 1.1 | 1.3 | 2.1 | 4.6 | 4.6 |
| 50 | 1.1 | 1.2 | 2.3 | 4.6 | 4.7 |
| 100 | 1.0 | 1.2 | 2.5 | 4.6 | 4.8 |
| 150 | 1.2 | 1.3 | 3.2 | 4.8 | 4.6 |
| 200 | 1.4 | 1.5 | 3.8 | 4.7 | 4.7 |
| SE | | ±0.04[a] | | ±0.12[b] | |

[a,b]See Table 1.

TABLE 7

Effect of N fertilization on percentage of total nitrogen translocated to seed

| Nitrogen applied (kg/ha) | Sorghum | | | Groundnut | | | |
|---|---|---|---|---|---|---|---|
| | CSH 8R | M 35-1 | Mean | Non-nod | Robut 33-1 | J 11 | Mean |
| 0 | 54 | 57 | 55 | 52 | 61 | 71 | 61 |
| 50 | 63 | 59 | 61 | 56 | 60 | 66 | 60 |
| 100 | 70 | 63 | 66 | 47 | 65 | 67 | 60 |
| 150 | 70 | 63 | 66 | 53 | 67 | 58 | 60 |
| 200 | 69 | 56 | 62 | 58 | 54 | 61 | 57 |
| SE | | ±3.4[a] | ±2.4 | | ±3.4[b] | | ±2.4 |
| Mean | 65 | 60 | | 53 | 61 | 64 | |
| SE | | ±1.5 | | | ±1.4 | | |

[a,b] See Table 1.

earlier experiments (unpubl. data, 1983) indicated negligible root nitrate reductase in sorghum and groundnut. Seasonal changes in the NRA/plant and NRA/mg dry leaf at 200 kg N/ha are shown in Figs. 2A and 3A. In all genotypes, NRA/plant and NRA/mg leaf dry weight increased to a maximum at about 75 DAS. NRA/plant remained at near maximum levels until about 90 DAS then decreased to a minimum at about 100 DAS, before increasing slightly again at about harvest (Fig. 2A). The sorghum genotype CSH 8R showed higher NRA/plant than genotype M35-1 from 70 DAS to 90 DAS, perhaps enabling this genotype to respond to N fertilizer better than M 35-1. However, the NRA-/plant of the nodulating groundnut was higher than that of Non-nod at 85 and 92 DAS. Changes in NRA/plant of both groundnut and sorghum showed a more or less similar pattern, irrespective of differences in flowering and fruit growth. NRAs/plant of both groundnut genotypes were very much lower than

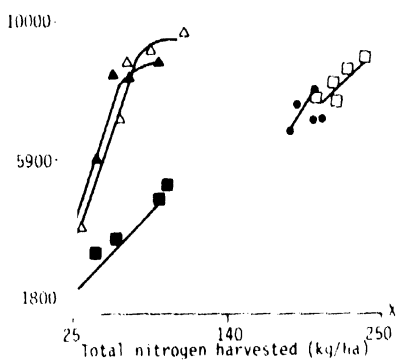


Fig. 1. Regression analyses of total biomass production as a function of total N assimilated by sorghum genotypes CSH 8R (△) and M 35-1 (▲), and groundnut genotypes Robut 33-1 (□), J 11 (•) and Non-nod (■) during the 1983-84 postrainy season.

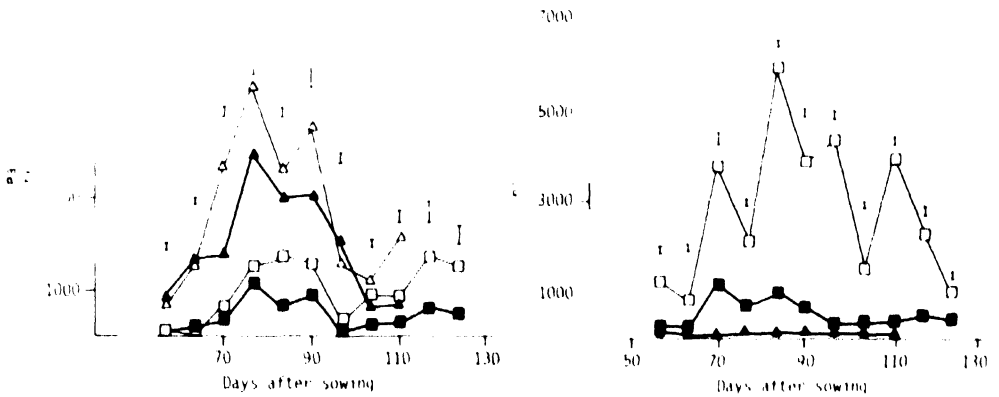


Fig. 2. Changes in NRA/plant (A) and nitrate content/plant (B) of sorghum and groundnut genotypes fertilized with 200 kg N/ha during the 1984-postrainy season. Notations are the same as in Fig. 1. Values of both sorghum genotypes are superimposed in B, hence only one is represented. Bars represent SE.

those of sorghum. NRA/mg leaf dry weight decreased after about 75 DAS to reach a minimum at about 100 DAS before increasing slightly again at about harvest (Fig. 3A). Both sorghum genotypes had more or less similar NRA/mg leaf (Fig. 3A). In general, leaves of both sorghum genotypes reduced nitrate faster than those of the groundnuts (Fig. 3A). The maximum NRA observed in groundnut genotypes was 0.8 nmoles nitrite/mg dry leaf per hour while sorghum genotypes exhibited 2.6–3.3 nmoles nitrite/mg dry leaf per hour. However, both groundnut genotypes retained considerble NRA during the later

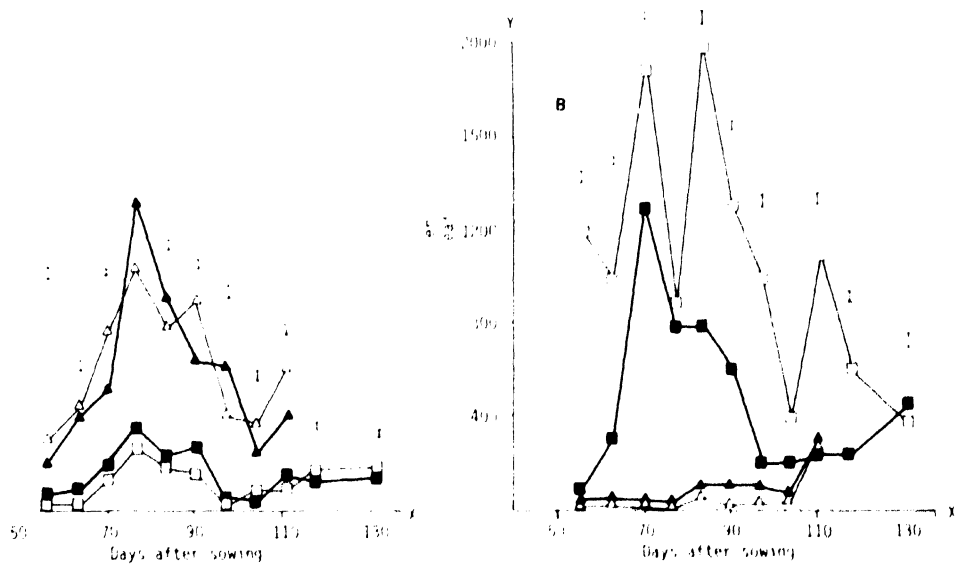


Fig. 3. Changes in NRA (nmoles nitrite/mg dry leaf per h) and nitrate content ($\mu$g/g dry leaf) of sorghum and groundnut genotypes fertilized with 200 kg N/ha during the 1984-85 postrainy season. Sorghum genotypes CSH 8R (△) and M 35-1 (▲), groundnut genotypes Robut 33-1 (□) and Non-nod (■).

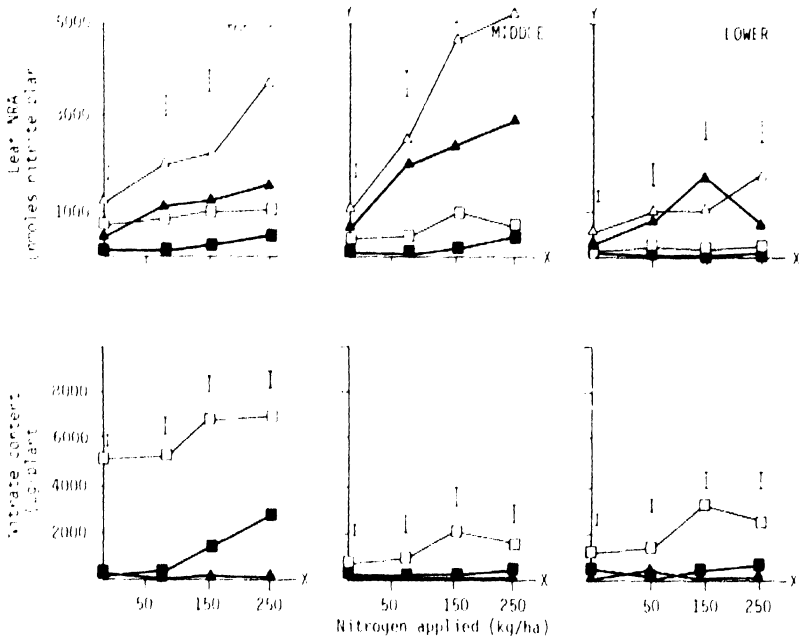


Fig. 4. Leaf NRA and leaf nitrate content in the top (A), middle (B) and lower (C) leaves of sorghum and groundnut genotypes at different N fertilizer levels, 71 DAS. Sorghum genotypes CSH 8R (△) and M 35-1 (▲), groundnut genotypes Robut 33-1 (□) and Non-nod (■).

stages of plant growth, though NRA was low compared to that of the sorghum genotpyes.

In Robut 33-1, the top and middle leaves had higher NRA than the lower leaves, but there was no significant difference in NRA of leaves from different canopy positions in the Non-nod genotype (Fig. 4). In both sorghum genotypes, higher NRA was observed in the middle leaves than in the top leaves.

*Nitrate concentration in leaves*

Leaf nitrate reductase is an inducible enzyme in many plant species, the enzyme being induced by the substrate, $NO_3^-$. Although the leaves of sorghum genotypes had higher NRA, groundnut genotypes had higher nitrate concentrations than sorghum genotypes throughout the growth cycle at all N levels. Representative data at 200 kg N/ha are shown in Figs. 2B and 3B. When fertilized with 200 kg N/ha, genotype Robut 33-1 had around 6 mg nitrate/plant at 85 DAS. Sorghum genotypes had most nitrate (0.3 mg/g dry leaf) at 110 DAS but levels were never higher than 0.2 mg/g dry leaf during the rest of the growth period (Fig. 3B). The slightly higher nitrate content/g dry leaf in sorghum M 35-1 than in CSH 8R was not significantly different.

Nitrate concentrations in the top leaves of genotypes Robut 33-1 and Non-nod were higher than in the middle or lower leaves (Fig. 4). There was no significant difference in the nitrate level of top, middle and lower leaves of

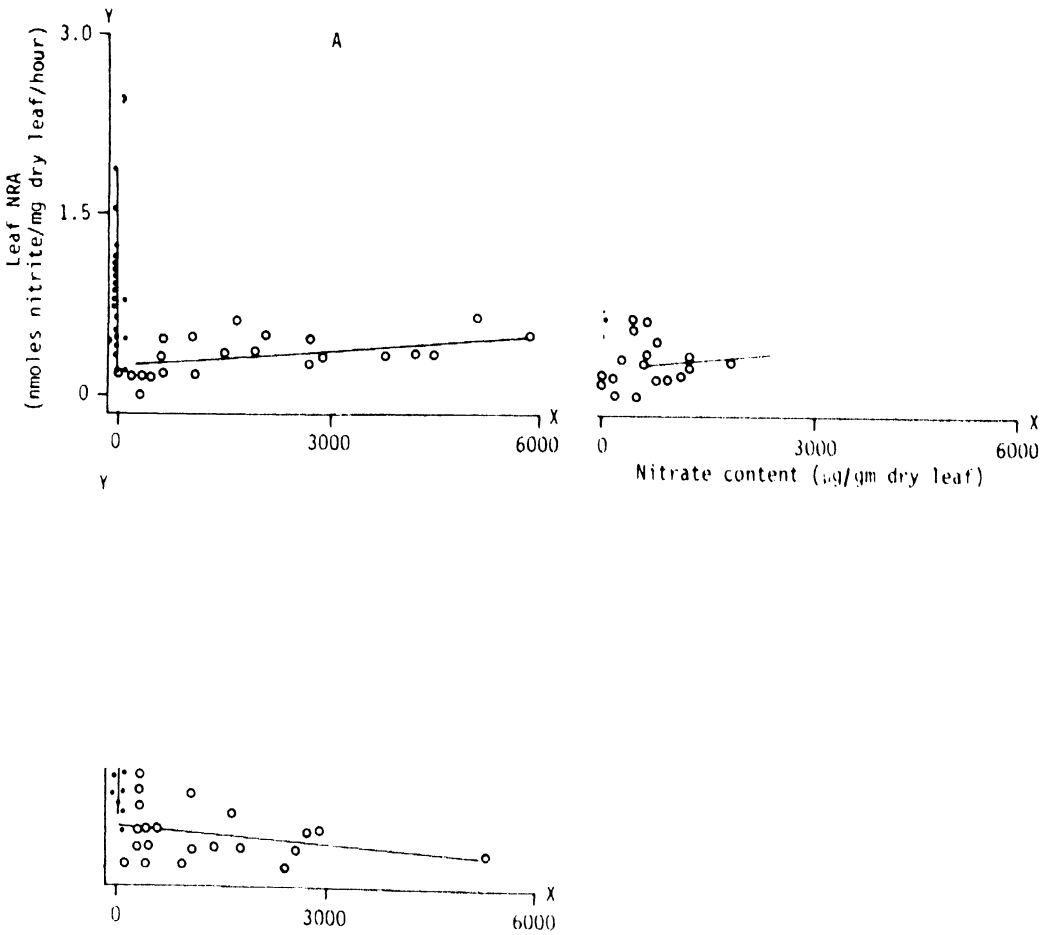


Fig. 5. Relationship between the NRA/mg leaf per h and nitrate/g leaf in the top (A), middle (B), and lower (C) leaves of sorghum (●) and groundnut (○) genotypes.

sorghum genotypes. Regression analyses of nitrate content in the leaves vs NRA of the three canopy portions 71 DAS are shown in Fig. 5. Although slope differences between genotypes of sorghum and those of groundnut are significant, slope differences within groundnut genotypes and sorghum genotypes were not significant. Hence only pooled data for groundnut (Robut 33-1 and Non-nod) and sorghum (M 35-1 and CSH 8R) are presented in Fig. 5. These data indicate high NRA activity at low nitrate content in the leaves of sorghum, and low NRA, even at high nitrate content, in the leaves of groundnut. Regression analyses carried out at other growth stages showed a similar pattern (analyses not presented).

## DISCUSSION

### *Dry matter yield and N uptake*

Responses of sorghum to fertilizer nitrogen, especially in India, are well documented (Tandon and Kanwar, 1984). The hybrid genotype CSH 8R pro-

duced a higher grain yield than the variety M 35-1. In general, improved sorghum genotypes respond more to fertilizer nitrogen than traditional cultivated varieties (reviewed by Tandon and Kanwar, 1984). Genotypic differences in the pod yield of nodulating groundnut in response to N have also been reported earlier (Gutstein, 1978). N fertilization increased harvest index in sorghum but decreased that of groundnut. The percentage of N translocated to seed in sorghum increased with N application. In contrast, less N was translocated to kernels of groundnut J 11 at higher N levels and there were no significant effects on Non-nod and Robut 33-1, up to 150 kg N/ha.

Recent studies indicate that the energy requirement for nitrogen fixation in legumes is greater than that required for mineral nitrogen utilization (Silsbury, 1977; Mahon, 1979; Ryle et al., 1979). Therefore, genotypes of legumes which depend only on mineral N, if adequately fertilized, should grow and yield better than genotypes of the same crop species which are dependent on symbiotic nitrogen fixation. Within the context of this experiment, the growth of the nitrogen-fertilized Non-nod groundnut should be greater than that of non-fertilized (no added N) nodulating genotypes. However, growth of the Non-nod genotype at high nitrogen levels was very poor when compared to that of the non-fertilized nodulating genotypes. Several other studies on nodulating and non-nodulating genotypes of groundnut and soybean have reported similar results (Weber, 1966; Johnson et al., 1975; Pancholy et al., 1983; Selamat and Gardner, 1983; Walker et al., 1983). Hence the findings from the present experiment, and from many previous studies, are at variance with the hypothesis that adequately fertilized non-nodulating legumes which depend on uptake of inorganic N for their N requirements, should out-yield nodulated genotypes which depend on the less energy-efficient symbiotic process of $N_2$ fixation for their N requirements. Although this result might be explained by the fact that the nodulating and non-nodulating groundnut genotypes used in the present study were not isolines, and could therefore be expected to have different inherent growth rates and rates of uptake and utilization of N, further study is needed to reveal the cause of the lower yields and lower efficiency of biomass production per unit of N harvested of fertilized non-nodulating genotypes of groundnut.

*Estimations of $N_2$ fixation and N utilization*

Estimations on nitrogen fixation using non-fixing control (sorghum or Non-nod groundnut grown without any nitrogen fertilizer) indicated that groundnut could fix large amounts of nitrogen (up to 190 kg N/ha). Based on experiences for breeding efforts to increase the yields of legumes, Arnon (1980) argued that "because of the high energy requirements of nitrogen fixation, plant breeders have not been able to raise the level of yields of legumes, despite considerable breeding efforts". However, the results presented in Fig. 1 indi-

cate that high protein content in legumes is not a consequence of nitrogen fixation, but apparently legumes acquired symbiotic nitrogen fixation because of their high N requirement to produce an equivalent biomass. Hence, it may not be possible to obtain high biomass yields in legumes by substituting fertilizer N application for nitrogen fixation. Brown (1978) hypothesized that among the cereals $C_4$ plants (like sorghum) have a greater N use efficiency (biomass production per unit of N in the plant) than do $C_3$ plants. N use efficiency of both sorghum genotypes reached a plateau at around 100 kg N harvested/ha, while that of Non-nod groundnut increased even up to 200 kg N/ha, indicating that even at this fertilization level, N limits the growth of this genotype (Fig. 1). At maximum N use level, sorghum genotypes showed greater N use efficiency (120 kg biomass/kg N harvested) than groundnut genotypes (36 kg biomass/kg N harvested). Though NRA/mg leaf of Non-nod was consistently more than in Robut 33-1, the differences were small and not significant. Therefore the dependence of the Non-nod on soil nitrogen did not greatly modify the N metabolism of this genotype.

The reduction of nitrate ($NO_3^-$ to $NO_2^-$) is believed to be the rate-limiting step in plants growing on mineral nitrogen, and the enzyme NR in the leaves is inducible by the substrate $NO_3^-$ in many plant species (Beevers and Hageman, 1969; Srivastava, 1980). Significant positive correlations between NRA and plant growth have been obtained in many plant species (Zieserl et al., 1963; Croy and Hageman, 1970; Dykstra, 1974; Singh et al., 1976). Hence we examined the relationship between leaf nitrate content and leaf nitrate reductase activity of groundnut and sorghum. Both groundnut genotypes showed low leaf NRA, even at very high levels of nitrate content in the leaves, whereas sorghum genotypes showed higher NRA at low levels of leaf nitrate (Fig. 5) relative to groundnut genotypes. This could be because of (a) higher nitrate concentration being required to induce nitrate reductase in groundnut than in sorghum, or (b) nitrate reductase in groundnut having a lower affinity for the substrate (higher *Km*, the Michaelis-Menten constant) compared to that of sorghum. This implies that groundnut is a poor utilizer of mineral nitrogen at all stages of growth. This may be one of the reasons for the poor response of groundnut to N application; differences in symbiotic $N_2$ fixation in different soil types could also contribute to the erratic responses of groundnut to N application.

As nitrogenase activity of groundnut root nodules declines during the pod-fill stage (Hardy et al., 1973; Nambiar et al., 1982), it is generally thought that mineral nitrogen application during this stage could increase the yields. However, in an Alfisol field at ICRISAT, nitrogen application (0, 20, 40, and 60 kg N/ha) during the pod-filling stage did not significantly influence the pod yield of the groundnut genotype Robut 33-1 (authors, unpubl., 1983). Although both considerable NRA and nitrate content were detected during the later stages of plant growth in the N-fertilized (200 kg/ha) Robut 33-1 (Fig. 2A and 2B), the

NRA of this genotype was much lower than that observed in sorghum, indicating low enzyme efficiency. Hence it is unlikely that one may replace symbiotic $N_2$ fixation by nitrogen application during the pod-fill stage in groundnut.

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