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# Nodule Formation and Function in Six Varieties of Cowpea (*Vigna unguiculata* L. Walp.) Grown in a Nitrogen-Rich Field Soil in South Africa

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## Abstract

This study, which was conducted in the field during the 1997–1998 cropping season, assesses growth and symbiotic performance of six cowpea varieties (namely, Pan 311, Glenda, Bechuana white, Chappy, Encore and NWK) cultivated in a rich mineral N soil environment. The experiment was carried out on a sandy loam, Hutton form soil using randomized complete block design. At 42 and 63 d after emergence (DAE), the plants were assessed for nodulation, $N_2$ fixation, dry matter yield and tissue N concentration. Estimates of N fixed were determined using the $^{15}N$ natural abundance technique with maize as reference plant. Except variety Encore, which grew significantly ($p<0.05$) better than Pan 311, Glenda and NWK, the rest maintained similar plant growth. Nodulation varied significantly ($p<0.05$) among the six varieties, Pan 311 showing the lowest number of nodules per plant. In fact, a number of plants sampled from Pan 311 had no root nodules at 42 and 63 DAE. By contrast, Glenda, Chappy and NWK produced significantly ($p<0.05$) many more nodules under the same field conditions. At 42 DAE, the $\delta^{15}N$ values for plant

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roots were between 4.77±0.3‰ and 5.46±0.7‰, while at 63 DAE they varied from 5.05±2.6‰ to 5.89±1.6‰. However, shoot $\delta^{15}N$ values at 42 DAE were high, ranging from 7.09±0.4‰ to 7.35±1.8‰, and decreased at 63 DAE to lower levels, ranging from 5.12±4.7‰ to 5.80±1.1‰. The $\delta^{15}N$ value of the maize reference plant was 8.06±0.45‰ at 42 DAE and 7.34±0.68‰ at 63 DAE. The high $\delta^{15}N$ values obtained for these cowpea varieties were consistent with a low dependence on symbiotic fixation for their N nutrition. The proportion of plant N obtained from atmospheric fixation ranged from 32.3±10.1 to 48.8±14.8% at 42 DAE, and 23.5±2.4 to 41.1±10.5% at 63 DAE, clearly showing strong varietal differences in nodule functioning. Estimates of N fixed at 63 DAE show that Chappy and Encore fixed the highest amounts of N, while Pan 311 (the commercial variety) fixed the least. The marked differences ($p<0.05$) in nodulation and $N_2$ fixation exhibited by the six cowpea varieties was due to inhibition by mineral N. Analysis of soil at planting and at 63 DAE revealed very high concentrations of available N ($NO_3$-N + $NH_4$-N) in soil solution. About 13–40 mM was obtained for soil within 0–30 cm depth at planting, and 63.9–98.9 mM for the same depth at 63 DAE. The presence of such large concentrations of available N in soils of the experimental site was responsible for the poor nodulation and low level of $N_2$ fixation in root nodules of the six cowpea cultivars. Nodule formation and function in the commercial variety (Pan 311) was for example very sensitive to soil mineral N, whereas Bechuana White, Encore and Chappy were more tolerant of this anion. Identifying legumes with relatively high rates of $N_2$ fixation in N-rich soils has potential for sustainably increasing yields of cereals and non-legume crops from the soil N spared by legume and from that fixed by symbiosis.

Keywords: Cowpea varieties, soil mineral N, nodulation, $\delta^{15}N$ values, %Ndfa, N fixed

## 1. Introduction

Cowpea is indigenous to Africa and is the major grain legume grown by many small-scale farmers in the continent. Recent attempts at improving the productivity of this grain legume in South Africa have included the development and testing of several distinct varieties in the diverse agroecological zones of the country, and the evaluation of the $N_2$ fixing efficiency of various varieties. How much N is contributed by cowpea plants, whether in sole or intercrop, is still not known in South Africa. Additionally, practices such as grazing the legume residue by livestock may deplete the soil N reserves in the long term if the $N_2$ fixing capacity of the legume crop is not enhanced (Powel et al., 1998). Also, with the intensive cultivation of small holdings in South Africa and the need to fertilize non-legume crops in order to optimize yields, large pools of N could easily accumulate in the soil with consequent detrimental effects on the establishment of legume symbioses with $N_2$ fixing rhizobia.

In South Africa, commercial farmers have historically applied heavy doses of N fertilzer to maize crops in the field, a practice which disregards legume culture and its associated benefits of biological N to the ecosystem. To date, it is unclear whether these maize fields, which are heavily fertilized with mineral N, can support adequate nodulation and effective $N_2$ fixation in symbiotic legumes. A priority area in $N_2$ fixation research today is the development of legume symbioses that have the ability to fix large amounts of $N_2$ in the presence of high concentrations of nitrate ($NO_3^-$) and ammonium ($NH_4^+$) ions in the soil solution. This is because the nodulation and $N_2$ fixation in many legume-*Rhizobium* symbioses are highly sensitive to combined N (Pate et al., 1980; Hardarson et al., 1984; Senaratne et al., 1987). It has been shown, however, that nodulation in some soybean varieties can moderately tolerate high concentrations of soil $NO_3^-$, especially when inoculated with high numbers of rhizobia (Betts and Herridge, 1987; Herridge and Betts, 1988). Nitrogenase activity in root nodules of some African legumes also exhibits tolerance of $NO_3^-$ inhibition (Dakora et al., 1992). A recent study (Dakora, 1998) has provided further evidence for $NO_3^-$ tolerance of nodule function in landraces of two African legumes. Compared to many agricultural legumes, nitrogenase activity in root nodules of Bambara groundnut and Kersting's bean was found to be tolerant of $NO_3^-$ supplied in the root medium (Dakora, 1998). Layzell and Moloney (1994) have indicated that increases in $N_2$ fixation of up to 300% could be achieved in soils with large concentrations of $NO_3^-$ if highly effective $NO_3^-$-tolerant symbioses were identified. Clearly, the findings of all these studies (Betts and Herridge, 1987; Herridge and Betts, 1988; Dakora et al., 1992; Dakora, 1998) show that $NO_3^-$-tolerant symbioses exist. Initiating an aggressive program of plant testing in high N soils could identify additional symbiotic partnerships that are tolerant of soil mineral N.

The aim of this study was to assess nodulation and $N_2$ fixation in six distinct cowpea varieties under field conditions in a N-rich soil.

## 2. Materials and Methods

*Plant varieties*

Six cowpea cultivars of varying morphology were used in this study. They include Pan 311, Bechuana White, Chappy, Glenda, Encore, and NWK. Pan 311 is a commercially marketed variety whereas the others represent landraces collected from different locations in southern Africa and improved by the Agricultural Research Council.

### *Soil characteristics and N fertility history*

The soil type was a sandy loam (Glenrosa, Hutton form). Prior to planting, soil samples were taken from the upper 0–15 cm, and 15–30 cm layer and analyzed. The 0–15 cm zone contained 44 mg $kg^{-1}$ P, 323 mg $kg^{-1}$ K, and 13 mg $kg^{-1}$ mineral N ($NO_3^-$-N + $NH_4^+$-N) with pH ($H_2O$) 7.5. Soil from the 15–30 cm depth was characterized by 54 mg $kg^{-1}$ P, 298 mg $kg^{-1}$ K, 40 mg $kg^{-1}$ mineral N ($NO_3^-$-N + $NH_4^+$-N) and pH ($H_2O$) 7.8.

The high content of soil mineral N (mainly $NO_3$ and $NH_4$ ions) at planting reflects the history of the experimental site. The field was previously planted to an active $N_2$ fixing population of lucerne for six years, followed by maize cropping and fertilization with $NH_4NO_3$ (120 kg N $ha^{-1}$ $yr^{-1}$) for another two years before this study was conducted.

### *Field planting*

Seeds of the six varieties were inoculated with a peat-based culture of *Bradyrhizobium* strain CB756 and planted at the University of the North experimental farm on 16 December 1997 in a randomized complete block design with four replications. Each variety was planted in a three-row plot, 6 m long, and seeded at a density of 70000 plants $ha^{-1}$. Rows within plots were spaced 90 cm apart. Phosphorus was incorporated to the soil at a rate of 20 kg P $ha^{-1}$ at planting. Weeds were controlled manually by hand, twice during the experimental period.

### *Mineral N analysis*

As plants were sampled for $^{15}N/^{14}N$ isotopic analysis at 63 DAE, soil samples were simultaneously taken from each plot at depths of 0–15 cm and 15–30 cm using an auger. Soil cores taken from three separate sites within each plot were pooled on the basis of depth, and duplicate subsamples extracted with 1 M KCl for analysis of mineral N ($NO_3^-$-N and $NH_4^+$-N) as described by Keeney and Nelson (1982).

### *Plant sampling and $^{15}N$ analysis*

Four plants were selected from the middle row of each plot at 42 DAE which corresponded to late vegetative stage, and at 63 DAE corresponding to mid-flowering to early pod-filling stage of the cowpea varieties. The plants were dug up, and the roots washed in water to remove bound soil. Any fibrous roots detached during washing were collected by sieving. Nodules were plucked from roots and counted on per plant basis. The four plants from each replicate plot were pooled and separated into nodules, roots and shoot, and oven-dried to constant dry weight at 60°C. The shoots of four maize plants from each of the four replicate plots were similarly harvested, pooled, and oven-dried at 60°C for $^{15}N/^{14}N$ isotopic analysis as reference plant. The dried plant parts were weighed, oven-dried and ground into fine powder.

Total N content and $\delta^{15}N$ values were determined using a mass spectrometer (Model NA 15000 NC (CHNN) analyzer). The proportion of plant N derived from atmospheric fixation (%Ndfa) was calculated as described by Unkovich et al. (1993) using the following equation:

$$P = \frac{(\delta^{15}\text{Nref} - \delta^{15}\text{Nlegume})}{(\delta^{15}\text{Nref} - B)}$$

where $\delta^{15}$Nref is the value of a non-leguminous reference plant grown in close proximity to the legume, and of the same age as the legume; and B is the $\delta^{15}N$ value of cowpea plants grown in the glasshouse, and which depended solely on symbiotic fixation for their N nutrition. The reference plant used in this study was maize grown with the cowpea cultivars during the period of experimentation. The B value was obtained by growing inoculated (*Bradyrhizobium* strain CB756) seeds of the cowpea varieties in sand culture under glasshouse conditions and supplying them with N-free Hoagland nutrient solution until harvest.

*Statistical analysis*

All data were analyzed using the Statistical Analysis System (SAS) software. Analysis of variance was used to test significance of parameters measured and differences due to variety were compared using LSD (Steel and Torrie, 1980).

## 3. Results

*Mineral N concentrations in soil profile*

Analysis of top soil collected from plots at mid reproductive stage (63 DAE) of cowpea plants showed large concentrations of available N ($NO_3^-$-N + $NH_4^+$-N) at both 0–15 and 15–30 cm depth (Table 1). Available N content ranged from 63.9 to 88.7 mg kg$^{-1}$ in the 0–15 cm depth, and 70.9 to 98.9 mg kg$^{-1}$ for the 15–30 cm depth (Table 1).

Table 1. Available soil N ($NO_3^-$-N + $NH_4^+$-N) concentrations at 0–15 and 15–30 cm depth in field soil sampled at 63 d after emergence of cowpea seedlings

| Cowpea variety | Soil N content ($mg.kg^{-1}$) at 2 depths | |
|---|---|---|
| | 0–15 cm | 15–30 cm |
| Pan 311 | 72.8 | 78.4 |
| Glenda | 63.9 | 86.8 |
| Bech. White | 77.0 | 98.9 |
| Chappy | 66.3 | 70.9 |
| Encore | 88.7 | 72.3 |
| NWK | 84.9 | 92.9 |
| LSD(0.05) | ns | ns |

ns = non significant

Table 2. Nodule number and nodule DM of field-grown cowpea varieties harvested at 42 and 63 d after emergence (DAE)

| Cowpea variety | Nodule number (nod. $plant^{-1}$) | | Nodule DM (mg DM $plant^{-1}$) | |
|---|---|---|---|---|
| | 42 DAE | 63 DAE | 42 DAE | 63 DAE |
| Pan 311 | 1.5 | 2.0 | 3.8 | 15.5 |
| Glenda | 11.1 | 5.9 | 30.8 | 29.2 |
| Bech. White | 1.9 | 2.7 | 10.1 | 45.8 |
| Chappy | 8.0 | 8.5 | 28.6 | 29.2 |
| Encore | 5.9 | 5.0 | 22.5 | 50.0 |
| NWK | 7.2 | 5.3 | 17.1 | 33.3 |
| LSD(0.05) | 5.0 | 4.3 | 21.0 | 22.5 |

*Nodulation*

The six cowpea varieties differed significantly ($p<0.05$) in their nodulation response to field cultivation. Nodulation in Pan 311, the widely grown commercial variety, was severely inhibited at both 42 and 63 DAE (Table 2), with some plants having no nodules at all. Glenda, Chappy and NWK varieties produced significantly more nodules per plant than Pan 311 and Bechuana White at 42 DAE (Table 2). At 63 DAE, Chappy was the only

Table 3. Total DM, root and shoot N content of field-grown cowpea varieties harvested at 42 and 63 d after emergence (DAE)

| Cowpea variety | Total DM (g DM $plant^{-1}$) | | Root N (mg N $plant^{-1}$) | | Shoot N (mg N $plant^{-1}$) | |
|---|---|---|---|---|---|---|
| | 42 DAE | 63 DAE | 42 DAE | 63 DAE | 42 DAE | 63 DAE |
| Pan 311 | 42 | 106 | 26.4 | 19.6 | 473.6 | 1967.0 |
| Glenda | 40 | 101 | 23.2 | 28.6 | 704.8 | 2461.2 |
| Bech.White | 53 | 101 | 22.8 | 18.8 | 676.8 | 2093.6 |
| Chappy | 55 | 111 | 33.8 | 32.1 | 558.4 | 2967.3 |
| Encore | 64 | 114 | 28.6 | 24.9 | 713.4 | 2560.3 |
| NWK | 46 | 96 | 26.3 | 21.1 | 754.5 | 2431.3 |
| LSD(0.05) | 14 | ns | ns | 8.0 | ns | 670.0 |

ns = not significant

Table 4. $\delta^{15}N$ values of roots and shoots of field-grown cowpea varieties harvested at 42 and 63 d after emergence (DAE)

| Cowpea variety | $\delta^{15}N$ (‰) | | | |
|---|---|---|---|---|
| | Root | | Shoot | |
| | 42 DAE | 63 DAE | 42 DAE | 63 DAE |
| Pan 311 | 5.36±3.2 | 5.89±1.6 | 7.35±1.8 | 5.56±0.4 |
| Glenda | 5.46±0.7 | 5.81±0.5 | 7.27±1.0 | 5.80±1.1 |
| Bech.White | 4.77±0.3 | 5.09±4.1 | 7.20±1.3 | 5.12±4.7 |
| Chappy | 4.94±2.0 | 5.05±2.6 | 7.22±4.3 | 5.74±1.2 |
| Encore | 4.95±.0.6 | 5.57±2.9 | 7.29±1.0 | 5.41±4.0 |
| NWK | 5.27±0.5 | 5.77±2.7 | 7.09±0.4 | 5.69±1.4 |
| Maize (ref. plant) | nd | nd | 8.06±0.45 | 7.34±0.68 |

nd = not determined. Values are Means±SD (n = 4). B values obtained were 0.04‰ for shoot, and 1.42‰ for roots of glasshouse plants dependent solely on symbiotic fixation.

cultivar that produced more nodules than all the rest. As a result of their significantly higher nodulation, Glenda and Chappy also yielded the greatest nodule dry matter (DM), especially at 42 DAE. Although Bechuana White and Encore produced relatively fewer nodules at 63 DAE, these were large, and resulted in significantly ($p<0.05$) greater nodule DM (Table 2).

*Accumulation of DM and N*

Total DM yields differed significantly among the six cowpea varieties at 42 DAE, but were similar 20 d later (Table 3). The variety Encore produced significantly ($p<0.05$) more DM than Pan 311, Glenda and NWK at 42 DAE; Pan 311 and Glenda on the other hand showed the lowest DM accumulation. By 63 DAE, however, there were no significant differences in DM yield among the six varieties (Table 3).

The N content of nodulated roots and shoots of cowpea plants were similar in all the varieties at 42 DAE (Table 3). However, at 63 DAE, root N of Chappy was significantly ($p<0.05$) higher than those of Pan 311, Bechuana White and NWK (Table 3). Similarly, the root N of Glenda was greater than that of Pan 311 and Bechuana White (Table 3). As with root N, the shoot N of Chappy was again markedly higher than those of Pan 311 and Bechuana White (Table 3). However, the remaining varieties did not differ in their shoot N content. Compared to shoots, the amount of N in roots was much lower in all the six cowpea varieties at both 42 and 63 DAE (Table 3).

*$\delta^{15}N$ values*

The $\delta^{15}N$ value can indicate whether a plant is dependent on soil N or symbiotic fixation for its N nutrition. Greater difference between the $\delta^{15}N$ of a nodulated legume and that of a non-legume reference crop is an indication of greater dependence on symbiotic fixation by the legume (Unkovich et al., 1993). The data presented in Table 4 show that the $\delta^{15}N$ values of cowpea roots were similar at both 42 and 63 DAE, although those of Pan 311, Glenda and NWK were slightly higher. Also, the $\delta^{15}N$ values obtained at 63 DAE ranged from 5.05±2.6‰ for Chappy to 5.89±1.6‰ for Pan 311, and were greater in magnitude than those at 42 DAE, which varied from 4.77±0.3‰ for Bechuana White to 5.36±3.2‰ for Pan 311 (Table 4). However the $\delta^{15}N$ values for shoot showed the opposite pattern, in that those of 42 DAE were much higher than their counterparts at 63 DAE (Table 4). The $\delta^{15}N$ values of shoots sampled at 42 DAE were strikingly high, ranging from 7.09±0.4‰ for NWK to 7.35±1.8‰ for Pan 311. However, the $\delta^{15}N$ values of shoots at 63 DAE ranged from 5.12±4.7‰ for Bechuana White to 5.80±1.1‰ for Glenda (Table 4). The $\delta^{15}N$ value of the maize reference plant was 8.06±0.45‰ and 7.34±0.68‰ for shoots sampled at 42 and 63 DAE, respectively.

*Proportion of N derived from fixation and amounts of N fixed*

Estimates of the proportion of N derived from fixation in roots and shoots at 63 DAE ranged from 24.6±3.9% for Pan 311 to 51.7±15.7% for Chappy root, and

Table 5. Proportion of N derived from symbiotic fixation of atmospheric $N_2$ in shoots and roots of field-grown cowpea varieties harvested at 42 and 63 d after emergence (DAE)

| Cowpea variety | % N derived from fixation | | | |
|---|---|---|---|---|
| | Root | | Shoot | |
| | 42 DAE | 63 DAE | 42 DAE | 63 DAE |
| Pan 311 | 40.6±4.9 | 24.6±3.9 | 23.9±15.3 | 24.3±1.5 |
| Glenda | 53.8±13.7 | 25.8±2.2 | 24.7±15.3 | 21.1±2.6 |
| Bech. White | 71.8±15.7 | 51.7±15.7 | 25.8±14.0 | 30.4±5.4 |
| Chappy | 47.0±3.9 | 38.7±5.0 | 40.4±19.6 | 21.9±2.7 |
| Encore | 58.9±12.7 | 30.0±5.3 | 25.6±15.3 | 26.5±5.0 |
| NWk | 42.1±1.9 | 26.5±5.0 | 12.1±1.4 | 22.6±3.0 |

Values are Means±SD (n = 4).

Table 6. Amount of N fixed and proportion of N derived from fixation at whole-plant level at 42 and 63 d after emergence (DAE)

| Cowpea variety | %Ndfa at whole-plant level | | N fixed (kg $ha^{-1}$) |
|---|---|---|---|
| | 42 DAE | 63 DAE | |
| Pan 311 | 32.3±10.1 | 24.5±2.7 | 35.7±3.0 |
| Glenda | 39.3±14.5 | 23.5±2.4 | 40.9±7.6 |
| Bechuana White | 48.8±14.8 | 41.1±10.5 | 46.7±7.0 |
| Chappy | 43.7±11.7 | 30.3±3.8 | 47.0±4.5 |
| Encore | 42.3±13.9 | 28.3±5.2 | 50.7±9.1 |
| NWK | 34.2±1.7 | 24.6±4.0 | 40.6±5.2 |

Values are Mean±SD (n = 4).

21.1±2.6% for Glenda to 30.4±5.4% for Bechuana White shoot (Table 5). These values are relatively low compared to what have been reported for other grain legumes (Amstrong et al., 1994; Unkovich, 1995; Kohl et al., 1980) and they indicate the reduced dependence of the plants on symbiotic fixation for their N nutrition. More of the symbiotically fixed N was found in the roots (an average of 52%) at 42 DAE compared to shoot which had only 25% during the same period. By 63 DAE, the proportions of tissue N derived from fixation were similar for both roots and shoots, and averaged 29% (Table 5). At 42 DAE, the

%Ndfa values for whole plants were low, ranging from 32.3±10.1% for Pan 311 to 48.8±14.5% for Bechuana White (Table 6). By pod-filling stage (63 DAE), however, these values had decreased to 24.5±2.7% for Pan 311 and 41.1±10.5% for Bechuana White.

Because the %Ndfa values were low, the actual amounts of N fixed by the six cowpea varieties were also low (Table 6), especially when compared to published data. Bechuana White, Chappy and Encore fixed 46.7±7.0, 47.0±4.5 and 50.7±9.1 kg N $ha^{-1}$, respectively, and were the highest in symbiotic performance. Interestingly, Pan 311 (the commercial variety) could fix only 35.7±3.0 kg N $ha^{-1}$, and therefore performed the least among the six cowpea cultivars (Table 6).

## 4. Discussion

The commercial cowpea variety (Pan 311) and five other cultivars (Glenda, Chappy, Encore, NWK and Bechuana White) showed strong symbiotic differences in response to field cultivation, even though plant growth was similar at pod-filling stage for all cultivars (Table 3). Compared to the others, the commercial variety formed the lowest number of root nodules per plant (Table 2). Glenda and Chappy, on the other hand, had significantly ($p<0.05$) more nodules on their roots, which resulted in greater nodule DM at 42 DAE (Table 2). Although Bechuana White was the next poor nodulator after Pan 311, its nodules were larger and hence yielded significantly greater biomass, especially at 63 DAE (Table 2), suggesting higher efficacy in nodule function. Relative to the other cultivars, roots and shoot of Pan 311 showed the highest $\delta^{15}N$ values at both 42 and 63 DAE (Table 4), an indication of reduced $N_2$ fixing activity. In contrast, Bechuana White had relatively lower $\delta^{15}N$ values for roots and shoots at both harvests (Table 4), indicating greater nodule function which is consistent with the observation of fewer but larger and more effective nodules on this cultivar.

The B values (i.e. the $\delta^{15}N$ of glasshouse-grown plants solely dependent on fixation of atmospheric $N_2$ for their N nutrition) were 0.04‰ for shoots and 1.42‰ for roots. The accuracy involved in estimating $N_2$ fixation using the $^{15}N$ natural abundance technique depends, to some extent, on variations associated with the B value. Although the B values of the six cowpea cultivars can vary when partnered with *Bradyrhizobium* strain CB756, Unkovich et al. (1994) have shown that such differences are insignificant to affect the accuracy of the technique. From testing five genotypes of subterranean clover (one with three cultivars), three cultivars of lupin and six cultivars of pea, they found only slight differences in the B values of those genotypes and cultivars, indicating that such variations are unlikely to influence the accuracy of fixed-N estimation. The use of soil inocula from 243 sites also showed that the rhizobia in many of those soils were not significantly different from each other in terms of shoot and root B values of plants nodulated by them (Unkovich et al., 1994). Consequently, the use of only *Bradyrhizobium* strain CB756 in this study (without any soil inoculum from the experimental field) to determine the B value in glasshouse plants should not affect the accuracy of the measured N fixed.

Estimates of %Ndfa using $\delta^{15}N$ values of field plants and B values of glasshouse-grown plants revealed differences among the cultivars in terms of dependence on symbiotic fixation for their N nutrition. The %Ndfa was highest in Bechuana White for roots, shoot and the whole plant, while Pan 311 recorded the lowest %Ndfa for whole plant and its organs at 42 and 63 DAE (Tables 5 and 6). The %Ndfa values for the other four varieties were intermediate between those of Pan 311 and Bechuana White. Consequently, the actual amounts of N fixed were higher in Bechuana White, Chappy and Encore varieties (46.7±7.0, 47.0±4.5, and 50.7±9.1 kg N $ha^{-1}$, respectively), followed by Glenda and NWK (40.9±7.6 and 40.6±5.2 kg N $ha^{-1}$), and least in Pan 311 (35.7±3.0 kg N $ha^{-1}$).

Averaged across all varieties, only about 29% of the N in cowpea plant was obtained from symbiotic fixation, indicating that these varieties acquired relatively low amounts of N from fixation of atmospheric $N_2$. Although the %Ndfa values and measures of N fixed obtained in this study are quite low compared to levels reported in the literature (Dakora and Keya, 1996), they are nevertheless similar to those of van Kessel and Roskoski (1988) for cowpea grown sole or as an intercrop with maize. This low dependence on $N_2$ fixation for N nutrition could be due to a number of factors. These include nodulation of the plants by ineffective but highly competitive indigenous rhizobia, genetic factors of the host plant which restrict nodulation, inherently low nitrogenase activities in these cowpea varieties, and/or inhibition of nodule formation and nodule function by high levels of soil N (Ayisi et al., 1992; Eaglesham et al., 1981). The concentration of endogenous mineral N in most fertile agricultural soils falls in the range of 1.0–5.0 mM, with 5 mM being the upper threshold (Marschner, 1995). This suggests that tolerance of soil mineral N by symbiotic legumes is also likely to fall within that range. Thus, concentrations above 5 mM would be expected to inhibit nodule formation and nodule functioning (Streeter, 1988).

Although at the onset of experimentation the available N concentration in the soil used for this study was 13.0 mM for 0–15 cm depth and 40.0 mM for 15–30 cm depth, these values changed with time and plant development (Table 1). Consequently, by pod-filling stage, the available N concentrations had increased markedly to levels that would rapidly inhibit nodulation and nitrogenase activity in any legume symbiosis. For example, at 63 DAE, the soil solution N concentration ranged from 63.9–88.7 mM for 0–15 cm depth and 70.9–98.9 mM for the 15–30 cm depth, which is a significant increase from the 13.0–40.0 mM measured at planting for 0-30 cm depth. These high concentrations of available N in the soil are no doubt responsible for the very low level of nodulation and $N_2$ fixation obtained for the six cowpea cultivars in this study. This is because, even at relatively low N concentrations (e.g. 5 mM) in the root medium, many legumes are known to show inhibition of both nodule formation and $N_2$ fixation (Oghoghorie and Pate, 1971; Pate et al., 1980; Hardarson et al., 1984; Minchin et al., 1986; Senaratne et al., 1987; Hansen et al., 1989; Wu and Harper, 1990). At the molecular level, high concentrations of soil $NH_4^+$ are known to repress the induction of nodulation (nod) genes in symbiotic rhizobia (Wang and Stacey, 1990), although bacterial mutants exist which can nodulate legumes in the presence of $NH_4^+$ (Dusha et al., 1989). Ammonia represses the expression of nodABC and nodD genes, the common nod genes in symbiotic rhizobia (Dakora, 1994). Because the nodD gene product controls the synthesis of all other nod genes in $N_2$ fixing rhizobia (Banfalvi et al., 1988), its suppression by high concentrations of $NH_4^+$ in soil can adversely affect nodule formation by the microsymbiont. Similarly, the synthesis and release of host-plant nodulation signals (isoflavonoid nod gene inducers) are decreased when legume roots are exposed to high concentrations of $NO_3^-$ in soil (Cho and Harper, 1991a), this leads to reduced nodulation and nitrogenase activity (Cho and Harper, 1991b). Consequently, the 63.9–98.9 mM mineral N ($NO_3^-$ + $NH^+$) experienced by cowpea roots and rhizobia within the 0–30 cm depth of soil at the study site was more than adequate to suppress nodulation and nodule function.

It however appears that some landraces and ecotypes of indigenous legumes are capable of nodulating and fixing $N_2$ optimally in soils containing large concentrations of $NO_3^-$. Such symbioses have the ability to overcome $NO_3^-$ inhibition of nodule development and nitrogenase activity. A recent report (Dakora, 1998) has in fact shown that, compared to other legume symbioses, nitrogenase activity in root nodules of Bambara groundnut and Kersting's bean (two underutilized African legumes) is tolerant of $NO_3^-$. So, although in this study the six cowpea cultivars showed significant inhibition of nodulation (Table 2) and $N_2$ fixation (Tables 4–6) caused by high concentrations of endogenously available N in the soil (Table 1), the individual varieties differed significantly in the extent of N damage to nodulation and nodule function. Compared to Pan 311 (the commercial variety), Bechuana White, Chappy and Encore seem relatively tolerant of mineral N, in that, they could still form functional nodules when their roots were exposed to very high concentrations of available N in the soil. Selecting such symbioses has the potential for promoting sustainable yields of crops under intensive cultivation as greater dependence on symbiotic fixation would spare soil N for cereal crops.

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REFERENCES

Amstrong, E.L., Pate, J.S., and Unkovich, M.J. 1994. Nitrogen balance of field pea crop in S.W. Australia, studies using the $^{15}N$ natural abundance technique. *Australian Journal of Plant Physiology* **21**: 533–549.

Ayisi, K.K., Putnam, D.H., Vance, C.P., and Graham, P.H. 1992. Dinitrogen fixation, nitrogen and dry matter accumulation, and nodulation in white lupin. *Crop Science* **32**: 1197–1202.

Banfalvi, Z., Nieuwkoop, A., Shell, M., Besl, L., and Stacey, G. 1988. Regulation of nod gene expression in *Bradyrhizobium japonicum. Molecular and General Genetics* **214**: 420–424.

Betts, J.H. and Herridge, D.F. 1987. Isolation of soybean capable of nodulation and nitrogen fixation under high levels of nitrate supply. *Crop Science* **27**: 1156–1161.

Cho, M.-J. and Harper, J.E. 1991a. Effect of inoculation and nitrogen on isoflavonoid concentration in wild-type and nodulation-mutant soybean roots. *Plant Physiology* **95**: 435–442.

Cho, J.-M. and Harper, J.E. 1991b. Effect of localized nitrate application on isoflavonoid concentration and nodulation in split-root systems of wild-type and nodulation-mutant soybean plants. *Plant Physiology* **95**: 1106–1112.

Dakora, F.D. 1994. Nodulation gene induction and genetic control in the legume-*Rhizobium* symbiosis. *South African Journal of Science* **90**: 596–599.

Dakora, F.D. 1998. Nodule function in symbiotic Bambara groundnut (*Vigna unguiculata* L.) and Kersting's bean (*Macrotyloma geocarpum* L.) is tolerant of nitrate in the root medium. *Annals of Botany* **82**: 687–690.

Dakora, F.D. and Keya, S.O. 1997. Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. *Soil Biology and Biochemistry* **29**: 809–817.

Dakora, F.D., Atkins, C.A., and Pate, J.S. 1992. Effect of $NO_3$ on $N_2$ fixation and nitrogenous solutes of xylem in two nodulated West African geocarpic legumes, Kersting's bean (*Macrotyloma geocarpum* L.) and Bambara groundnut (*Vigna subterranea* L.). *Plant and Soil* **140**: 255–262.

Dusha, I., Bakos, A., Kondorosi, A., de Bruijin, F.J., and Schell, J. 1989. The *Rhizobium meliloti* early nodulation genes (nodABC) are nitrogen-regulated: Isolation of a mutant strain with efficient nodulation capacity on alfalfa in the presence of ammonium. *Molecular and General Genetics* **219**: 89–96.

Eagelsham, A.R.J., Ayanaba, A., Rao, V.R., and Eskew, D.L. 1981. Improving the nitrogen nutrition of maize by intercropping with cowpea. *Soil Biology and Biochemistry* **13**: 169–171.

Hansen, A.P., Peoples, M.B., Gresshoff, P.M., Atkins, C.A., Pate, J.S., and Carroll, B.L. 1989. Symbiotic performance of supernodulating soybean (*Glycine max* L. Merr.) mutants during development on different nitrogen regimes. *Journal of Experimental Botany* **40**: 715–724.

Hardarson, G., Zapata, F., and Danso, S.K.A. 1984. Effect of plant genotype and nitrogen fertilizer on symbiotic fixation by soybean cultivars. *Plant and Soil* **82**: 397–405.

Herridge, D.F. and Betts, J.H. 1988. Field evaluation of soybean genotypes selected for enhanced capacity to nodulate and fix $N_2$ in the presence of nitrate. *Plant and Soil* **110**: 129–135.

Keeney, D.R. and Nelson, D.W. 1982. Nitrogen: Inorganic forms. In: *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, 2nd Edition. Agron. Monogr. 9. ASA and SSSA, Madison, USA, pp. 643–693.

Kohl, D.H., Shearer, G., and Harper, J.E. 1980. Estimates in $N_2$ fixation based on differences in the natural abundance of $^{15}N$ in nodulating and non-nodulating isolines of soybeans. *Plant Physiology* **66**: 61–65.

Layzell, D.B. and Moloney, A.H.M. 1994. Dinitrogen fixation. In: *Physiology and Determination of Crop Yield*, K.J. Boote, J.M. Bennett, T.R. Sinclair, G.M. Paulsen, eds. ASA-CSs-SSCA Inc., Madison, USA, pp. 311–335.

Marschner, H. 1995. *Mineral Nutrition in Higher Plants*, 2nd Edition. Academic Press, London.

Minchin, F.R., Minguez, M.I., Sheehy, J.E., Witty, J.F., and Skot, L.L. 1986. Relationships between nitrate and oxygen supply in symbiotic nitrogen fixation by white clover. *Journal of Experimental Botany* **37**: 1103–1113.

Oghoghorie, C.G.O. and Pate, J.S. 1971. The nitrate stress syndrome of the nodulated pea (*Pisum arvense* L.). Techniques for measurement and evaluation in physiological terms. In: *Proceedings of the Technical Meeting on Biological Nitrogen Fixation of the International Programme*, T.A. Lie and E.G. Mulder, eds. *Plant and Soil* special volume, pp. 185–202.

Pate, J.S., Atkins, C.A., White, S.T., Rainbird, R.M., and Woo, K.C. 1980. Nitrogen nutrition and xylem transport in nitrogen in ureide-producing grain legumes. *Plant Physiology* **65**: 961–965.

Powel, J.M., Pearson, R.A., and Hopkins, J.C. 1998. Impact of livestock on crop production. In: *Proceedings of Food, Lands and Livelihood – Setting Research Agenda for Animal Science*, Nairobi, January 1998. *Occasional Publication of the British Society of Animal Scientists* No. 21, pp. 53–66.

Senaratne, R., Amornpimol, C., and Hardarson, G. 1987. Effect of combined nitrogen on nitrogen fixation of soybean (*Glycine max* L. Merrill) as affected by cultivar and rhizobial strain. *Plant and Soil* **103**: 45–50.

Steel, R.G.D. and Torrie, J.H. 1980. *Principles and Procedures of Statistics, a Biometrical Approach*, 2nd Edition. McGraw-Hill, New York, pp. 172–194.

Streeter, J. 1988. Inhibition of legume nodule formation and $N_2$ fixation by nitrate. *CRC Critical Reviews in Plant Science* **7**: 1–23.

Unkovich, M.J., Pate, J.S., Amstrong, E.L., and Sanford, P. 1995. Nitrogen economy of annual crops and pasture legumes in Southwest Australia. *Soil Biology and Biochemistry* **27**: 585–588.

Unkovich, M.J., Pate, J.S., Sanford, P., and Amstrong, E.L. 1993. Potential precision of the $^{15}N$ natural abundance method in field estimation of nitrogen fixation by crop and pasture legumes in Southwest Australia. *Australian Journal of Agricultural Research* **45**: 119–132.

Van Kessel, C. and Roskoski, J.P. 1988. Row spacing effects in $N_2$ fixation, N yield and soil N uptake of intercropped cowpea and maize. *Plant and Soil* **111**: 17–23.

Wang, S.-P. and Stacey, G. 1990. Ammonia regulation of nod genes in *Bradyrhizobium japonicum. Molecular and General Genetics* **223**: 329–331.

Wu, S. and Harper, J.E. 1990. Nitrogen fixation of nodulation mutants of soybean as affected by nitrate. *Plant Physiology* **92**: 1142–1147.