*SYMBIOSIS* (2009) 48, 47–56  ISSN 0334-5114

# Measurement of $N_2$ fixation in 30 cowpea (*Vigna unguiculata* L. Walp.) genotypes under field conditions in Ghana, using the $^{15}N$ natural abundance technique

Alphonsus K. Belane and Felix D. Dakora*

Department of Crop Science, Faculty of Science, Arcadia Campus, Room 3-614E, 175 Nelson Mandela Drive, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa, Tel. +27-12-382 6120, Fax. +27-12-382 6286, Email. dakorafd@tut.ac.za

*(Received June 1, 2008; Accepted September 12, 2008)*

**Abstract**

In 2005 and 2006, 30 and 15 cowpea genotypes were respectively evaluated for plant growth and symbiotic performance at Manga in Northern Ghana, in order to identify $N_2$-fixing potential of these cowpea genotypes as source of N for cropping systems. The results showed differences in biomass production by the 30 or 15 cowpea genotypes. In 2005, cultivars Fahari, Mchanganyiko, IT97K-499-39, IT93K-2045-29 and IT84S-2246 produced the most shoot biomass, while Apagbaala, Brown Eye, ITH98-46, Vita 7 and Iron Grey produced the least. Of the 15 genotypes tested in 2006, cv. TVu11424 produced the largest amount of biomass, and ITH98-46, the least. Isotopic analysis of $^{15}N$ in plant parts also revealed significant differences in $\delta^{15}N$ of the cowpea genotypes studied. As a result, the percent N derived from fixation (% Ndfa) also differed among the cowpea genotypes tested in 2005, with only 5 out of the 30 cultivars obtaining over 50% of their N from symbiotic fixation. Whether expressed as mg N.plant$^{-1}$ or kg N.ha$^{-1}$, the levels of $N_2$ fixation by the cowpea genotypes varied considerably during 2005 and 2006, with values of N contribution ranging from 14.1 kg N.ha$^{-1}$ by cv. TVu1509 to 157.0 kg N.ha$^{-1}$ by IT84S-2246 in 2005. The amounts of N-fixed in 2006 ranged from 16.7 kg N.ha$^{-1}$ by cv. ITH98-46 to 171.2 kg N.ha$^{-1}$ by TVu11424, clearly indicating genotypic differences in symbiotic N yield. Re-evaluating 15 out of the 30 cowpea genotypes for $N_2$ fixation in 2006, revealed higher % Ndfa values (>50%) in all (15 cowpea genotypes) relative to those tested in 2005, indicating greater dependence on $N_2$ fixation for their N nutrition even though, the actual amounts of fixed-N were lower in 2006. This was due, in part, to reduced plant biomass as a result of very late sampling in 2006, close to physiological maturity (72 DAP in 2006 vs. 46 DAP in 2005) when considerable leaf matter was lost. The amount of N-fixed in 2006 can therefore be considered as being under-estimated.

**Keywords**: Cowpea genotypes, plant growth, $\delta^{15}N$, % Ndfa, N-fixed

## 1. Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is the most important food grain legume in Africa. As a result, it has very high genetic diversity and wide adaptation to different soil ecologies through out the continent (Ehlers and Hall, 1997). Dietarily, the grain contains about 22–25% protein (Oyenuga, 1959; Platt, 1962), and 57% carbohydrate (Platt, 1962), while the green leaves which are eaten as vegetables in many African countries, contain up to about 27–34.3% protein (Jasper and Norman, 1983; Ahenkora et al., 1998).

Cowpea plays a major role in tropical cropping systems, as it has been estimated to fix as much as 201 kg N.ha$^{-1}$ per season and contribute up to about 42 kg N.ha$^{-1}$ to the N nutrition of a following maize crop (Dakora et al., 1987). Because of its ability to improve the N economy of cropping systems, cowpea is traditionally grown as an intercrop with millet, sorghum, maize, and more recently cassava (Ehlers and Hall, 1997).

Symbiotic $N_2$ fixation in legumes can be measured using the $^{15}N$ natural abundance technique (Shearer and Kohl, 1986). However, the major problem limiting the use of this method is the choice of suitable reference plants that accurately measure the $^{15}N$ natural abundance of soil N

*The author to whom correspondence should be sent.

*Presented at the 15th International Congress on Nitrogen Fixation, January 21–26, 2007, Cape Town, South Africa*

taken up by the test legume (Shearer and Kohl, 1986; Bremer et al., 1993; Pate et al., 1994; Unkovich et al., 1994; Nyemba and Dakora, 2005). Fortunately, a number of studies have successfully used the $^{15}N$ natural abundance method to estimate $N_2$ in a range of grain and pasture legumes (Peoples et al., 1997; Unkovich et al., 1994; Pate et al., 1994). In Africa, the $^{15}N$ natural abundance has been used to estimate $N_2$ fixation in food grain legumes such as cowpea, groundnut, Bambara groundnut and pigeon pea (Ayisi et al., 2000; Adu-Gyamfi et al., 2007; Ncube et al., 2007; Ojiem et al., 2007; Adjei-Nsiah et al. 2008). Other methods such as the N difference and the $^{15}N$ fertilizer techniques have also been used to quantify $N_2$ fixation in cowpea (Eaglesham et al., 1981; Dakora et al., 1987; Dakora and Keya, 1997; Carsky et al., 2001; Ofori et al., 1987; Peoples and Herridge, 1990; Bado et al., 2006).

The data so far obtained have been variable, suggesting the need for a broader program of selecting cowpea for enhanced $N_2$ fixation. Thus, there is a need to identify high $N_2$-fixing cowpea genotypes that can contribute large amounts of biological N towards increased yields in traditional African cropping systems. This need for high $N_2$-fixing cowpea genotypes in African agriculture is heightened not only by the low levels of N in many of Africa's agricultural soils, but also by the fact that cowpea plays a critical role in household food security in Africa, and thus in the dietary quality of plant protein intake.

This study, conducted over a 2-year period during 2005 and 2006, examined plant growth, symbiotic performance, and N yield in as many as 30 cowpea genotypes collected from Ghana, South Africa, Tanzania, and the International Institute for Tropical Agriculture (IITA) in Nigeria, with the hope of identifying high $N_2$-fixing genotypes for use as biofertilizers in traditional cropping systems. To ensure high symbiotic performance, the cowpea collection had a good mix of both breeder-improved cultivars and farmer-selected varieties. The aim of the study was to screen and select cowpea genotypes that fix high levels of $N_2$ for use in enhancing N nutrition in the cropping systems of Africa.

## 2. Methods and Materials

*Field design and planting*

Field experiments were conducted at Manga Station of the Savanna Agricultural Research Institute, Ghana, in 2005, using 30 cowpea genotypes collected from Ghana, South Africa, Tanzania, and the International Institute for Tropical Agriculture (IITA) in Nigeria. In 2006, 15 of the 30 cowpea genotypes were re-planted at Manga for further evaluation of $N_2$ fixation. A randomized complete block design was used with four replicate plots for each cowpea genotype.

Each plot measured 15 $m^2$ (3 m × 5 m), with inter-row spacing of 60 cm in 2005, and 75 cm in 2006. Cowpea seeds were planted 20 cm apart within each row. Weeds were manually controlled with hand hoes. Two sprays of lambda cyhalothrin (Karate 2.5 EC) insecticide were applied at flowering and at pod formation as minimum protection against insect pests.

*Plant harvest and processing*

In 2005, plants were sampled from the middle rows of each plot at 46 DAP corresponding to about 50% flowering, while in 2006, plants were similarly sampled from the middle rows at 72 DAP corresponding to early physiological maturity. Each plant was separated into shoots, roots and nodules, oven-dried (70°C) to constant weight, ground (0.85 mm sieve), and stored prior to $^{15}N$ analysis.

*Determination of $^{15}N$ in plant samples, $\delta^{15}N$ and % Ndfa values*

The ratio of $^{15}N/^{14}N$, the $^{15}N$ natural abundance (‰), and %N of both cowpea and reference plant material were analyzed using a Carlo Erba NA 1500 elemental analyzer (Fisions Instruments SpA, Strada Rivoltana, Italy) coupled to a Finnigan MAT 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) via a Conflo II open-split device. Finely ground plant material was weighed (1.999 to 2.006 mg per shoot sample and 2.1 to 2.5 mg per root or non-fixing reference plant sample) into aluminum capsules, and loaded onto the Carlo-Erba system. *Nasturtium* spp. (4.62% N) was included as internal standard. All plant samples were combusted in evacuated quartz tubes in the presence of cupric oxide and metallic copper and the resultant gases cleaned on-line before entering the mass spectrometer. The $\delta^{15}N$ values of the *Nasturtium* spp. was used to correct machine errors.

As described by Unkovich et al. (1994), the $^{15}N$ abundance is usually expressed in a relative, δ (delta) notation, which is the ‰ deviation of the $^{15}N$ natural abundance of the sample from atmospheric $N_2$ (= 0.36637 atom % $^{15}N$):

$$\delta^{15}N = \frac{atom\%\,^{15}N\,sample - atom\%\,^{15}N\,air}{atom\%\,^{15}N\,air} * 1000$$

The proportion of legume N derived from atmospheric $N_2$ fixation was estimated as (Shearer and Kohl 1986; Unkovich et al., 1993; Boddey et al 2000):

$$\%\,Ndfa = [(\delta^{15}N_{ref} - \delta^{15}N_{leg}) / (\delta^{15}N_{ref} - B)] \times 100$$

where $\delta^{15}N_{ref}$ is the $^{15}N$ natural abundance of reference plant, $\delta^{15}N_{leg}$ is the $^{15}N$ natural abundance of legume and the B value is the $^{15}N$ natural abundance of cowpea

genotype which is dependent solely on symbiotic nitrogen fixation for its N nutrition. The *B* value used for cowpea was -1.759‰ and -0.940‰ for shoots and roots, respectively.

## 3. Results

*Plant growth*

Data collected on plant growth, symbiotic performance, and $\delta^{15}N$ are shown in Tables 1–6. The dry matter yield obtained in 2005 revealed significant differences among the 30 cowpea genotypes. At organ level, the genotype Fahari produced the largest amount of shoot dry matter, followed by the cvs. IT97K-499-39, IT84S-2246, and Mchanganyiko (Table 1). In contrast, two farmer varieties, Apagbaala and Bensogla, produced the least amount of shoot dry matter, generally about 3.4-fold less shoot biomass compared to cv. Fahari. The shoot dry matter yield of the remaining cowpea genotypes fell in between the two extremes (Table 1).

In 2005, root dry mass also differed among the 30 cowpea genotypes, with cvs. IT93K-2045-29, Brown Eye, Ngonji and Mchanganyiko showing greater root growth compared to cvs. Vuli-1, ITH98-46, TVu 1509, Iron Grey, ITH98-20, and Vita 7, which produced the least root dry mass (Table 1).

Because of the variation in organ biomass, whole-plant dry matter yield was also found to differ significantly among the 30 cowpea genotypes in 2005 (Table 1). For example, the cvs. Fahari, Mchanganyiko, IT97K-499-39, IT93K-2045-29 and IT84S-2246 produced the most biomass in 2005 (Table 1), while the genotypes Apagbaala, Brown Eye, Iron Grey, ITH98-46 and Vita 7 produced the least biomass on a per-plant basis (Table 1).

Similar to the 2005 study, there were large genotypic differences in the dry matter yield of the 15 cowpea genotypes evaluated in 2006. For example, cowpea cv. TVu11424 produced the greatest amount of shoot and plant total biomass in 2006, followed by the genotype Apagbaala, both of which were respectively 9- and 5-fold higher in biomass than ITH98-46, the cultivar with least dry matter yield (Table 2). Whole-plant (shoots + roots + nodules) dry matter yield of the remaining genotypes fell in between those of cvs. TVu11424 and ITH98-46.

*Field nodulation in cowpea genotypes*

There were strong differences in nodule numbers per plant in 2005. As shown in Table 1, Omondaw exhibited the highest nodulation, followed by Mchanganyiko, Fahari, Ngonji and Sanzie (all farmer-selected varieties) when compared to the other cowpea cultivars. In contrast, Apagbaala (a farmer variety) and four inbred cultivars (namely, CH14, IT82D-889, IT90K-76 and ITH98-20) showed the least nodulation (Table 1). Nodulation levels of the remaining genotypes were interspersed between the low and highest nodulating genotypes. The nodule dry matter in 2005 followed a similar pattern as nodule number per plant. The cultivars Omondaw and Mchanganyiko, which produced the highest number of nodules per plant, also had the largest nodule dry mass per plant (Table 1). In contrast, cowpea genotypes TVu11424 and Apagbaala, which were poorly nodulated, showed the lowest nodule dry matter per plant (Table 1).

In 2006, nodulation in the 15 genotypes tested was highest in cvs. Brown Eye and CH14 compared to the rest, with cv. Glenda showing the least nodule number per plant (Table 2). Because of the high nodulation of cv. Brown Eye, its nodule dry matter weight was significantly greater in 2006 (Table 2). Cultivar IT93K-2045-29 showed the lowest nodule dry weight in 2006 (Table 2).

*Amounts of N in organs and whole plants of cowpea genotypes*

There were genotypic differences in the N content of shoots, roots and whole plants in 2005 (Table 1). For example, the N content of shoots and roots were highest in cultivars Fahari, Mchanganyiko, and IT93K-2045-29 and thus resulted in markedly greater total biomass of the three genotypes (Table 1). This was in contrast to cultivars Apagbaala, Bensogla, ITH98-46 and Omondaw, which generally had reduced shoot and root dry matter yield, resulting in much smaller total plant biomass (Table 1). The remaining genotypes produced intermediate plant biomass.

The N content of shoots and whole plants were markedly greater in cowpea cv. TVu11424, followed by Apagbaala in 2006 (Table 2). In contrast, the N content of shoots and whole plants of cv. ITH98-46 were significantly lower than those of all the other cowpea varieties (Table 2).

*$\delta^{15}N$ values (‰) of reference plants*

In 2005, maize, sorghum and the weed *Hyptis specifigera* were the reference plant species sampled from the experimental site. The mean shoot and root $^{15}N$ natural abundance values ($\delta^{15}N$) of these reference plants were +5.123‰ and +3.57‰, respectively. The reference plant species sampled in 2006 included *Metracarpus villosus, Cynodon dactylon, Pennisetum pedicellatum, Eleusine indica, Cenchrus ciliaris* and *Hyptis specifigera*; and their mean shoot and root $\delta^{15}N$ values were +4.31‰ and +2.44‰, respectively. These averaged mean $\delta^{15}N$ values of the reference plants sampled for each year were used to estimate % Ndfa in the different cowpea genotypes, as earlier studies have shown that the combined mean $\delta^{15}N$ value of herbaceous weeds from a site can provide an accurate estimate of N-fixed (Pate et al., 1994; Nyemba and Dakora, 2005).

Table 1. Dry matter and grain yield, and N content of field-grown cowpea plants sampled at 46 DAP at Manga, Ghana in 2005. Mean values followed by dissimilar letters in a column are significant at P≤0.05. *ps = photosensitive genotypes, therefore not podded at physiological maturity of the other genotypes.

| Genotype | Nodule no. per plant | Dry matter | | | | Grain yield ($kg.ha^{-1}$) | N content | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Nodule dry mass ($mg.plant^{-1}$) | Shoots ($g.plant^{-1}$) | Roots ($g.plant^{-1}$) | Shoots+ roots+ nodules ($g.plant^{-1}$) | | Shoots ($mg.plant^{-1}$) | Roots ($mg.plant^{-1}$) | Shoots+ roots ($mg.plant^{-1}$) |
| Apagbaala | 6.4n | 300.0o | 14.1d | 6.3de | 20.9d | 458de | 639.3c | 65.5def | 704.8cd |
| Bengsogla | 26.3h | 1500.0ef | 14.9d | 5.6de | 55.2abc | 680bc | 633.7c | 166.4cde | 800.2bcd |
| Botswana White | 54.8d | 1333.3fg | 17.0cd | 15.0bc | 33.9cd | 903ab | 571.5c | 71.5def | 642.9d |
| Brown Eye | 19.9i | 2466.7cd | 29.8abcd | 22.7ab | 22.0cd | 637bcd | 1306.7abc | 317.9a | 1624.6ab |
| CH14 | 10.9m | 1300.0fgh | 24.2cd | 10.3cd | 36.1cd | 473cde | 954.1abc | 118.1def | 1072.3bcd |
| Fahari | 65.5c | 1633.3e | 47.8a | 18.4abc | 68.2a | 444def | 1823.9a | 231.5abc | 2055.4a |
| Glenda | 33.4g | 1133.3ghi | 34.5abc | 12.9cd | 48.8abc | 622bcd | 1389.8abc | 161.7cde | 1551.4abc |
| Iron Grey | 17.4ij | 500.0n | 19.2cd | 5.9de | 25.8cd | 221g | 715.4bc | 70.1cde | 785.4cd |
| IT82D-889 | 12.8lm | 1500.0ef | 29.4bcd | 16.3bc | 47.4abcd | 533cd | 1277.5abc | 173.4cde | 1450.9abc |
| IT84S-2246 | 17.2ij | 2500.0c | 43.3ab | 14.5bc | 60.5abc | 444def | 1598.0ab | 170.4cde | 1768.4ab |
| IT90K-59 | 32.3g | 2300.0d | 30.5abcd | 17.2bc | 50.3abc | 436def | 1115.1abc | 206.9bcd | 1322.0abcd |
| IT90K-76 | 13.1klm | 866.7jkl | 31.2abcd | 15.2bc | 47.5abcd | 488def | 1311.6abc | 178.7cde | 1490.3abc |
| IT93K-2045-29 | 36.4f | 1000.0jk | 36.0abc | 23.6a | 60.9abc | 324efg | 1412.2abc | 290.4ab | 1702.6ab |
| IT93K-452-1 | 43.2e | 700.0lm | 34.2abc | 10.9cd | 46.1abcd | 429ef | 1258.9abc | 123.4def | 1382.3abc |
| IT94D-437-1 | 16.0jk | 500.0n | 27.1bcd | 16.1bc | 43.8abcd | *ps | 1053.3abc | 204.4bcde | 1257.6abc |
| IT97K-499-39 | 19.2i | 1200.0ghi | 44.9ab | 15.5bc | 62.1abc | 429ef | 1678.1ab | 181.1cde | 1859.2ab |
| ITH98-20 | 13.2klm | 566.7mn | 21.3cd | 5.4de | 27.4cd | 518cde | 971.1abc | 60.0ef | 1031.0bcd |
| ITH98-46 | 36.7f | 800.0kl | 16.3cd | 4.9de | 22.2cd | 1036a | 668.0c | 60.4ef | 728.4cd |
| Line 2020 | 37.3f | 1066.7ij | 20.4cd | 13.5cd | 35.2cd | *ps | 888.9bc | 166.1cde | 1055.1bcd |
| Mamlaka | 24.6h | 800.0kl | 18.3cd | 13.3cd | 32.6cd | 470cde | 790.8bc | 186.5cde | 977.2bcd |
| Mchanganyiko | 73.6b | 3133.3b | 42.9ab | 20.0abc | 66.2ab | *ps | 1773.0ab | 252.4abc | 2025.4a |
| Ngonji | 52.2d | 1066.7ij | 27.9bcd | 21.0abc | 50.3abc | 429ef | 972.5abc | 236.1abc | 1208.6abcd |
| Omondaw | 85.0a | 4133.3a | 15.9cd | 8.5de | 28.6cd | 673bc | 599.4c | 101.7def | 701.1cd |
| Pan 311 | 15.6jkl | 800.0kl | 31.2abcd | 8.5de | 40.6bcd | 666bc | 1330.5abc | 102.5def | 1433.0abcd |
| Sanzie | 52.1d | 1100.0hi | 34.3abc | 12.8cd | 48.4abc | 888abc | 1201.5abc | 171.2cde | 1372.8abcd |
| TVu11424 | 16.9ij | 100.0p | 28.9bcd | 6.5de | 35.8cd | 251fg | 1207.7abc | 79.8def | 1287.5bcd |
| TVu1509 | 39.1f | 2300.0d | 17.9cd | 5.9de | 26.3cd | 251fg | 785.6bc | 82.0def | 867.6cd |
| TVX3236 | 37.7f | 1333.3fg | 29.9abcd | 9.1de | 40.5bcd | 547cd | 1218.3abc | 102.1def | 1320.3abcd |
| Vita 7 | 25.1h | 833.3kl | 17.8cd | 5.0de | 23.9cd | 429ef | 812.6bc | 61.1ef | 873.7cd |
| Vuli-1 | 25.4h | 1633.3e | 23.1cd | 4.3e | 29.2cd | 606bcd | 997.2abc | 51.1f | 1048.2bcd |
| $LSD_{0.05}$ | 398.5*** | 177.4*** | 2.2** | 3.2*** | 3.2*** | 6.5** | 1.8** | 5.9*** | 2.8*** |
| C.V. (%) | 5 | 9 | 4 | 32 | 30 | 11 | 13 | 27 | 12 |

*$\delta^{15}N$ values (‰) and % Ndfa of cowpea plants*

The $^{15}N$ natural abundance ($\delta^{15}N$ values) of shoots, roots, and whole plants differed significantly for the 30 cowpea cultivars in 2005. Farmer varieties such as Bensogla, Sanzie, Ngonji, Mamlaka, and the improved cv. IT84S-2246, showed the lowest $\delta^{15}N$ values in shoots in contrast to cvs. Apagbaala, Botswana White, TVu1509, TVX3236, IT90K-76, ITH98-20, IT94D-437-1, IT97K-499-39 and Fahari which generally exhibited the highest $\delta^{15}N$ in shoots (Table 3). Root $\delta^{15}N$ values also varied significantly among the 30 cowpea genotypes. Estimates of N derived from fixation (% Ndfa) in the 2005 trial revealed large genotypic differences in plant dependence on $N_2$ fixation for N nutrition. Only 5 out of the 30 cowpea genotypes derived over 50% of their N from symbiotic $N_2$ fixation (Table 3).

In 2006, there were significant differences in the $\delta^{15}N$ of shoots, roots and whole plants of field-grown cowpea at Manga. The $\delta^{15}N$ values of shoots and whole plants were generally very low compared to 2005 data (Table 4). Data on estimates of N derived from symbiotic $N_2$ fixation showed that genotypes with low $\delta^{15}N$ at organ level (e.g. TVu11424 and Botswana White) also had the highest % Ndfa values. In contrast, genotypes with high $\delta^{15}N$ values (e.g. Soronko, IT90K-59 and Glenda) exhibited the lowest % Ndfa values (Table 4).

Table 2. Dry matter yield and N content of cowpea varieties sampled at 72 DAP at Manga, Ghana, in 2006. Mean values with different letters in a column are significant at $P \leq 0.05$.

| Genotype | Nodule no. per plant | Dry matter | | | | | N content | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Nodule dry mass ($mg.plant^{-1}$) | Shoots ($g.plant^{-1}$) | Roots ($g.plant^{-1}$) | Shoots+ roots+ nodules ($g.plant^{-1}$) | Grain yield ($kg.ha^{-1}$) | Shoots ($mg.plant^{-1}$) | Roots ($mg.plant^{-1}$) | Shoots+ roots ($mg.plant^{-1}$) |
| Apagbaala | 7.8e | 73.3cd | 17.4b | 2.6c | 20.5b | 772.6cd | 564.8b | 61.0bcde | 625.8b |
| Botswana white | 8.3d | 97.3bc | 12.3de | 2.5c | 15.4d | 688.2d | 382.6cd | 57.6cdef | 440.2cd |
| Brown Eye | 11.9a | 137.3a | 13.0cde | 3.7b | 17.2bcd | 1007.1abcd | 389.7bcd | 68.2abcd | 457.9bcd |
| CH14 | 11.3b | 74.3cd | 15.4bcd | 1.59d | 17.9bcd | 1494.7a | 525.4bc | 33.9fghi | 559.3bc |
| Glenda | 3.7j | 42.3f | 15.3bcd | 2.67c | 18.4bcd | 1147.8abcd | 559.8b | 53.2defg | 613.0bc |
| IT82D-889 | 8.7c | 103.7b | 14.3bcd | 4.59a | 19.5bcd | 1264.6abcd | 431.0bcd | 84.6ab | 515.6bc |
| IT84S-2246 | 4.5hi | 60.7def | 14.5bcd | 1.3de | 16.2cd | 915.4bcd | 509.5bc | 24.6hi | 534.1bc |
| IT90K-59 | 5.6g | 47.7ef | 9.9ef | 1.3de | 11.7e | 1360.5ab | 312.7d | 23.5hi | 336.1d |
| IT93K-2045-29 | 4.7h | 16.3g | 14.2bcd | 4.6a | 19.4bcd | 1332.9abc | 516.4bc | 88.8a | 605.2bc |
| IT97K-499-39 | 7.5e | 54.7def | 15.7bc | 2.5c | 18.7bcd | 734.1d | 542.1bc | 55.0def | 597.1bc |
| ITH98-46 | 6.5f | 62.3def | 3.1g | 0.8e | 4.3f | 749.9d | 111.0e | 14.8i | 125.9e |
| Sanzie | 6.7f | 65.3def | 13.6cd | 2.0cd | 16.1cd | 880.4cd | 412.8bcd | 43.0efgh | 455.7bcd |
| Soronko | 5.5g | 104.0b | 13.7cd | 3.5b | 17.6bcd | 771.6cd | 427.9bcd | 81.3abc | 509.2c |
| TVu11424 | 5.6g | 85.3bcd | 33.0a | 2.0cd | 35.5a | 839.5bcd | 1177.1a | 40.6efgh | 1217.7a |
| Vuli-1 | 4.3i | 91.7bc | 7.6f | 1.5de | 9.5e | 993.3abcd | 265.1d | 28.7ghi | 293.8d |
| $LSD_{0.05}$ | 308.2*** | 14.1*** | 29.7*** | 21.0*** | 30.3*** | 2.3** | 19.4*** | 9.1*** | 20.6*** |
| C.V. (%) | 8 | 10 | 14 | 18 | 13 | 21 | 20 | 28 | 18 |

*Amounts of N-fixed in cowpea genotypes*

Estimates of N-fixed in whole cowpea plants differed significantly, and ranged from 84.5 to 941.7 $mg.plant^{-1}$ in 2005 (Table 5). Cultivars IT84S-2246, Mchanganyiko and Sanzie fixed significantly more N on per-plant basis in 2005 compared to the other genotypes (Table 5). The lowest amount of N-fixed was observed in cowpea cv. TVu1509 at both organ and whole-plant level (Table 5). In 2006, the amounts of N-fixed in the 15 cowpea genotypes ranged from 111.0 to 1177.1 $mg.plant^{-1}$, with cv. TVu11424 showing significantly more fixed-N in shoots and whole plants compared to the other 14 genotypes. Cowpea cv. ITH98-46 indicated the least amount of N-fixed (Table 6). Genotypes intermediate between low and high levels of $N_2$ fixation included cvs. Apagbaala, CH14, IT84S-2246, IT93K-2045-29 and IT97K-499-39 (Table 6).

Whether expressed on a per-hectare or per-plant basis, the amount of N-fixed in 2005 remained significantly greater in cowpea genotypes IT84S-2246, Mchanganyiko and Sanzie, with cv. TVu1509 being the lowest, followed by Apagbaala, Bensogla and IT90K-76 (Table 5). As with the per-plant data, cowpea cv. TVu11424 fixed more N on a per-hectare basis in 2006 relative to the other genotypes (Table 6), and produced 10.3-fold more fixed-N per hectare than ITH98-46, the lowest $N_2$-fixing genotype in 2006 (Table 6).

## 4. Discussion

In this study, there were strong variations in organ development and overall plant growth of the 30 or 15 cowpea genotypes tested in 2005 and 2006, respectively (Tables 1 and 2). In 2005, the cowpea genotypes Fahari, Mchaganyiko, IT97K-499-39, IT93K-2045-29 and IT84S-2246 produced significantly more biomass of the 30 cultivars (about 3.3, 3.2, 2.97, 2.91 and 2.89-fold respectively greater than cv. Apagbaala, the least in dry matter production). However, the data also showed that, in 2005, the same genotypes (i.e. Fahari, Mchaganyiko, IT97K-499-39, IT84S-2246 and IT93K-2045-29) exhibited the highest N content per plant, which were respectively 2.9, 2.87, 2.63, 2.51 and 2.41-fold greater than that of Apagbaala, the cultivar with least N yield (Table 1). Similar findings were obtained in 2006, with genotypes TVu11424, Apagbaala and IT82D-889 being the highest in both dry matter production and whole-plant N content compared to cv. ITH98-46, which was least (Table 1). More specifically, the genotypes TVu11424, Apagbaala and IT93K-2045-29 produced 8.26, 4.77 and 4.51-fold more biomass, and had 9.7 4.97 and 4.80-fold more N than cv. ITH98-46. As symbiotic parameters are reported to correlate with plant biomass (Herridge et al., 1990), the level of growth of a legume crop is therefore a function of its symbiotic N nutrition. In that regard, our data on plant growth and plant N yield of the best performing genotypes probably reflect their symbiotic performance. The higher nodulation (nodule

Table 3. $\delta^{15}N$ values and % Ndfa and B values of field-grown cowpea genotypes sampled at 46 DAP at Manga, Ghana, in 2005. Mean values followed by dissimilar letters in a column are significant at $P \leq 0.05$. Mean $\delta^{15}N$ values of shoots (5.12‰) and roots (3.57‰) of three plant species (i.e. maize, grain sorghum and the weed *Hyptis specifigera*) were used as reference plant values for estimating % Ndfa in cowpea.

| Genotype | $\delta^{15}N$ (‰) | | B values | | % Ndfa | |
|---|---|---|---|---|---|---|
| | Shoots | Roots | Shoots | Roots | Shoots | Roots |
| Apagbaala | 4.3bc | 3.8ab | -1.759 | -0.940 | 19.3jk | 16.1ghi |
| Bengsogla | 1.3l | 2.0bcd | -1.759 | -0.940 | 19.7jk | 6.9i |
| Botswana White | 4.2bc | 3.9ab | -1.759 | -0.940 | 59.6a | 36.4cd |
| Brown Eye | 3.8cd | 3.6ab | -1.759 | -0.940 | 25.3hijk | 13.6i |
| CH14 | 3.5ef | 3.2ab | -1.759 | -0.940 | 30.2ghi | 24.2fgh |
| Fahari | 4.5b | 2.3bcd | -1.759 | -0.940 | 25.3hijk | 25.8ef |
| Glenda | 3.4ef | 3.7ab | -1.759 | -0.940 | 30.6ghi | 15.3hi |
| Iron Grey | 3.8de | 2.4bcd | -1.759 | -0.940 | 26.1hijk | 15.9ghi |
| IT82D-889 | 3.1fg | 3.1ab | -1.759 | -0.940 | 34.3gh | 32.6cdef |
| IT84S-2246 | 1.5kl | 2.5bcd | -1.759 | -0.940 | 56.0abc | 27.2def |
| IT90K-59 | 2.1ij | 2.6bcd | -1.759 | -0.940 | 48.0bcd | 31.6cdef |
| IT90K-76 | 5.1a | 3.8ab | -1.759 | -0.940 | 8.5l | 16.2ghi |
| IT93K-2045-29 | 3.4ef | 2.6bcd | -1.759 | -0.940 | 30.8ghi | 26.4ef |
| IT93K-452-1 | 2.3ij | 2.8bc | -1.759 | -0.940 | 45.6cd | 24.6fg |
| IT94D-437-1 | 4.5b | 3.5ab | -1.759 | -0.940 | 16.3kl | 25.1efg |
| IT97K-499-39 | 4.2bc | 3.4ab | -1.759 | -0.940 | 20.5ijk | 26.2ef |
| ITH98-20 | 4.5b | 3.2ab | -1.759 | -0.940 | 16.3kl | 15.1hi |
| ITH98-46 | 3.8cd | 1.2d | -1.759 | -0.940 | 25.3hijk | 76.1a |
| Line 2020 | 3.6ef | 2.8bc | -1.759 | -0.940 | 27.7hij | 25.6ef |
| Mamlaka | 1.9jk | 2.7bc | -1.759 | -0.940 | 51.1abcd | 24.2fgh |
| Mchanganyiko | 2.4ij | 2.1cd | -1.759 | -0.940 | 44.0cde | 26.0ef |
| Ngonji | 1.5kl | 2.8bc | -1.759 | -0.940 | 56.0abc | 38.6c |
| Omondaw | 2.0ij | 2.2cd | -1.759 | -0.940 | 49.5bcde | 29.3cdef |
| Pan 311 | 4.3bc | 4.3a | -1.759 | -0.940 | 19.1jk | 8.7i |
| Sanzie | 1.4l | 2.5bcd | -1.759 | -0.940 | 58.2ab | 34.5cde |
| TVU11424 | 3.3ef | 2.5bcd | -1.759 | -0.940 | 32.6gh | 33.8cdef |
| TVU1509 | 5.1a | 2.2cd | -1.759 | -0.940 | 7.9l | 47.6b |
| TVX3236 | 5.1a | 2.2cd | -1.759 | -0.940 | 8.6l | 53.4b |
| Vita 7 | 2.8hi | 2.4bcd | -1.759 | -0.940 | 39.6efg | 50.4b |
| Vuli-1 | 3.1gh | 2.6bcd | -1.759 | -0.940 | 35.0fgh | 32.4cdef |
| $LSD_{0.05}$ | 39.6*** | 1.7** | nd | nd | 39.6*** | 1.7** |
| C.V. (%) | 10 | 28 | nd | nd | 16 | 17 |

number and weight) observed in genotypes with better plant growth in 2005 (e.g. Mchanganyiko, Fahari and IT84S-2246, see Table 1), and cowpea cultivars that showed improved growth in 2006 (e.g. TVu11424 and IT93K-2045 in 2006, see Table 6) would seem to suggest that plant growth benefits from nodule functioning in symbiotic legumes (Table 6).

Evaluating $N_2$ fixation in legumes using the $^{15}N$ natural abundance technique usually requires isotopic analysis of both legume and reference plants (Shearer and Kohl, 1986; Unkovich et al., 1993; Ramos et al., 2001; Teixeira et al., 2006). In this study, different plant species were sampled as non-$N_2$-fixing reference plants for measuring soil N uptake by the cowpea genotypes during both 2005 and 2006. Due to the large volume of data collected in this study, the mean $\delta^{15}N$ values of reference plant (shoots and roots) in 2005 and 2006 were used for estimating $N_2$ fixation in the cowpea genotypes. Because there were large differences in $\delta^{15}N$ values of organs and whole cowpea plants, estimates of N derived from fixation in 2005 and 2006 were also found to vary considerably at both organ and whole-plant level (Tables 3 and 4). As predicted above, the cowpea genotypes with greater biomass accumulation were generally among those with the lowest whole-plant $\delta^{15}N$ values (see cvs. IT84S-2246 and IT93K-2045-29 in Table 3, or TVu11424 and IT93K-2045-29 in Table 4) and also showed significantly greater Ndfa values (whether measured as a percentage; see Tables 3 and 4, or in mg $N.plant^{-1}$, see Tables 5 and 6). Interestingly, in 2005, only 5 out of the 30 cowpea genotypes obtained about 50% or more of their N from symbiotic fixation of atmospheric $N_2$ (Table 3), in contrast to 2006 where all 15 genotypes obtained over 50% of their N nutrition from symbiosis (Table 4). This difference in symbiotic functioning of the

Table 4. $\delta^{15}N$ (‰) and % Ndfa and B values of cowpea varieties sampled at 72 DAP at Manga, Ghana, in 2006. Mean values with dissimilar letters in a column are significant at $P \leq 0.05$. Mean $\delta^{15}N$ values of shoots (4.31‰) and roots (2.44‰) of six plant species (namely, *Metracarpus villosus, Cynodon dactylon, Pennisetum pedicellatum Eleusine indica, Cenchrus ciliaris* and *Hyptis specifigera*) were used as reference plant values for estimating % Ndfa in cowpea genotypes.

| Genotype | $\delta^{15}N$ (‰) | | B values | | % Ndfa | |
|---|---|---|---|---|---|---|
| | Shoots | Roots | Shoots | Roots | Shoots | Roots |
| Apagbaala | -0.2d | 1.1de | -1.759 | -0.940 | 74.0d | 38.7ab |
| Botswana white | -0.8ef | 1.1de | -1.759 | -0.940 | 83.7bc | 39.4ab |
| Brown Eye | -0.4d | 1.6bc | -1.759 | -0.940 | 76.8d | 26.2bcd |
| CH14 | -0.4d | 0.8e | -1.759 | -0.940 | 76.8d | 48.3a |
| Glenda | 0.9a | 1.4cd | -1.759 | -0.940 | 56.7g | 31.6b |
| IT82D-889 | 0.1c | 2.1a | -1.759 | -0.940 | 69.3e | 10.7e |
| IT84S-2246 | -0.9f | 1.5cd | -1.759 | -0.940 | 85.0b | 28.4bc |
| IT90K-59 | 0.4b | 2.0a | -1.759 | -0.940 | 65.0f | 13.7de |
| IT93K-2045-29 | -0.6e | 1.3cd | -1.759 | -0.940 | 81.0c | 32.4b |
| IT97K-499-39 | 0.2bc | 2.1a | -1.759 | -0.940 | 67.4ef | 9.4e |
| ITH98-46 | -1.1g | 2.2a | -1.759 | -0.940 | 89.0a | 7.8e |
| Sanzie | -0.8f | 1.1de | -1.759 | -0.940 | 84.4bc | 39.1ab |
| Soronko | 0.9a | 1.9ab | -1.759 | -0.940 | 55.7g | 15.1de |
| TVu11424 | -0.9fg | 1.2cde | -1.759 | -0.940 | 85.8ab | 37.6ab |
| Vuli-1 | -0.9f | 1.5cd | -1.759 | -0.940 | 85.0b | 28.1bc |
| $LSD_{0.05}$ | 80.6*** | 9.1*** | nd | nd | 12.0*** | 111.8*** |
| C.V. (%) | 5 | 16 | nd | nd | 3 | 27 |

cowpea genotypes between 2005 and 2006 at Manga was due to an earlier history of very high N fertilization of cereal crops in that same field prior to its use in this experiment. Thus, while soil N may not have limited nodule formation in this study (Herridge and Betts, 1988; Streeter, 1988), it probably decreased nodule functioning in some genotypes (Dakora et al., 1992; Dakora and Keya, 1997; Ayisi et al., 2000), hence the low levels of N-fixed.

Whether expressed as mg $N.plant^{-1}$ or kg $N.ha^{-1}$, the levels of $N_2$ fixation by the cowpea genotypes varied considerably during 2005 and 2006 (Tables 5, and 6), with values of symbiotic N contribution ranging from 14.1 kg $N.ha^{-1}$ by cv. TVu1509 to 157.0 kg N $ha^{-1}$ by IT84S-2246 in 2005. The amounts of N-fixed in 2006 also ranged from 16.7 kg $N.ha^{-1}$ by cv. ITH98-46 to 171.2 kg $N.ha^{-1}$ by TVu11424, clearly indicating genotypic differences in symbiotic N yield. When 15 out of the 30 cowpea genotypes were re-evaluated for $N_2$ fixation in 2006, all 15 genotypes showed higher shoot % Ndfa values relative to those tested in 2005 (Tables 3 and 4), indicating greater dependency on $N_2$ fixation for N nutrition (i.e. greater % Ndfa), even though the actual amounts of N-fixed per hectare were generally lower in 2006. This was due to the fact that the late sampling of plants in 2006 (at 72 DAP), resulted in loss of nodules and leaves from senescence, leading to reduced biomass and N accumulation (even though % Ndfa values and grain yield were much greater in 2006; see Tables 1–4). The data on N-fixed in 2006 can therefore be considered as being under-estimated. However, despite these differences in the age of cowpea plants (46 DAP in 2005 vs. 72 DAP in 2006), the amounts of N-fixed per shoot were generally similar for genotypes such as Botswana White, Brown Eye, IT82D-889, IT93K-2045-29, Glenda, and ITH98-46 during both 2005 and 2006.

In 2005, cowpea genotypes Fahari, Mchanganyiko, IT84S-2246 and Ngonji showed better plant growth and N-fixed in contrast to cvs. Apagbaala, ITH98-46, TVu1509 and Iron Grey, which exhibited the least plant growth and $N_2$ fixation (Tables 1 and 5). Interestingly, cowpea cv. Sanzie was not only the highest $N_2$-fixing genotype, it also produced the highest amount of grain yield compared to the other cowpea genotypes (Table 1). A similar pattern was observed in 2006, where cvs. TVu11424, Apagbaala, IT93K-2045-29 and CH4 showed greater plant growth and N-fixed, which led to significantly more grain yield by cvs. CH4 and IT93K-2045-29 compared to genotypes that produced low biomass and N-fixed (Tables 2 and 6). For example, cowpea cvs. ITH98-46, Vuli-1, IT90K-59 and Botswana White showed the least plant growth and N-fixed, which resulted in ITH98-46 and Botswana White producing the least grain yield (Tables 2 and 6). In a number of instances, there was thus a direct relationship between plant growth, $N_2$ fixation and grain yield, with the best performing genotypes showing greater plant growth, higher $N_2$ fixation and increased grain yield in contrast to low performing genotypes which showed poor plant growth, low $N_2$ fixation and low grain yields (Tables 1, 2, 5 and 6).

Compared to this study, cowpea has been shown to fix 24–29 kg $N.ha^{-1}$ in Kenya (Ssali and Keya, 1984), 122.0 kg

Table 5. Amount of N derived from fixation in field-grown cowpea genotypes sampled at 46 DAP at Manga, Ghana, in 2005. Mean values followed by dissimilar letters in a column are significant at P≤0.05.

| Genotype | N-fixed | | | | | |
|---|---|---|---|---|---|---|
| | Shoots | Roots (mg.plant$^{-1}$) | Shoots+Roots | Shoots | Roots (kg N.ha$^{-1}$) | Shoots+Roots |
| Apagbaala | 122.1cd | 10.7f | 132.8d | 20.3cd | 1.8f | 22.1d |
| Bengsogla | 123.5cd | 11.1f | 134.6d | 20.6cd | 1.8f | 22.4d |
| Botswana White | 337.8bcd | 26.8def | 364.6bcd | 56.3bcd | 4.5def | 60.8bcd |
| Brown Eye | 330.7bcd | 42.9cde | 373.6bcd | 55.1bcd | 7.1cde | 62.3bcd |
| CH14 | 283.7bcd | 28.7def | 312.3bcd | 47.3bcd | 4.8def | 52.1bcd |
| Fahari | 640.1abc | 59.0bcd | 699.1abc | 106.7abc | 9.8bcd | 116.5abc |
| Glenda | 432.7bcd | 24.3def | 457.0bcd | 72.1bcd | 4.0def | 76.2bcd |
| Iron Grey | 186.2cd | 11.4f | 197.7cd | 31.0cd | 1.9f | 32.9cd |
| IT82D-889 | 434.3bcd | 56.0bcd | 490.3bcd | 72.4bcd | 9.3bcd | 81.7bcd |
| IT84S-2246 | 895.5a | 46.2bcd | 941.7a | 149.3a | 7.7bcd | 157.0a |
| IT90K-59 | 531.3abc | 65.4abc | 596.7abc | 88.6abcd | 10.9abc | 99.5abc |
| IT90K-76 | 112.2cd | 29.0def | 141.2d | 18.7cd | 4.8def | 23.5d |
| IT93K-2045-29 | 445.8bcd | 75.5ab | 521.3bcd | 74.3bcd | 12.6ab | 86.9bcd |
| IT93K-452-1 | 572.9abc | 29.4def | 602.3abc | 95.5abcd | 4.9def | 100.4abc |
| IT94D-437-1 | 184.8cd | 51.2bcd | 236.0cd | 30.8cd | 8.5bcd | 39.3cd |
| IT97K-499-39 | 345.4bcd | 47.3bcd | 392.8bcd | 57.6bcd | 7.9bcd | 65.5bcd |
| ITH98-20 | 158.5cd | 9.1f | 167.6cd | 26.4cd | 1.5f | 27.9cd |
| ITH98-46 | 168.6cd | 43.4cde | 212.0bcd | 28.1cd | 7.2cde | 35.3cd |
| Line 2020 | 245.7bcd | 42.6cde | 288.3bcd | 40.9bcd | 7.1cde | 48.0bcd |
| Mamlaka | 422.0bcd | 43.7cde | 465.7bcd | 70.3cd | 7.3cde | 77.6bcd |
| Mchanganyiko | 767.8ab | 65.5abc | 833.3ab | 128.0ab | 10.9abc | 138.9a |
| Ngonji | 544.2abc | 91.2a | 635.4abc | 90.7abcd | 15.2a | 105.9ab |
| Omondaw | 297.0bcd | 28.3def | 325.3bcd | 49.5bcd | 4.7def | 54.2bcd |
| Pan 311 | 244.8bcd | 9.0f | 253.8bcd | 40.8bcd | 1.5f | 42.3bcd |
| Sanzie | 701.2abc | 59.3bcd | 760.6abc | 116.9abc | 9.9bcd | 126.8ab |
| TVu11424 | 396.4bcd | 27.1def | 423.5bcd | 66.1bcd | 4.5def | 70.6bcd |
| TVu1509 | 48.2d | 36.4def | 84.5de | 8.0d | 6.1def | 14.1d |
| TVX3236 | 105.3cd | 54.4bcd | 159.7cd | 17.6cd | 9.1bcd | 26.6cd |
| Vita 7 | 322.0bcd | 31.0def | 353.1bcd | 53.7bcd | 5.2def | 58.8bcd |
| Vuli-1 | 365.6bcd | 16.3ef | 381.9bcd | 60.9bcd | 2.7ef | 63.7bcd |
| $LSD_{0.05}$ | 3.2*** | 19.2*** | 2.0** | 3.2*** | 19.2*** | 2.0** |
| C.V. (%) | 19 | 12 | 18 | 6 | 4 | 6 |

N.ha$^{-1}$ in Nigeria (Eaglesham et al., 1981), and 201.0 kg N.ha$^{-1}$ in Ghana (Dakora et al., 1987). It has also been shown in Australia that cowpea can derive about 53–69% of its N supply from $N_2$ fixation, and contribute about 87 kg N.ha$^{-1}$ in monoculture (Ofori et al., 1987). Because a large number of cowpea genotypes were evaluated in this study, the proportions and amounts of N derived from symbiotic fixation were within the range of values obtained in similar studies in Southern Ghana, Western Kenya and Southeastern Zimbabwe where the $^{15}N$ natural abundance method was used to measure $N_2$ fixation in field-grown cowpea plants (Ncube et al., 2007; Ojiem et al., 2007; Adjei-Nsiah et al., 2008).

In conclusion, our results show that cowpea genotypes differ in their levels of $N_2$ fixation. This indicates that the choice of a high $N_2$-fixing genotype is important where biofertilization is the major objective for legume inclusion in the cropping system. The data further show that the low-N status of African soils can easily be overcome if high $N_2$-fixing cowpea genotypes such as IT84S-2246, Mchanganyiko, Sanzie, IT93K-2045-29, IT93K-452-1, Ngonji and TVu11424 are used in cropping systems to provide symbiotic N, which can range from 61.0 to 171.2 kg N.ha$^{-1}$ (Tables 5 and 6).

**Acknowledgement**

We are grateful to the McKnight Foundation for the grant on South Africa Legumes Project, which supported this study as well as the student bursary of Alphonsus Belane. The National Research Foundation and the South African Research Chair on Agrochemurgy and Plant Symbioses are duly acknowledged for supporting the research of Felix Dakora.

Table 6. Amount of N-fixed in field-grown cowpea varieties sampled at 72 DAP at Manga, Ghana, in 2006. Mean values followed by dissimilar letters in a column are significant at P≤0.05.

| Genotype | N-fixed | | | | | |
|---|---|---|---|---|---|---|
| | Shoots | Roots ($mg.plant^{-1}$) | Shoots+Roots | Shoots | Roots ($kg\ N.ha^{-1}$) | Shoots+Roots |
| Apagbaala | 564.8b | 61.0bcde | 625.8b | 69.7b | 3.9ab | 73.7b |
| Botswana white | 382.6cd | 57.6cdef | 440.2cd | 53.2bcd | 3.8ab | 57.0bcd |
| Brown Eye | 389.7bcd | 68.2abcd | 457.9bcd | 50.0bcd | 3.0abc | 53.0bcd |
| CH14 | 525.4bc | 33.9fghi | 559.3bc | 67.2b | 2.7bcd | 70.0b |
| Glenda | 559.8b | 53.2defg | 613.0bc | 53.0bcd | 2.8bc | 55.7bcd |
| IT82D-889 | 431.0bcd | 84.6ab | 515.6bc | 50.0bcd | 1.5cde | 51.5bcd |
| IT84S-2246 | 509.5bc | 24.6hi | 534.1bc | 72.1b | 1.1cde | 73.2b |
| IT90K-59 | 312.7d | 23.5hi | 336.1d | 33.8de | 0.5e | 34.3de |
| IT93K-2045-29 | 516.4bc | 88.8a | 605.2bc | 69.7b | 4.7a | 74.4b |
| IT97K-499-39 | 542.1bc | 55.0def | 597.1bc | 60.9cd | 0.8de | 61.7bc |
| ITH98-46 | 111.0e | 14.8i | 125.9e | 16.5e | 0.2e | 16.7e |
| Sanzie | 412.8bcd | 43.0efgh | 455.7bcd | 58.0bc | 3.0abc | 61.0cd |
| Soronko | 427.9bcd | 81.3abc | 509.2bc | 39.7cd | 2.0bcde | 41.7cd |
| TVu11424 | 1177.1a | 40.6efgh | 1217.7a | 168.5a | 2.7bcd | 171.2a |
| Vuli-1 | 265.1d | 28.7ghi | 293.8d | 37.4cde | 1.4cde | 38.8cd |
| $LSD_{0.05}$ | 9.1*** | 20.6*** | 6.89** | 21.7*** | 14.0*** | 6.9** |
| C.V. (%) | 27 | 20 | 22 | 20 | 5 | 19 |

## REFERENCES


Ahenkora, K., Adu Dapaah, H.K., and Agyemang A. 1998. Selected nutritional components and sensory attributes of cowpea (*Vigna unguiculata* (L.) Walp) leaves. *Plant Foods for Human Nutrition* **52**: 221–229.

Ayisi, K.K., Nkgapele, R.J., and Dakora, F.D. 2000. Nodule formation and function in six varieties of cowpea (*Vigna unguiculata* L. Walp) grown in a nitrogen-rich field soil in South Africa. *Symbiosis* **28**: 17–31.

Boddey, R.M., Peoples, M.B., Palmer, B., and Dart, P.J. 2000. Use of the $^{15}N$ natural abundance technique to quantify biological nitrogen fixation by woody perennials. *Nutrient Cycling in Agroecosystems* **57**: 235–270.

Bado, B.V., Bationo, A., and Cescas, M.P. 2006. Assessment of cowpea and groundnut contributions to soil fertility and succeeding sorghum yields in the Guinean savannah zone of Burkina Faso (West Africa). *Biology and Fertility of Soils* **43**: 171–176.

Bergersen, F.J. and Turner, G.L. 1983. An evaluation of $^{15}N$ methods for estimating nitrogen fixation in a subterranean clover-perennial ryegrass sward. *Australian Journal of Agricultural Research* **34**: 391–401.

Bremmer, E., Gehlen, H., Swerhone, G.D.W., and van Kessel, C. 1993. Assessment of reference crops for the quantification of $N_2$ fixation using natural and enriched levels of $^{15}N$ abundance. *Soil Biology and Biochemistry* **25**: 1197–1202.

Carsky, R.J., Singh, B.B., and Oyewole, B. 2001. Contribution of early season cowpea to late season maize in the savanna of West Africa. *Biological Agriculture and Horticulture* **18**: 303–315.

Dakora, F.D., Aboyinga R.A., Yahaya, M., and Apaseku, J. 1987. Assessment of $N_2$ fixation in groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L. Walp) and their relative N contribution to succeeding maize crop in Northern Ghana. *MIRCEN Journal* **3**: 389–399.

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.

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.

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

Ehlers, J.D. and Hall, A.E. 1997. Cowpea (*Vigna unguiculata* L. Walp). *Field Crops Research* **53**: 187–204.

Jasper, K.I. and Norman, N.P. 1983. Nutrient contents of raw and cooked cowpea leaves. *Journal of Food Science* **48**: 1252–1254.

Ncube, B., Twomlow, S.J., Van Wijk, M.T., Dimes, J.P., and Giller, K.E. 2007. Productivity and residual benefits of grain legumes to sorghum under semi-arid conditions in southwestern Zimbabwe. *Plant and Soil* **299**: 1–15.

Nyemba, R.C. and Dakora, F.D. 2005. Identifying suitable arable weeds as reference plants for measuring $N_2$ fixation in grain legumes in Zambia using $^{15}N$ natural abundance. *Symbiosis* **40**: 79–86.

Ofori, F. and Stern, W.R. 1987. Cereal-legume intercropping systems. *Advances in Agronomy* **41**: 41–90.

Ojiem, J.O., Vanlauwe, B., de Ridder, N., and Giller, K.E. 2007. Niche-based assessment of contribution of legumes to nitrogen economy of Western Kenya smallholder farm. *Plant and Soil* **292**: 119–135 .

Oyenuga, V.A. 1959. *Nigerian Feeding Stuffs: Their Compositions and Nutritional Value.* 2nd Edition. University Press, 120 pp.

Pate, J.A., Unkovich, M.J., Amstrong, E.L., and Sanford, P. 1994. Selection of reference plants for $^{15}N$ natural abundance assessment of $N_2$ fixation by crop and pasture legumes in

South-west Australia. *Australian Journal of Agricultural Research* **45**: 133–147.

Pausch, R.C., Mulchi, C.L., Lee, E.H., and Meisinger, J.J. 1996. Use of $^{13}C$ and $^{15}N$ isotopes to investigate $O_3$ effects on C and N metabolism in soybeans. Part II. Nitrogen uptake, fixation and partitioning. *Agriculture, Ecosystems and Environment* **60**: 61–69.

People, M.B. and Herridge, D.F. 1990. Nitrogen fixation by legumes of tropical and subtropical agriculture. *Advances in Agronomy* **44**: 155–223.

Peoples, M.B., Turner, G.L., Shah, Z., Shah, S.H., Aslam, M., Ali, S., Maskey, S.L., Bhattarai, S., Afandi, F., Schwenke, G.D., and Herridge, D.F. 1997. Evaluation of the $^{15}N$ natural abundance technique to measure $N_2$ fixation in experimental plots and farmers fields. In: *Extending Nitrogen Fixation to Farmers Fields.* Rupela, O.P., Johansen, C., and Herridge, D.F., eds. ICRISAT, Patancheru, India. pp. 57–76.

Platt, B.S. 1962. Tables of representative values of food commonly used in tropical countries. *Medical Research Council* special report, series 20. MRC, London.

Ramos, M.G., Villatora, M.A.A., Urquiaga, S., Alves, B.J.R., and Boddey, R.M. 2001. Quantification of the contribution of biological nitrogen fixation to tropical green manure crops and residual benefits to subsequent maize crop using $^{15}N$-isotope dilution techniques. *Journal of Biotechnology* **91**: 105–115.

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

Shearer, G. and Kohl, D.H. 1986. $N_2$-fixation in field settings: Estimations based on $^{15}N$ natural abundance. *Australian Journal of Plant Physiology* **13**: 699–756.

Teixera, C.P., Reinert, F., Rumjanek, N.G., and Boddey, R.M. 2006. Quantification of biological nitrogen fixation to *Cratylia mollis* using the $^{15}N$ natural abundance technique in the semi-arid Caatinga region of Brazil. *Soil Biology and Biochemistry* **38**: 1989–1993.

Unkovich, M.J., Pate, J.S., Sanford, P., and Amstrong, E.L. 1994. Potential precision of the $\delta^{15}N$ natural abundance method in field estimation of nitrogen fixation by crop and pasture legumes in South-west Australia. *Australian Journal of Agricultural Research* **45**: 119–132.

Ssali, S. and Keya, S.O. 1984. Nitrogen level and cultivar effects on nodulation, dinitrogen fixation and yield of grain legumes. I. Cowpea cultivars. *East Africa Journal of Agriculture and Forestry* **45**: 247–254.