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Full Length Research Paper

Assessment of N_2 fixation in 32 cowpea (*Vigna unguiculata* L. Walp) genotypes grown in the field at Taung in South Africa, using ^{15}N natural abundance

Alphonsus K. Belane[1], Joseph Asiwe[2] and Felix D. Dakora[3]*

[1]Department of Crop Science, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa.
[2]Agricultural Research Centre (ARC)-Grain Crop Research Institute, Pochefstroom 2522, South Africa.
[3]Faculty of Science, Arcadia Campus, Nelson Mandela Drive, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa.

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The aim of this study was to evaluate plant growth, grain yield and symbiotic N contribution by 32 cowpea genotypes, at Taung in South Africa. The data from a 2-year field study conducted in 2005 and 2006 showed that genotypes Fahari, Pan 311 and Glenda exhibited the highest dry matter yield and N contribution as they produced 2.9-, 2.7- and 3.5-fold more dry matter than cv. ITH98-46 and yielded 2.7-, 2.2- and 3.2-fold more N than cultivar ITH98-46 from IITA. Except for Benpila, all the 32 cowpea genotypes derived between 52.0 and 80.9% of their N nutrition from symbiotic fixation in 2005, with IT82D-889, Botswana White, IT93K-2045-29 and Ngonji exhibiting the highest %Ndfa values. The genotype Fahari showed the highest amount N-fixed (182 kg N-fixed/ha), followed by Pan 311, Glenda, TVu11424 and Mamlaka which contributed 160, 146, 130 and 125 kg N/ha, respectively. Genotypes Pan 311, Fahari and Glenda were among those that produced highest grain yield in 2005 and except for CH14 and IT86S-2246 (which produced 131 kg N/ha each), Fahari, Glenda and Pan 311, were again the highest in symbiotic N contribution (112, 106 and 105 kg N/ha, respectively). Grain yield was similarly high in Glenda, Pan 311 and Fahari (3.3, 3.1 and 2.9 t/ha, respectively) in 2006. In general, these data show that genotypes that fixed more N also produced more biomass and grain yield and are therefore, the best candidates for inclusion in cropping systems as biofertilizers.

Key words: Symbiotic performance, N nutrition, biomass, N-fixed, cowpea varieties.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is the most important food legume indigenous to Africa and is used extensively in cropping systems of the continent (Eaglesham et al., 1981; Vandermeer, 1992). This legume is mycorrhizal (Kwapata and Hall, 1985) and highly adapted to different soil ecologies (Hall and Patel, 1985), including low soil fertility (Elowad and Hall, 1987) as well as acidic and alkaline soil conditions (Fery, 1990). The crop is grown mainly as a grain legume but the leaves and fresh green pods are also eaten as vegetables in many parts of Sub-Saharan Africa. Nutritionally, the grain contains 23.4% protein, 56.8% carbohydrates and 3.6% ash (Platt, 1962), while the green leaves eaten as vegetables contain between 27.1 and 34.7% protein (Jasper and Norman, 1983; Ahenkora et al., 1998).

An earlier study that evaluated N_2 fixation in cowpea using the N difference method reported N contribution of about 201 kg N/ha from the symbiosis, with an estimated benefit of 42 kg N-fixed/ha to a succeeding maize crop (Dakora et al., 1987). Recent studies have used the ^{15}N natural abundance technique to assess N_2 fixation in field-grown cowpea plants. However, the data obtained have shown rather varied levels of symbiotic N contribution. For example, cowpea fixed about 32 to 67 kg

*Corresponding author. E-mail: dakorafd@tut.ac.za Tel: 27-12-382 6120. Fax: 27-12-382 6286.

N/ha in the forest and transitional zones of Ghana (Adjei-Nsiah et al., 2008), while in southwestern Zimbabwe, the species contributed only about 4 to 28 kg N-fixed/ha (Ncube et al., 2007). In Australia, this legume has also been reported to contribute about 87 kg N/ha (Ofori and Stern, 1987).

Past programmes on cowpea improvement in South Africa led to the commercial release of varieties such as Glenda and Pan311. However, these improved varieties have never been assessed for their symbiotic N contribution. Except for one report (Ayisi et al., 2000), which selected cowpea genotypes for nitrate tolerance, little is known about the N_2-fixing capacity of both local and improved cowpea varieties in South Africa. As a result, the contribution of cowpea to the N economy of cropping systems also remains unknown in South Africa. Yet low soil nutrient fertility is the major constraint to increased crop yields in Africa, including South Africa. Because chemical fertilizers are expensive and inaccessible to small-scale farmers, selecting high N_2-fixing and high-yielding cowpea genotypes could be one way to improve soil N fertility in traditional cropping systems for resource-poor farmers in Africa. This study assessed plant growth, symbiotic performance and N contribution by 32 cowpea genotypes planted in the field at Taung, South Africa, in 2005 and 15 of them re-evaluated in 2006 using the ^{15}N natural abundance technique.

MATERIALS AND METHODS

Site description and soil characteristics

The experiments were conducted at the Agricultural Research Council (ARC)-Grain Crop Research Institute farm at Taung, in 2005 and 2006. The site is located between latitudes 27°30′ N, 30°S; and longitudes 24°30′ W and 25°E. The agro-ecology is a grassland savanna with a unimodal rainfall (mean annual 560 mm) pattern that begins in October and lasts until June/July. The dominant soil type is structureless and freely drained AC red-yellow Apedal (SCWG, 1991). The experimental site was previously cropped to maize (*Zea mays L.*) with the application of NPK fertilizer. The plants were surface irrigated during period of dry spell. Soil was sampled from experimental plots and pooled. Sub-samples were taken from pool samples and analyzed for nutrient status before planting. Soil properties before planting were: pH (H_2O) 7.02, NO_3-N 1.3 mg/kg, NH_4-N 2.8 mg/kg, P 21.4 mg/kg, K 158 mg/kg, Ca 756 mg/kg, Mg 320 mg/kg, Na 29.2 mg/kg and 0.73% C.

Field design

The experimental treatments in this study consisted of 32 cowpea genotypes collected from Ghana, South Africa, Tanzania and the International Institute for Tropical Agriculture (IITA) in Nigeria. A randomized complete block design was used with four replicate plots for each cowpea genotype. Each experimental plot consisted of four rows and measured 3 m × 5 m. Cowpea genotypes were sown in mid-November in 2005 and early December in 2006. Inter and intra-row spacing was 60 cm and 20 cm, respectively. Weeds were manually controlled by hand hoe.

Plant sampling and processing

Ten plants were sampled from the two middle rows of each plot at 76 days after planting (DAP) in 2005 which corresponded to late vegetative growth phase (that is, 50% flowering to early pod-fill stage) and at physiological maturity in 2006. In 2005, the plants were dug out and then separated into shoots, roots and nodules. Nodules number per plant was determined and plant parts (nodules, roots and shoots) pooled per plot, oven-dried (60°C) and weighed for dry matter determination. The samples were then ground (0.85 mm sieve) and stored prior to ^{15}N analysis using mass spectrometry. In 2006, however, only the plant shoots and grain were harvested and analyzed for ^{15}N.

$^{15}N/^{14}N$ isotopic analysis

The isotopic ratio of $^{15}N/^{14}N$ and the concentration of N in plant organs and whole plants were determined using a Thermo Finnigan delta plus XP stable light isotope mass spectrometer coupled to Carlo Erba NA1500 elemental analyzer (Fisons Instrument SpA, Strada Rivolta, Italy) via Conflo II open-split device. Weighed samples of finely ground plant material (1.999 to 2.006 mg for shoots and 2.100 to 2.500 mg for roots of legume and non-fixing reference plant) were loaded onto the Carlo-Erba system, where they were combusted in evacuated quartz tubes in the presence of cupric oxide and metallic copper and the resultant gases cleaned on-line before being introduced to the mass spectrometer.
The isotopic composition ($\delta^{15}N$) was measured as the difference in the number of atoms of ^{15}N to ^{14}N in atmospheric (atm) N_2:

$$\delta^{15}N = \frac{\left({}^{15}N/{}^{14}N\right)_{sample} - \left({}^{15}N/{}^{14}N\right)_{atm}}{\left({}^{15}N/{}^{14}N\right)_{atm}} \times 1000$$

Where, whole plant ^{15}N natural abundance was calculated as an average of the ^{15}N natural abundance values of all plant parts weighted by their respective total N content (Robinson et al., 2000):

$$\delta^{15}N_{whole plant} = \frac{\sum\left(\delta^{15}N_{root} \times N_{root} + \delta^{15}N_{shoot} \times N_{shoot}\right)}{\sum\left(N_{root} + N_{shoot}\right)}$$

N content

The N content in organs or whole plant was determined from %N and sample weight using the equation (Pausch et al., 1996):

Amount of N (mg.plant^{-1}) = % N_{organ} x dry $mass_{organ}$

Percentage of Ndfa

The proportion of legume N derived from fixation of atmospheric N_2 (%Ndfa) was estimated as (Unkovich et al., 1994):

$$\%Ndfa = 100 \times \frac{x - y}{x - B}$$

Where, is the 15N natural abundance of non-N2-fixing reference plant; y is the ^{15}N natural abundance of legume; B is the ^{15}N natural abundance of test legume deriving all of its N nutrition from N_2

fixation. The B value represents isotopic fractionation associated with atmospheric N_2 fixation.
The amount of N-fixed was calculated as (Maskey et al., 2001):

N-fixed = %Ndfa x legume biomass N

Where, legume biomass N was obtained from shoots and roots.

Statistical analysis

All data were analyzed using software of STATISTICA 2 program 2007 (Statsoft Inc., Tulsa, OK, USA). Analysis of variance (ANOVA) was done to test significance levels of the variables and the Duncan multiple range (DMRT) test was used to compare treatment means at $p \leq 0.05$. In order to determine the relationship between dry matter yield and N-fixed or nodule-specific activity and plant growth, Pearson correlation analysis was done.

RESULTS

Field nodulation in cowpea

There were significant differences ($p \leq 0.05$) in nodule numbers and nodule dry matter among the 32 cowpea genotypes planted in 2005 and harvested at 76 DAP (Table 1). There was significantly better nodulation in cowpea cultivars (cvs.) IT93K-2045-29, Line 2020, Bensogla, Fahari, Vuli-1 and IT90K-76 relative to CH14, Brown Eye, Encore, IT94D-437-1, Omondaw, Soronko and TVu11424. The remaining genotypes produced intermediate nodules between the high and low nodulators. However, contrary to expectation, nodule dry matter was greater in cvs. IT82D-889, Benpila and Fahari and not those showing higher nodule numbers. Cowpea cvs. Apagbaala, Bensogla, Botswana White, CH14, IT94D-437-1, IT96D-1951, Line2020, Mamlaka, Mchan-ganyiko and Vallenga exhibited low nodule dry matter (Table 1). In 2006, the cowpea genotypes were harvested at physiological maturity.

Dry matter yield

There were large differences in plant growth (measured as dry matter yield) among the 32 cowpea genotypes in 2005 (Table 1). Cultivars Fahari from Tanzania, Pan311 and Glenda from South Africa and TVu11424 from IITA accumulated the largest amounts of shoot biomass, while cvs. ITH98-46, Line 2020 and Benpila showed the least shoot dry matter (Table 1). Cowpea root biomass also differed significantly, with genotypes CH14 and Fahari accumulating 3.2-fold root dry matter over cv. ITH98-46. As with shoot dry matter, cvs. Fahari, Glenda and Pan311 showed the highest dry matter yield at whole-plant level, with Fahari accumulating 3.6-fold more total biomass than cv.ITH98-46, which had the least dry matter (Table 1). Plant growth also differed significantly among the 15 cowpea genotypes planted in 2006. For example, cvs. CH14, Botswana White and Mamlaka produced more dry matter compared with Bensogla, which was the least in growth (Table 3).

N content

In 2005, N yield was markedly different among the 32 cowpea varieties at both organ and whole-plant level. As shown in Table 1, the genotypes with the highest N accumulation were also the genotypes that produced the highest dry matter yield. Thus, cv. Fahari (which showed the best growth) accumulated more N in organs and whole plants relative to ITH98-46 (which had the least growth). In fact, Fahari accumulated 3.7-fold more N than ITH98-46. The amount of N in cowpea roots also varied with genotype (Table 1). The 15 cowpea genotypes planted in 2006 also exhibited differences in N content, with Bensogla, showing the least N (Table 3).

$\delta^{15}N$ values of reference plants

In this study, *Eragrostis* sp. and a broad-leafed weed were sampled as reference plants from experimental sites in 2005 and 2006 and used to determine soil N uptake and isotopic fractionation associated with that process. The combined mean $\delta^{15}N$ values of shoots of reference plants were +5.03 and +3.17% in 2005 and 2006 respectively, and roots +3.56% in 2005 (as only shoots were harvested in 2006). It is these averaged $\delta^{15}N$ values of reference plants that were used to estimate %Ndfa in the 32 cowpea genotypes in 2005 and the 15 genotypes reassessed in 2006. This approach was based on the finding that the combined mean $\delta^{15}N$ values of different herbaceous weeds from a site can be used to accurately estimate %Ndfa in grain legumes (Pate et al., 1994; Nyemba and Dakora, 2005). Furthermore, the greater the differences in magnitude between the $\delta^{15}N$values of a nodulated legume and that of the non-legume reference plant, the more accurate the estimation of %Ndfa (Shearer and Kohl, 1986; Unkovich et al., 1994).

$\delta^{15}N$ values of cowpea genotypes

The $\delta^{15}N$ value of a nodulated legume is a good indicator of whether a plant is dependent on soil N or N_2 fixation for its N nutrition. The lower the $\delta^{15}N$ value of legume, the greater the N_2 fixation. Just like other parameters, the 32 cowpea genotypes differed significantly in the $\delta^{15}N$ values of shoots and roots in 2005 (Table 2). The estimated $\delta^{15}N$ values of whole plants using the equation by Robinson et al. (2000) also differed among the cowpea genotypes (Table 2). The shoot $\delta^{15}N$ values varied widely, ranging from -1.2(‰) in cv. CH14 to 1.8(‰) in cv. Benpila (Table 2). Most of the cowpea genotypes

Table 1. Dry matter yield and N level of field-grown cowpea plants sampled at 76 days after planting at Taung, South Africa, in 2005.

Cowpea genotype	Nodulation (no/plant)	Dry matter				Grain yield (t/ha)	N concentration			N content		
		Nodule (mg/plant)	Root (g/plant)	Shoot	Whole plant		Root (%)	Shoot	Whole plant	Root (mg/plant)	Shoot	Whole plant
Apagbaala	24^{fgh}	132^{ij}	12.7^{efg}	1.0f^{g}	13.9^{fgh}	0.6^{ef}	1.4^{a}	4.6^{bcd}	3.0^{bc}	15^{efg}	582^{efg}	597^{efg}
Benpila	33^{cd}	665^{b}	9.9^{gh}	1.4^{ef}	11.4^{gh}	0.2^{f}	1.4^{a}	5.7^{a}	3.7^{a}	29^{bcd}	601^{efg}	621^{def}
Bensogla	45^{b}	113^{j}	15.6^{def}	1.4^{ef}	17.2^{efg}	3.1^{ab}	1.2^{a}	4.7^{bc}	2.9^{cd}	20^{efg}	718^{cdef}	735^{cde}
Botswana White	25^{fgh}	171^{hij}	18.9^{bcd}	2.4^{bcd}	21.9^{bcd}	1.6^{de}	1.2^{a}	4.0^{de}	2.7^{cde}	17^{efg}	778^{cdef}	807^{cde}
Brown Eye	16^{ij}	362^{efg}	13.2^{efg}	2.2^{cd}	15.8^{efg}	0.5^{ef}	1.6^{a}	4.2^{cde}	2.9^{cd}	35^{abc}	550^{efg}	585^{efg}
CH14	15^{j}	137^{ij}	15.3^{def}	2.9^{a}	18.3^{efg}	2.2^{bcd}	1.4^{a}	4.3^{cde}	2.8^{cd}	42^{a}	652^{def}	693^{def}
Encore	17^{ij}	321^{fg}	18.0^{cde}	1.5^{ef}	19.8^{cd}e	1.5^{de}	1.5^{a}	4.6^{bcd}	3.0^{bc}	23de	806^{cde}	830^{cde}
Fahari	43^{b}	539^{c}	28.6^{a}	2.9^{a}	32.0^{a}	1.5^{de}	1.2^{a}	4.6^{bcd}	2.9^{cd}	37^{ab}	1294^{a}	1331^{a}
Glenda	33^{cd}	399^{def}	22.5^{bc}	1.8^{ef}	24.7^{bc}	1.9^{cd}	1.6^{a}	5.3^{a}	3.5^{ab}	29^{bcd}	1189^{a}	1218^{a}
Iron Gray	17^{ij}	335^{efg}	12.8^{efg}	1.5^{ef}	14.7^{efg}	2.1^{cd}	1.5^{a}	4.6^{bcd}	3.0^{bc}	23de	581^{efg}	604^{efg}
IT82D-889	20^{hi}	1437^{a}	14.2^{efg}	2.2^{cd}	17.9^{efg}	1.2^{de}	1.1^{a}	4.0^{de}	2.6^{de}	27^{cd}	565^{efg}	591^{efg}
IT84S-2246	16^{ij}	415^{cde}	15.9^{def}	2.17^{cd}	18.5^{def}	1.5^{de}	1.3^{a}	4.8^{b}	3.1^{bc}	30^{bcd}	764^{cdef}	794^{cde}
IT86D-2075	33^{cd}	216^{ghi}	13.0^{efg}	2.0^{de}	15.2^{efg}	0.5^{ef}	1.3^{a}	5.0^{ab}	3.2^{abc}	26de	649^{def}	675^{def}
IT90K-59	34^{cd}	339^{efg}	15.9^{def}	1.6^{ef}	17.8^{efg}	1.6^{de}	1.4^{a}	4.5^{cd}	2.9^{cd}	23de	722^{cdef}	745^{cde}
IT90K-76	36^{c}	158^{hij}	18.1^{cde}	1.8^{ef}	20.0^{cde}	1.6^{de}	1.2^{a}	5.0^{ab}	3.1^{bc}	22de	899^{cd}	921^{cd}
IT93K-2045-29	53^{a}	469^{cde}	13.6^{efg}	1.9^{de}	15.9^{efg}	3.4^{a}	1.2^{a}	5.0^{ab}	3.1^{bc}	24de	680^{def}	704^{def}
IT93K-452-1	27^{efg}	364^{efg}	10.8^{fgh}	1.3^{fg}	12.4^{gh}	0.6^{ef}	1.3^{a}	4.8^{b}	3.1^{bc}	17^{efg}	515^{efg}	532^{efg}
IT94D-437-1	13^{j}	168^{hij}	11.3^{fgh}	2.5^{abc}	14.0^{fgh}	0.9^{de}	1.3^{a}	4.8^{b}	3.0^{bc}	32^{bcd}	538^{efg}	570^{efg}
IT96D-1951	26^{fg}	157^{hij}	12.3^{fgh}	1.1^{fg}	13.5^{fgh}	0.9^{de}	1.3^{a}	4.5^{cd}	2.9^{cd}	14^{fg}	542^{efg}	556^{efg}
IT97K-499-39	35^{c}	514^{cd}	17.0^{def}	2.1^{cd}	19.6^{cde}	1.0^{de}	1.3^{a}	5.1^{ab}	3.2^{abc}	27^{cd}	865^{cde}	892^{cd}
ITH98-46	23^{gh}	263^{ghi}	7.8^{h}	0.9^{g}	9.0^{h}	1.5^{de}	1.4^{a}	4.4^{cde}	2.9^{cd}	13^{g}	347^{g}	360^{g}
Line 2020	46^{b}	119^{ij}	9.7^{gh}	1.4^{ef}	11.2^{gh}	0.2^{f}	1.3^{a}	4.9^{ab}	3.1^{bc}	19^{efg}	474^{fg}	493^{efg}
Mamlaka	25^{fgh}	132^{ij}	17.3^{def}	1.7^{ef}	19.1^{def}	2.5^{bc}	1.3^{a}	5.1^{ab}	3.2^{abc}	22de	882^{cd}	904^{cd}
Mchanganyiko	24^{fgh}	167^{hij}	16.4^{def}	2.4^{bcd}	19.0^{def}	1.6^{de}	1.2^{a}	4.1^{de}	2.7^{cde}	31^{bcd}	677^{def}	707^{def}
Ngonji	34^{cd}	323^{fg}	17.5^{def}	1.7^{ef}	19.5^{def}	2.8^{ab}	1.1^{a}	4.6^{bcd}	2.8^{cd}	19^{efg}	794^{cde}	813^{cde}
Omondaw	16^{ij}	237^{ghi}	12.1^{fgh}	1.4^{ef}	13.8^{fgh}	2.2^{bcd}	1.1^{a}	4.7^{bc}	2.9^{cd}	17^{efg}	563^{efg}	580^{efg}
Pan311	30^{def}	236^{ghi}	23.4^{b}	2.4^{bcd}	26.1^{b}	2.0^{cd}	1.4^{a}	4.9^{ab}	3.2^{abc}	36^{ab}	1154^{ab}	1191^{ab}
Soronko	17^{ij}	223^{ghi}	13.4^{efg}	1.6^{ef}	15.2^{efg}	1.4^{de}	1.3^{a}	3.7^{e}	2.5^{de}	22de	492^{efg}	514^{efg}
TVu11424	17^{ij}	286^{fg}	19.6^{bc}	1.9^{de}	21.8^{bcd}	1.2^{de}	1.6^{a}	4.9^{ab}	3.2^{abc}	30^{bcd}	945^{bc}	974^{ab}
TVX3236	27^{efg}	264^{ghi}	18.0^{cde}	1.5^{ef}	19.7^{cde}	0.5^{ef}	1.2^{a}	4.7^{bc}	3.0^{bc}	18^{efg}	845^{cde}	863^{cde}
Vallenga	30^{def}	132^{ij}	18.2^{cde}	2.1^{cd}	20.4^{cde}	1.5^{de}	1.1^{a}	4.7^{bc}	2.9^{cd}	25de	844^{cde}	868^{cde}
Vuli-1	36^{c}	227^{ghi}	15.8^{def}	1.9^{de}	17.9^{efg}	1.9^{cd}	1.3^{a}	4.5^{cd}	2.9^{cd}	26de	710^{cdef}	736^{cde}
C.V. (%)	8	23	17	14	15	13	41	30	27	21	18	17

Mean values followed by dissimilar letters in a column are significant at $P \leq 0.05$.

Table 2. δ^{15}N values, ‰ Ndfa and N-fixed in field-grown cowpea genotypes harvested at 76 days after planting (DAP) at Taung, South Africa, in 2005.

Genotype	δ^{15}N			Ndfa			N-fixed					
	Root (‰)	Shoot	Whole plant	Root (%)	Shoot	Whole plant	Root (mg/plant)	Shoot	Whole plant	Root (kg/ha)	Shoot	Whole plant
Apagbaala	0.4^{cd}	0.7^{b}	0.9^{c}	51^{cd}	61.2^{de}	57.7^{cdef}	376^{fgh}	19^{de}	384^{fgh}	1. 1.3^{e}	63^{fgh}	64^{fgh}
Benpila	0.5^{bc}	1.8^{a}	1.6^{a}	51^{cd}	50.7^{fg}	49.6^{f}	290^{h}	9^{ef}	300^{h}	1.7^{cd}	48^{h}	50^{h}
Bensogla	-0.3^{fg}	-0.1^{efg}	-0.3^{klm}	58^{bc}	74.1^{cd}	66.7^{bcd}	537^{efg}	8^{f}	547^{efg}	3.4^{abc}	90^{efg}	91^{efg}
Botswana white	-0.1^{ef}	-0.7^{fghi}	0.01^{ij}	67.5^{ab}	85.4^{ab}	78.7^{ab}	701^{cde}	30^{abc}	721^{cde}	1.7^{cd}	117^{cde}	120^{cde}
Brown eye	0.4^{cd}	0.6^{bc}	0.3^{gh}	67^{ab}	72.6^{cd}	61.2^{cde}	302^{h}	12^{ef}	326^{h}	4.0^{a}	50^{h}	54^{h}
CH14	0.4^{cd}	-1.2^{i}	-0.7^{qr}	54.7^{bc}	86.0^{ab}	73.5^{abc}	602^{def}	31^{ab}	625^{de}	3.8^{a}	100^{def}	104^{def}
Encore	1.3^{a}	-0.4^{efgh}	0.4^{fg}	35.8^{ef}	73.9^{cd}	59.1^{cdef}	666^{cde}	28^{bc}	674^{cde}	1.4^{de}	111^{cde}	112^{cde}
Fahari	0.2^{de}	-0.9^{ghi}	-0.6^{nq}	58^{bc}	77.0^{bc}	70.6^{abc}	1069^{a}	32^{a}	1089^{a}	3.4^{abc}	178^{a}	182^{a}
Glenda	0.7^{bc}	0.5^{bc}	1.8^{a}	52^{cd}	59.3^{ef}	62.3^{cde}	859^{bc}	18^{de}	874^{bc}	2.6^{bc}	143^{bc}	146^{bc}
Iron-Grey	0.4^{cd}	0.5^{bc}	0.6^{def}	60.6^{bc}	74.5^{cd}	65.7^{cde}	410^{fgh}	13^{ef}	424^{efgh}	2.3^{bcd}	68^{fgh}	71^{fgh}
IT82D-889	0.1^{ef}	-0.2^{efg}	0.7^{cde}	69^{ab}	92.9^{a}	80.9^{a}	522^{efg}	27^{bc}	540^{efg}	3.1^{abc}	87^{efg}	90^{efg}
IT84S-2246	-0.2^{ef}	-0.7^{fghi}	-0.5^{mnq}	74^{ab}	80.4^{bc}	77.1^{abc}	610^{def}	16^{de}	632^{de}	3.7^{ab}	102^{def}	105^{def}
IT86D-2075	0.5^{bc}	-0.5^{efgh}	0.3^{gh}	68^{ab}	72.0^{cd}	75.1^{abc}	532^{efg}	23^{cd}	549^{efg}	3.0^{abc}	89^{efg}	92^{efg}
IT90K-59	1.0^{ab}	-0.1^{def}	1.0^{c}	58^{bc}	76.4^{bc}	69.0^{abc}	583^{def}	19^{de}	595^{def}	2.1^{cde}	97^{defg}	99^{defg}
IT90K-76	0.2^{de}	0.01^{cde}	0.01^{ij}	68^{ab}	71.1^{cd}	69.7^{abc}	647^{def}	23^{de}	661^{de}	2.4^{bc}	108^{def}	110^{def}
IT93K-2045-29	0.2^{de}	-1.1^{i}	-0.9^{r}	68^{ab}	83.9^{ab}	78.6^{ab}	609^{def}	18^{de}	625^{de}	2.7^{bc}	101^{def}	104^{def}
IT93K-452-1	-0.8^{g}	0.5^{bc}	0.8^{cd}	82^{a}	64.8^{de}	74.8^{abc}	348^{gh}	15^{de}	362^{gh}	2.3^{bcd}	58^{gh}	60^{gh}
IT94D-437-1	0.5^{bc}	-0.5^{efgh}	0.5^{efg}	28^{f}	62.7^{de}	52.0^{ef}	407^{fgh}	30^{abc}	416^{efgh}	1.5^{de}	68^{fgh}	69^{fgh}
IT96D-1951	0.8^{bc}	0.3^{bcde}	0.5^{efg}	56^{bc}	78.8^{bcd}	68.7^{bcd}	439^{fgh}	14^{de}	446^{efgh}	1.3^{e}	73^{fgh}	74^{fgh}
IT97K-499-39	-0.1^{ef}	0.5^{bc}	1.2^{b}	73^{ab}	60.6^{de}	72.3^{abc}	623^{def}	20^{de}	643^{de}	3.3^{abc}	104^{def}	107^{def}
ITH98-46	0.4^{cd}	-0.4^{efgh}	-0.4^{lmn}	67^{ab}	81.5^{bc}	73.6^{abc}	277^{h}	13^{ef}	286^{h}	1.4^{de}	46^{h}	48^{h}
Line 2020	0.2^{de}	-0.5^{efgh}	-0.7^{qr}	59^{bc}	43.1^{g}	59.2^{cdef}	282^{h}	22^{cd}	292^{h}	1.8^{cd}	47^{h}	49^{h}
Mamlaka	0.2^{de}	-0.5^{efgh}	0.6^{def}	64^{ab}	69.8^{cd}	73.2^{abc}	733^{cde}	29^{abc}	748^{cde}	2.4^{bc}	122^{cde}	125^{cde}
Mchanganyiko	0.5^{bc}	-0.8^{ghi}	0.5^{fg}	53^{cd}	73.2^{cd}	68.1^{bcd}	569^{def}	32^{abc}	586^{def}	2.8^{bc}	95^{defg}	98^{defg}
Ngonji	-0.3^{fg}	-0.9^{ghi}	-0.4^{klmn}	72^{ab}	82.5^{bc}	77.6^{abc}	663^{cde}	22^{cd}	676^{cde}	2.2^{cd}	111^{cde}	113^{cde}
Omondaw	-0.4^{gh}	-0.3^{efgh}	-0.2^{jk}	73^{ab}	77.0^{bc}	76.6^{abc}	449^{efgh}	24^{cd}	462^{efgh}	2.1^{cde}	75^{efgh}	77^{efgh}
Pan311	0.9^{ab}	-0.5^{efgh}	0.1^{hi}	46^{def}	72.7^{cd}	63.8^{cde}	947^{ab}	26^{bc}	963^{ab}	2.6^{bc}	158^{ab}	160^{ab}
Soronko	-0.4^{fg}	-0.5^{efgh}	-0.2^{jkl}	63^{bc}	78.8^{bc}	73.5^{abc}	415^{fgh}	26^{cd}	429^{efgh}	2.3^{bcd}	69^{fgh}	71^{fgh}
TVu11424	0.9^{ab}	0.4^{bcde}	1.2^{b}	64^{ab}	75.8^{cd}	72.4^{abc}	762^{bcd}	25^{cd}	781^{bcd}	3.2^{abc}	127^{bcd}	130^{bcd}
TVX 3236	0.6^{bc}	0.2^{cde}	0.6^{def}	60^{bc}	67.7^{cd}	65.0^{cde}	576^{def}	12^{ef}	587^{def}	1.8^{cd}	96^{defg}	98^{defg}
Vallenga	0.6^{bc}	0.5^{bc}	0.9^{c}	56^{bc}	64.0^{de}	62.2^{cde}	581^{def}	19^{de}	595^{def}	2.3^{bcd}	97^{defg}	99^{defg}
Vuli-1	0.7^{bc}	-1.0^{hi}	-0.6^{nq}	54^{cd}	80.3^{bc}	68.6^{bcd}	598^{def}	31^{ab}	611^{de}	2.3^{bcd}	100^{def}	102^{def}
C.V. (%)	9	24	30	18	10.5	6	22	27	22	26	22	22

Mean values followed by dissimilar letters in a column are significant at $P \leq 0.05$.

Table 3. Plant growth, grain yield and N level of 15 cowpea genotypes harvested at 164 days after planting at Taung, South Africa, in 2006.

Genotype	Dry matter	Grain yield	N concentration	N content
	Shoot + grain (g/plant)	(t/ha)	Shoot + grain (%)	Shoot + grain (mg/plant)
Bensogla	28.3[f]	1.8[bc]	3.5[a]	991.9[g]
Botswana White	46.8[a]	2.0[ab]	3.3[a]	1536.4[b]
Brown eye	31.8[e]	2.4[ab]	3.4[a]	1083.6[fg]
CH 14	46.3[a]	3.5[a]	3.7[a]	1689.8[a]
Encore	37.4[d]	1.2[f]	3.3[a]	1222.8[cde]
Fahari	35.8[cd]	2.9[a]	3.4[a]	1202.2[def]
Glenda	37.8[cd]	3.3[a]	3.3[a]	1261.9[cd]
IT84S-2246	31.7[e]	2.7[ab]	3.5[a]	1122.3[efg]
IT93K-2045-29	35.1[d]	2.0[bc]	3.2[a]	1262.9[cd]
IT90K-59	36.3[cd]	2.7[ab]	3.5[a]	1281.8[cd]
Mamlaka	41.0[b]	2.7[ab]	3.1[a]	1286.9[cd]
Ngonji	37.8[cd]	1.4[c]	3.4[a]	1324.9[cd]
Pan311	35.4[d]	3.1[a]	3.7[a]	1109.0[efg]
TVu 11424	31.8[e]	2.4[ab]	3.3[a]	1062.1[g]
Vuli-1	38.1[c]	2.7[ab]	3.5[a]	1345.8[c]
C.V. (%)	5	25	32	26

Mean values followed by dissimilar letters in a column are significant at $P \leq 0.05$.

showed negative $\delta^{15}N$ values of shoots, an indication of greater N_2 fixation. The $\delta^{15}N$ values of roots also varied from -0.8‰ in IT93K-452-1 to 1.3‰ in CH14. At whole-plant level, the $\delta^{15}N$ values ranged from -0.9‰ in cv. IT93K-2045-29 to 1.6‰ in Benpila (Table 2). The $\delta^{15}N$ values again differed among the 15 cowpea genotypes planted in 2006. Shoot $\delta^{15}N$ values ranged from -0.3 to 1.4‰ for Ngonji and Pan311, respectively (Table 4).

Proportion of N derived from atmospheric N_2 fixation (%Ndfa)

Estimates of the proportion of N derived from fixation in organs and whole plants revealed some significant differences among the 32 cowpea genotypes, as shoot %Ndfa values varied from 50.7% in Benpila to 92.9% in IT82D-889. At whole-plant level, the values were 49.6% for Benpila (the least fixer) and 80.9% for IT82D-889 (the highest fixer). Except for cv. Benpila (which obtained less than 50% of its N from symbiotic fixation), all the remaining 31 genotypes derived between 52 and 80.9% of their N nutrition from N_2 fixation in 2005 (Table 2). Similarly, in 2006, the %Ndfa varied among the 15 cowpea genotypes and ranged from 21% in Ngonji to 66% in Pan311 and Fahari. The cvs. Bensogla, Encore, IT90K-59 and Ngonji showed the lowest dependence on N_2 fixation for N nutrition (45, 30, 41 and 21%, respectively). The dependence of the other varieties on biological N_2 fixation was greater than 50% (Table 4).

Amounts of N-fixed in cowpea genotypes

As a result of the significant differences in the proportion of N derived from symbiotic fixation in 2005, the actual amounts of N-fixed also varied enormously among the 32 cowpea genotypes (Table 2). Whether expressed as mg N-fixed per plant or kg N-fixed per hectare in shoots and/or whole plants, the three genotypes Fahari, Pan311 and Glenda (in that order) were the most superior in symbiotic performance, contributing 182, 160 and 146 kg N-fixed/ha, respectively. Another three genotypes, namely, ITH98-46, Line 2020 and Benpila (in that order) were the lowest in symbiotic performance and contributed 48, 49 and 50 kg N-fixed/ha, respectively (Table 2). The amount of N-fixed in the 15 cowpea genotypes tested in 2006 showed marked differences that ranged from a low 24.2 to 131 kg N/ha for Ngonji, CH14 and IT84S-2246. Seven cowpea genotypes (Pan311, Mamlaka, IT93K-2045-29, IT84S-2246, Glenda, Fahari and CH14) out of the 15 cowpea genotypes revealed high N_2 fixation of 100 kg N/ha (Table 4).

Grain yield

Grain yield differed significantly ($p \leq 0.05$) among the 32 cowpea genotypes. Cultivar IT93K-2045-29 (the highest yielding genotype) produced 3.4 t/ha of grain relative to the 0.2 t/ha grain harvested from Benpila (the least yielding genotype) in 2005. Although, the top seven high

Table 4. Symbiotic performance and N-fixed in shoots and grain of 15 cowpea genotypes sampled at 164 days after planting (DAP) at Taung, South Africa, in 2006.

Genotype	$\delta^{15}N$			Ndfa	N-fixed
	Shoot (‰)	Grain	Shoot + grain	Shoot + grain (%)	Shoot + grain (kg/ha)
Bensogla	0.5^{abc}	2.1^{c}	1.9^{c}	45.1^{f}	74.6^{d}
Botswana White	0.4^{abc}	0.7^{g}	0.7^{h}	61.2^{b}	99.1^{c}
Brown Eye	0.7^{abc}	1.0^{f}	1.0^{f}	53.9^{d}	79.3^{d}
CH 14	0.2^{bc}	0.9^{f}	0.9^{g}	56.7^{c}	131.2^{a}
Encore	0.6^{abc}	2.4^{b}	2.2^{b}	30.4^{g}	41.8^{f}
Fahari	0.3^{bc}	0.4^{i}	0.4^{i}	66.1^{a}	112.5^{b}
Glenda	0.6^{abc}	0.7^{g}	0.7^{h}	60.6^{b}	106.3^{bc}
IT84S-2246	1.3^{a}	0.5^{gh}	0.6^{h}	62.9^{b}	131.1^{a}
IT93K-2045-29	0.4^{abc}	0.4^{i}	0.4^{i}	57.0^{c}	100.4^{c}
IT90K-59	-0.1^{bc}	1.0^{f}	0.9^{g}	41.4^{f}	66.9^{e}
Mamlaka	0.2^{bc}	1.8^{d}	1.6^{d}	56.8^{c}	100.4^{c}
Ngonji	-0.3^{c}	1.0^{f}	0.9^{g}	21.0^{h}	24.2^{g}
Pan311	1.4^{a}	3.2^{a}	2.6^{a}	66.7^{a}	104.8^{bc}
TVu 11424	0.5^{abc}	1.2^{e}	1.2^{e}	50.7^{e}	71.9^{de}
Vuli-1	0.5^{abc}	1.0^{f}	1.0^{fg}	54.8^{cd}	98.8^{c}
C.V. (%)	29	7	17	4	16

Mean values followed by dissimilar letters in a column are significant at $P \leq 0.05$

grain-yielding cowpea genotypes (namely, IT93K-2045-29, Bensogla, Ngonji, Mamlaka, Omondaw, CH14 and Iron Grey) did not include the best N_2-fixing cultivars, these were closely followed by Pan311 and Glenda, two of the three most high N_2-fixing genotypes (currently used as commercial varieties in South Africa). Significant differences ($p \leq 0.05$) were found among the 15 cowpea genotypes re-evaluated in 2006. For example, the cvs. Pan311, Fahari, CH14, Glenda, IT84S-2246, IT90K-59 and Mamlaka showed higher grain yield of between 2.7 and 3.5 t/ha (Table 3).

Correlation analysis

Correlation analysis of whole-plant biomass with whole-plant N-fixed was significant (r = 0.91***). Similarly, shoot dry matter significantly correlated with shoot N-fixed (r = 0.91***), just as plant growth (measured as total biomass) correlated significantly with nodule-specific activity (r = 0.31**) (data not shown).

DISCUSSION

The major constraints to increased crop yields in Africa include water and low nutrient fertility, especially N and P. Identifying nodulated grain legumes that can contribute large amounts of symbiotic N to cropping systems, while producing increased grain yield is currently the focus of our research. Evaluating 32 cowpea genotypes for plant growth and symbiotic performance at Taung, South Africa, in 2005 provided data that showed clear genotypic differences in plant growth, dry matter partitioning and N_2 fixation. The data reveal considerable variation in nodule numbers and nodule dry matter among the 32 cowpea genotypes in 2005. For example, although, cowpea genotype IT93K-2045-29 produced more nodules per plant compared with IT94D-437-1 and CH14, nodule DM was greater in genotype IT82D-889 and least in Bensogla. Cowpea cvs. Fahari, Pan311 and Glenda showed the highest whole-plant DM yield and symbiotic N contribution in 2005 and 2006, indicating that these genotypes from Southern Africa are locally-adapted, hence, they could respectively produce 2.9-, 2.7- and 3.5-fold more dry matter than cv. ITH98-46, and yielded 2.7-, 2.2- and 3.2-fold more N than cv. ITH98-46 from IITA in Nigeria (Table 1). There was therefore a direct relationship between symbiotic N accumulation and biomass production, with low N_2 fixation being associated with low plant biomass, and vice versa (r = 0.91***, data not shown). This interpretation was further supported by the positive correlation obtained between nodule-specific activity and whole-plant dry matter yield [r = 0.31** (data not shown), see also Herridge et al. (1990) and Maskey et al. (2001)], clearly indicating that in legumes, dry matter accumulation is directly linked to symbiotic N nutrition.

Our results also reveal significantly large differences in symbiotic performance among and between the 32 or 15 cowpea genotypes tested in 2005 and 2006 (Tables 2 and 4). Shoot $\delta^{15}N$ values varied considerably, ranging from -1.2‰ for cv. CH14 to 1.8‰ for cv. Benpila in 2005. Estimates of %Ndfa (the plant's proportional dependency on N_2 fixation for its N nutrition) using the combined mean $\delta^{15}N$ values of an *Eragrostis* sp. and a broad-leafed weed as non-N_2-fixng reference plants showed significant differences among the genotypes in 2005 and 2006. Those with the lowest $\delta^{15}N$ values expectedly exhibited the highest %Ndfa values (Tables 2 and 4). For example, of the 32 cowpea genotypes tested in 2005, cv. IT82D-889 showed a significantly greater %Ndfa value, followed by cvs. CH14, Botswana White and IT93K-2045-29, then IT84S-2246, Ngonji and Omondaw. Except for cowpea cv. Benpila, which derived only 49.6% of its N from N_2 fixation, the remaining 31 genotypes obtained between 52 and 80.9% of their N nutrition from symbiotic N_2 fixation in 2005. Our data are comparable to those of other studies, which showed %Ndfa values ranging from 12 to 92% in farmers' fields in the Upper West Region of Ghana (Naab et al., 2009), 61 to 77% in the forest and transitional zones of Southern Ghana (Adjei-Nsiah et al., 2008), 38 to 86% in the Ivory Coast (Becker and Johnson, 1998) and 69 to 74% in Western Australia (Ofori and Stern, 1987). In southwestern Zimbabwe, however, the dependency of cowpea on N_2 fixation for its N nutrition was very low and ranged from 15 to 17% in 2003, 16 to 56% in 2004 and 29 to 46% in 2005 (Ncube et al., 2007).

In this study, the amounts of N-fixed in the 32 cowpea genotypes in 2005 varied from 286 mg N/plant for ITH98-46 to 1089 mg N/plant in cowpea cv. Fahari. These values corresponded to 48 and 182 kg N-fixed/ha for ITH98-46 and Fahari, respectively. The amount of N-fixed in 2006 also ranged from 131.2 and 131.1 kg N/ha for CH14 and IT84S-2246, respectively, to 24.2 kg N/ha in Ngonji (Table 4). Cowpea grain yield also showed considerable variation among the 32 cowpea genotypes, with a range of 0.2 to 3.4 t/ha for cvs. Benpila and IT93K-2045-29, respectively. The genotypes that fixed more N tended to produce greater biomass, while those that fixed less N yielded low biomass. Within the African continent, cowpea has been shown to fix 24-29 kg N/ha in Kenya (Ssali and Keya, 1984), 122.0 kg N/ha in Nigeria (Eaglesham et al., 1981), 201.0 kg N/ha in Ghana's northern savanna (Dakora et al., 1987), 32 to 67 kg N/ha in the forest and transitional zones of southern Ghana (Adjei-Nsiah et al., 2008) and a low 4 to 28 kg N/ha in southwestern Zimbabwe (Ncube et al., 2008). In Australia, cowpea has also been shown to contribute about 87 kg N/ha (Ofori and Stern, 1987). The different levels of N_2 fixation obtained in those studies, as well as in this study, demonstrate the need to select superior N_2-fixing cowpea genotypes for use in cropping systems. Unlike the studies by Ncube et al. (2007), Ojiem et al. (2007) and Adjei-Nsiah et al. (2008), where few cowpea genotypes were assessed for their N contributions, this study evaluated as many as 32 cowpea genotypes in 2005 and 15 in 2006. The data clearly show that some cowpea genotypes such as Fahari, Pan311, IT84S-2246, CH14 and Glenda have the potential to contribute substantial amounts of symbiotic N in cropping systems, while providing relatively high grain yield.

In conclusion, many cowpea genotypes were found to depend largely on N_2 fixation for their N nutrition. For example, the cv. Fahari obtained 80.9% of its N from symbiotic fixation and fixed about 182 kg N/ha, followed by the cvs. Pan311, Glenda, TVu11424, Mamlaka, Botswana White, Ngonji, Encore, IT90K-76, IT84S-2246, IT93K-2045-29, CH14 and Vuli-1, which contributed 160, 146, 130, 125, 120, 113, 112, 110, 105, 104, 104 and 102 kg N/ha, respectively. In contrast, cowpea cv. ITH98-46 derived only 48.3% of its shoot N from symbiotic fixation, contributed about 49.6 kg N/ha and was therefore, the lowest N_2-fixing genotype. The high N-fixing genotypes identified in this study, are good candidates for use in cropping systems because of their high N contribution and relatively high grain yields, while the low N_2-fixing genotypes require further research to improve their symbiotic efficiency.

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