DOI: 10.1007/s11099-015-0144-z PHOTOSYNTHETICA 53 (4): 562-571, 2015

Assessing the relationship between photosynthetic C accumulation and symbiotic N nutrition in leaves of field-grown nodulated cowpea (*Vigna unguiculata* L. Walp.) genotypes

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Abstract

This study evaluated the relationship between photosynthetic carbon accumulation and symbiotic nitrogen nutrition in young fully expanded leaves of 30 nodulated cowpea genotypes grown in the field at Manga, Ghana, in 2005 and 2006. Estimates of fixed-N in photosynthetic leaves revealed greater symbiotic N in genotypes with higher photosynthetic rates and increased leaf transpiration rate/efficiency. There was also greater C accumulation in genotypes with higher symbiotic N and/or total N. Additionally, genotypes with high contents of C per unit of leaf total N exhibited greater C per unit of leaf N-fixed. The C/N and C/Rubisco-N ratios were generally similar in their magnitude when compared to the C/N-fixed ratio due possibly to the fact that Rubisco accounts for a high proportion of photosynthetic leaf N, irrespective of whether the enzyme was formed from soil N or symbiotic N. Cowpea genotypes that relied heavily on soil N for their N nutrition exhibited much higher C/N-fixed ratios, while conversely those that depended more on symbiosis for meeting their N demands showed markedly lower C/N-fixed values. For example, genotypes Omondaw, Bensogla, IT93K-2045-29, and Sanzie, which respectively derived 83.9, 83.1, 82.9, and 76.3% N from fixation, recorded lower C/N-fixed ratios of 10.7, 12.2, 12.1, and 13.0 mg mg^{-1} in that order in 2005. In contrast, genotypes Botswana White, IT94D-437-1, TVu1509, and Apagbaala, which obtained 14.8, 15.0, 26.4, and 26.0% of their N nutrition from fixation, showed high C/N-fixed values of 84.0, 69.0, 35.2, and 40.6 mg.mg^{-1}, respectively, in 2005. This clearly indicates that genotypes that obtained less N from symbiosis and more N from soil revealed very high C/N-fixed values, an argument that was reinforced by the negative correlations obtained between the three C/N ratios (*i.e.* C/N, C/Rubisco-N, and C/N-fixed) and leaf N concentration, percentage nitrogen derived from fixation, total N content, amount of N-fixed, and Rubisco N. These data suggest a direct link between photosynthetic C accumulation and symbiotic N assimilation in leaves of nodulated cowpea, and where genotypes derived a large proportion of their N from fixation, photosynthetic C yield substantially increased.

Additional key words: N_2 fixation; photosynthesis; photosynthetic fixed-N use efficiency; transpiration efficiency; $\delta^{15}N$.

Introduction

Fixation of carbon from atmospheric CO_2 accounts for over 90% of the biomass in terrestrial plants (Zelitch 1982, Khan *et al.* 2008), and is therefore the second most important biological process after photosynthesis (Drake *et al.* 1997, Moore *et al.* 1999). Because N is required for the synthesis of ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) and chlorophyll, N_2 fixation and photosynthesis are metabolically interlinked in nodulated legumes at the level of the plant N and C economy.

Many factors can influence photosynthetic rates in plants; these range from water needed for photolysis during photosynthesis (Drake *et al.* 1997) to mineral nutrition and source/sink strength (Heineke *et al.* 1999, Isopp *et al.* 2000, Ainsworth *et al.* 2004). Because of the N requirement for synthesis of Rubisco and chlorophyll (Chl), nodulated legumes have an advantage over non-legumes in their ability to meet their photosynthetic N requirements from symbiotic N_2 fixation (Evans 1989,

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Received 11 July 2014, *accepted* 25 March 2015.

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Abbreviations: Chl – chlorophyll; *E* – transpiration rate; g_s – stomatal conductance; %Ndfa – percentage of nitrogen derived from fixation of atmospheric N_2; P_N – net photosynthetic rate; PNUE – photosynthetic nitrogen use efficiency; DAP – days after planting; $\delta^{15}N$ – nitrogen isotopic composition.

Acknowledgements: 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 South African Research Chair in Agrochemurgy and Plant Symbioses, the National Research Foundation and Tshwane University of Technology are also acknowledged for supporting the research of FDD.

Hikosaka and Terashima 1995). Although photosynthetic activity and leaf N are sometimes positively correlated (Robertson *et al*. 2002), the photosynthetic machinery can potentially be deactivated if the leaf N concentration is above critical maxima (Mächler *et al*. 1988, Hikosaka and Terashima 1995, Cheng and Fuchigami 2000).

Since leaf stomata control leaf transpiration rates, metabolite flux, long-distance signaling (Lawson 2009), and the influx of CO_2 and water, differences in leaf stomatal density can also affect photosynthetic rates in land plants. Selection of crop species with high carbon yield in stress environments (Morison *et al.* 2007) would require a deeper understanding of stomatal functioning in relation to photosynthetic CO_2 exchange and leaf water relations (Wang *et al.* 2007, Lawson 2009). Where water is a limiting resource in cropping systems, especially under a climate change scenario (Rosenzweig and Parry 1994, Tubiello *et al.* 1999), plant species with high water-use efficiency are more likely to be preferred by growers over those with low water-use efficiency (Xu and Hsiao 2004).

Cowpea (*Vigna unguiculata* L. Walp.) is the major food legume cultivated by farmers in Africa. The young leaves, green pods, and grains are eaten as protein-rich food. Cowpea is adapted to growth in different environments and soil ecologies, including the drier areas of Sub-Saharan Africa. Although some cowpea genotypes are reported to be drought-tolerant (Elowad and Hall 1987), there is still a need to select genotypes for good biological traits, such as robust growth, grain yield, improved water relations, and N-fixed, all of which interface at the plant C and N economy. Cowpea genotypes can differ both in gas-exchange parameters (Aniya and Herzog 2004) and in photosynthetic carbon accumulation (Makoi *et al*. 2010).

So far, however, no link has been established between plant growth, carbon accumulation, symbiotic N yield, and photosynthesis in leaves of field-grown nodulated cowpea. The aim of this study was to assess the relationship between photosynthesis and symbiotic N_2 fixation in 30 cowpea genotypes through (*1*) measurement of photosynthetic rates, biomass, and carbon accumulation in young fully expanded cowpea leaves, (*2*) estimates of symbiotic N and Rubisco N in photosynthetic leaves, and (*3*) determination of the photosynthetic N-use efficiency in young fully expanded leaves of cowpea genotypes evaluated in the field in the Sudano-Sahelian savanna of Ghana over a two-year period in 2005 and 2006.

Materials and methods

Site description: Field experiments were conducted at the Savanna Agricultural Research Institute station at Manga, which is situated in the Sudano-Sahelian savanna (11°11′N, 0°61′E, 135 m a.s.l.), and has a unimodal rainfall (annual mean of 800 mm) that starts in May/June and ends in September/October. According to Driessen *et al.* (1990), the soils at Manga are classified as Gleyic Alfisols with pH 6.0 ($CaCl_2$), and contain 4.7 mg(P) kg^{-1}, 20.3 mg(K) kg^{-1}, 0.38% C, 0.07% N, 0.62% organic matter content, and a C/N ratio of 11.64.

Field design and planting: The experiments were conducted using a randomized complete block design with four replicate plots per cowpea genotype. Each plot measured 3 m × 5 m (15 m^2). Cowpea was planted in mid-July with inter-row and intra-row spacing of 60 cm and 20 cm, respectively. Weeds were manually controlled with hand hoes. The 30 cowpea genotypes used in this study exhibited different biological traits, including days to 50% flowering, number of days to physiological maturity, levels of N_2 fixation, pest resistance, and grain yield (Belane and Dakora 2009).

Gas-exchange measurements: At 46 and 72 days after planting (DAP) in 2005 and 2006, respectively, photosynthetic rates (P_N), stomatal conductance (g_s), and transpiration rates (E) were measured in each of 9 young fully expanded leaves per plot using a portable infrared gas analyzer (*CIRAS-2*, *PP System*, Hitchin, UK). Measurements were made from 8 to 11 h and from 14 to 16 h. for each replicate plot per day. Leaves were allowed 4 to 5 min to acclimate to the light environment in the chamber. Without trouble shooting, each measurement took approximately 2 min, which was the minimum time allowed for the reading to stabilize before they were recorded. The conditions prevailing in the leaf chamber during photosynthetic measurements included light intensity of 1,200 μmol(photon) m^{-2} s^{-1} (high enough to saturate leaf photosynthesis), CO_2 concentration of 400 ppm, flow rate of 400 μmol s^{-1}, leaf temperature of 25°C, and relative humidity of 44%. Water-use efficiency in cowpea was calculated as photosynthate produced per unit of water molecule transpired (Hamid *et al.* 1990).

Plant sampling, processing, and $^{15}N/^{14}N$ isotope analysis: The nine young fully expanded cowpea leaves used for gas-exchange studies were removed from field plants, oven-dried (60°C), weighed, ground to fine powder (0.85 mm sieve), and analyzed for $^{15}N/^{14}N$ ratios using mass spectrometry. About 2.0 mg of ground leaf sample was weighed into a tin capsule (*Elemental Microanalysis Ltd*., Okehampton, UK) and run on a *Thermo Finnigan Delta Plus XP* stable light isotope mass spectrometer (*Fisons Instrument SpA*, Strada Rivolta, Italy) coupled *via* a *Conflo II* device to *Thermo NA1500* flash elemental analyzer. In addition to running air an external standard from (IAEA, International Atomic Energy Agency) in Vienna, an internal standard consisting of material from a *Nasturtium* spp. was included in every five runs to correct for machine errors during isotopic fractionation.

The isotopic composition ($\delta^{15}N$) was measured as according to Junk and Svec (1958) and Mariotti *et al.* (1981):

$$\delta^{15}N = \frac{\left({}^{15}N/{}^{14}N\right)_{sample} - \left({}^{15}N/{}^{14}N\right)_{atm}}{\left({}^{15}N/{}^{14}N\right)_{atm}} \times 1{,}000$$

Determining N and C accumulation in cowpea leaves: The N [%] and C [%] of leaf dry matter were obtained directly from mass spectrometer, and used to estimate the N and C content of leaf tissue. Leaf N content [mg per leaf] was calculated as the product of N [%] and leaf dry mass, and leaf C [mg per leaf], the product of C [%] and leaf dry mass.

Percentage N derived from fixation (%Ndfa): The proportion of N derived from N_2 fixation was calculated as (Shearer and Kohl 1986, Unkovich *et al.* 2008):

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

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 plants depending solely on N_2 fixation for their N nutrition. The B value used for cowpea leaves in this study was –1.78 ‰ (Makoi *et al.* 2009). The plants from which the B value was obtained were grown under controlled glasshouse conditions with total reliance on N_2 fixation for their N nutrition. In 2005, maize (*Zea mays*), grain sorghum (*Sorghum bicolor*), late millet (*Pennisetum* spp), and a broad leaf weed (*Hyptis specifigera*) were sampled as reference plants and analyzed for their ^{15}N natural abundance. The combined mean $\delta^{15}N$ of shoots of these reference plants used for estimating cowpea leaf %Ndfa in 2005 was +7.12 ‰. In 2006, leaves of *Z. mays*, *S. bicolor*, *Andropogon gayanus, Rottboellia cochichininensis*, *H. specifigera*, and *Pennisetum* spp were collected as reference plants from the experimental site, and similarly analyzed for their ^{15}N natural abundance. The combined mean $\delta^{15}N$ value of these reference plants used to estimate %Ndfa in cowpea leaves in 2006 was +5.5 ‰.

Amount of N fixed in cowpea leaves: The amount of N-fixed was calculated as (Maskey *et al.* 2001):

N-fixed = %Ndfa × leaf N content

Photosynthetic nitrogen-use efficiency (PNUE) is a measure of C accumulated per unit N, and is directly related to Rubisco amount and functioning in leaves. Values of PNUE were obtained by dividing leaf C by leaf total N and leaf C by leaf N-fixed.

Leaf Rubisco N content: Field and Mooney (1986) reported an average value of 75% as the Rubisco N in photosynthetic leaves of wild plant species. Recently, Dordas and Sioulas (2008) also independently found 75% as the amount of Rubisco N in safflower leaves. As a result, leaf Rubisco N in this study was calculated as:

Leaf Rubisco N = α × leaf N content,

where α is the average percent N (75%) invested in Rubisco synthesis by photosynthetic leaves (Field and Mooney 1986, Dordas and Sioulas 2008).

Statistical analysis: The normality of the data was tested before analysis of variance (*ANOVA)* using *STATISTICA* program *version 7.1* (*Statsoft Inc*. 2005). One-way *ANOVA* was performed to compare gas-exchange parameters, leaf N content and C accumulation, as well as PNUE in cowpea genotypes. *Duncan*'s multiple range test was used to compare treatment means at $p \leq 0.05$.

Results

Photosynthetic rates, stomatal conductance and water-use efficiency of cowpea leaves: Gas-exchange studies showed significant differences among the 30 cowpea genotypes evaluated under field conditions at Manga in 2005. Some genotypes, such as IT84S-2246, Mchanganyiko, Brown Eye, and IT90K-59, revealed high P_N, g_s, and E compared with Apagbaala and TVu1509, which showed very low values of the gas-exchange characteristics (Table 1). In 2006, a similar pattern was obtained when 15 genotypes were re-evaluated at a different site at Manga. CH14, IT93K-2045-29, Apagbaala, and Soronko produced the highest P_N, g_s, and water-use efficiency (WUE) (Table 2). In contrast, Vuli-1 exhibited the lowest P_N, g_s, and WUE (Table 2). In both years, the photosynthetic parameters of the other genotypes were intermediate between the two extremes (Tables 1, 2).

Leaf dry mass, N [%], C [%], $\delta^{15}N$, and %Ndfa: The dry mass (DM) of the leaves used for photosynthetic measurements differed accordingly to genotypes. Leaves of IT97K-499-39 yielded the greatest biomass in 2005 followed by IT03K-2045-29, Ngonji, Botswana White, and Fahari, while ITH98-20, Bensogla, Pan 311, Omondaw, and IT94D-437-1 produced the least leaf biomass (Table 3). Although both leaf N and C concentrations were similar for the 30 genotypes, the $\delta^{15}N$ values were significantly different, ranging from –0.2 ‰ for IT93K-2045-29 to +6.4 ‰ for Botswana White (Table 3). As a result, IT93K-2045-29 and Omondaw (–0.3 ‰) derived the highest N from fixation (83% and 84%, respectively), while Botswana White, IT94D-437-1 (+5.6 ‰), and TVu1509 (+4.8 ‰) obtained the least N from fixation (15%, 15%, and 25%, respectively; Table 3).

Table 1. Photosynthetic rates (P_N), transpiration rates (E), stomatal conductance (g_s), and plant transpiration efficiency (WUE) measured in 46-d-old cowpea genotypes grown in the field at Manga, Ghana, in 2005. Means with *different letters* in a column are significant at $p \leq 0.01^{**}$ or $p \leq 0.001^{***}$

Genotype	P_N [μmol(CO$_2$) m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	E [mmol(H$_2$O) m^{-2} s^{-1}]	WUE [μmol(CO$_2$) mmol(H$_2$O)$^{-1}$]
Apagbaala	16.1^{e}	0.6^{e}	7.9^{f}	2.0^{a}
Bensogla	27.0^{cde}	4.0^{bc}	15.0^{cde}	1.8^{a}
Botswana White	18.7^{de}	3.7^{bc}	15.7^{cd}	1.1^{a}
Brown Eye	38.4^{abc}	3.7^{bc}	20.3^{ab}	1.9^{a}
CH14	21.0^{de}	3.3^{bc}	16.8^{bc}	1.3^{a}
Fahari	26.5^{cde}	1.4^{cde}	13.0^{de}	2.0^{a}
Glenda	24.9^{de}	1.3^{de}	11.7^{e}	2.1^{a}
Iron Gray	33.7^{cde}	2.6^{cde}	16.4^{cd}	2.0^{a}
IT82D-889	23.0^{de}	4.4^{abc}	11.7^{e}	2.7^{a}
IT84S-2246	51.7^{a}	6.1^{a}	19.3^{abc}	1.8^{a}
IT90K-59	36.8^{bc}	3.0^{bc}	20.9^{a}	2.3^{a}
IT90K-76	32.4^{cd}	2.5^{cde}	14.1^{cde}	1.9^{a}
IT93K-2045-29	22.3^{de}	1.4^{cde}	11.9^{e}	1.9^{a}
IT93K-452-1	39.4^{abc}	3.2^{bc}	16.8^{bc}	2.3^{a}
IT94D-437-1	31.8^{cd}	2.9^{bc}	15.1^{cde}	2.1^{a}
IT97K-499-39	25.2^{de}	2.0^{cde}	13.6^{cde}	1.8^{a}
ITH98-20	24.2^{de}	2.5^{cde}	13.1^{de}	1.3^{a}
ITH98-46	23.3^{de}	3.2^{bc}	16.9^{bc}	1.4^{a}
Line 2020	26.0^{cde}	1.4^{cde}	11.7^{e}	2.0^{a}
Mamlaka	19.9^{de}	2.5^{cde}	18.1^{abc}	2.4^{a}
Mchanganyiko	48.2^{ab}	3.7^{bc}	20.4^{ab}	1.6^{a}
Ngonji	33.0^{cd}	3.6^{bc}	15.1^{cde}	1.5^{a}
Omondaw	22.0^{de}	2.0^{cde}	14.2^{cde}	1.6^{a}
Pan 311	28.2^{cde}	2.3^{cde}	17.8^{abc}	1.7^{a}
Sanzie	28.0^{cde}	2.7^{cde}	16.3^{cd}	1.8^{a}
TVu11424	25.8^{de}	3.2^{bc}	14.4^{cde}	1.2^{a}
TVu1509	17.0^{e}	1.7^{cde}	14.3^{cde}	1.7^{a}
TVx3236	30.6^{cd}	4.9^{ab}	18.3^{abc}	1.7^{a}
Vita 7	26.7^{cde}	4.5^{abc}	16.9^{bc}	1.6^{a}
Vuli-1	25.8^{de}	3.9^{bc}	17.2^{bc}	1.5^{a}
F-statistics	2.9^{**}	5.2^{***}	2.7^{***}	1.1^{ns}
C.V. [%]	15	13	25	76

Table 2. Photosynthetic rates (P_N), transpiration rates (E), stomatal conductance (g_s), and plant transpiration efficiency (WUE) measured in 72-d-old cowpea genotypes grown in the field at Manga in Ghana in 2006. Means with *different letters* in a column are significant at $p \leq 0.05^{*}$ or $p \leq 0.01^{**}$.

Genotype	P_N [μmol(CO$_2$) m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	E [mmol(H$_2$O) m^{-2} s^{-1}]	WUE [μmol(CO$_2$) mmol^{-1}(H$_2$O)]
Apagbaala	17.8^{ab}	1.70^{abc}	3.5^{a}	0.30^{ab}
Botswana White	13.8^{e}	1.73^{abc}	3.2^{a}	0.28^{abc}
Brown Eye	14.0^{de}	1.85^{ab}	3.0^{a}	0.26^{bc}
CH14	19.6^{a}	1.64^{bc}	3.5^{a}	0.33^{a}
Glenda	15.9^{bcd}	1.83^{ab}	3.3^{a}	0.30^{ab}
IT82D-889	14.5^{cd}	1.80^{ab}	3.3^{a}	0.26^{bc}
IT84S-2246	15.6^{bcd}	1.73^{abc}	3.1^{a}	0.26^{bc}
IT90K-59	14.9^{cd}	1.78^{abc}	3.4^{a}	0.28^{abc}
IT93K-2045-29	17.8^{ab}	1.79^{abc}	3.5^{a}	0.32^{a}
IT97K-499-39	18.4^{ab}	1.56^{d}	3.3^{a}	0.30^{ab}
ITH98-46	15.8^{bcd}	1.59^{cd}	3.4^{a}	0.30^{ab}
Sanzie	15.6^{bcd}	1.81^{ab}	3.4^{a}	0.30^{ab}
Soronko	16.5^{abcd}	1.93^{a}	3.2^{a}	0.29^{ab}
TVU11424	17.0^{abc}	1.83^{ab}	3.4^{a}	0.28^{abc}
Vuli-1	16.3^{bcd}	1.48^{e}	3.1^{a}	0.23^{c}
F-statistics	2.2^{**}	2.0^{**}	1.2^{ns}	1.7^{*}
C.V. [%]	29	26	35	42

Table 3. Dry matter yield, N and C concentrations, and percentage N derived from fixation (Ndfa) in cowpea leaves sampled at 46 days after planting at Manga, Ghana, in 2005. Values with *different letters* in a column are significantly different at $p \leq 0.05$. Means with *different letters* in a column are significant at $p \leq 0.01^{**}$ or $p \leq 0.001^{***}$

Genotype	Leaf dry mass [mg leaf^{-1}]	N [%]	C [%]	δ^{15}N [‰]	Ndfa [%]
Apagbaala	23.5^{e}	4.0^{a}	39.6^{a}	4.7^{abc}	26.0^{j}
Bensogla	14.8^{f}	4.4^{a}	43.3^{a}	0.8^{h}	83.1^{a}
Botswana White	37.0^{b}	4.1^{a}	45.1^{a}	6.4^{a}	14.8^{k}
Brown Eye	27.2^{cde}	4.4^{a}	42.1^{a}	1.4^{efg}	63.9^{cde}
CH14	29.6^{c}	4.5^{a}	44.1^{a}	2.6^{cd}	50.5^{def}
Fahari	36.8^{b}	5.6^{a}	44.2^{a}	2.9^{cd}	46.1^{ef}
Glenda	27.2^{cde}	4.8^{a}	43.9^{a}	1.7^{de}	60.5^{cde}
Iron gray	24.7^{de}	4.1^{a}	40.7^{a}	3.3^{bc}	41.6^{fgh}
IT82D-889	27.2^{cde}	4.7^{a}	43.6^{a}	2.1^{cde}	62.5^{cde}
IT84S-2246	24.7^{de}	4.6^{a}	39.5^{a}	1.4^{efg}	63.8^{cde}
IT90K-59	36.5^{b}	4.9^{a}	44.8^{a}	1.5^{def}	64.9^{cd}
IT90K-76	24.7^{de}	5.0^{a}	44.0^{a}	4.2^{abc}	31.1^{ij}
IT93K-2045-29	38.5^{b}	4.4^{a}	43.4^{a}	-0.2^{j}	82.9^{a}
IT93K-452-1	34.3^{b}	5.4^{a}	42.5^{a}	4.2^{abc}	31.2^{hij}
IT94D-437-1	16.0^{f}	4.2^{a}	43.3^{a}	5.6^{ab}	15.0^{k}
IT97K-499-39	56.0^{a}	4.3^{a}	40.6^{a}	2.3^{cd}	53.0^{def}
ITH98-20	13.6^{f}	4.8^{a}	44.3^{a}	2.4^{cd}	48.8^{def}
ITH98-46	25.9^{cde}	4.6^{a}	44.4^{a}	2.7^{cd}	48.9^{def}
Line 2020	26.4^{cde}	5.1^{a}	45.9^{a}	3.4^{bc}	43.5^{ef}
Mamlaka	24.6^{de}	4.9^{a}	45.5^{a}	2.8^{cd}	45.0^{ef}
Mchanganyiko	25.1^{cde}	4.9^{a}	44.0^{a}	1.5^{def}	62.8^{cde}
Ngonji	38.3^{b}	5.0^{a}	40.7^{a}	2.0^{cde}	56.6^{def}
Omondaw	16.0^{f}	4.7^{a}	42.7^{a}	-0.3^{j}	83.9^{a}
Pan 311	14.8^{f}	4.1^{a}	44.9^{a}	4.6^{abc}	39.2^{ghi}
Sanzie	27.2^{cde}	4.4^{a}	43.5^{a}	0.4^{i}	76.3^{ab}
TVu11424	35.3^{b}	4.1^{a}	38.2^{a}	1.6^{def}	69.4^{bc}
TVu1509	24.7^{de}	4.4^{a}	38.2^{a}	4.8^{abc}	24.6^{jk}
TVx3236	17.3^{f}	5.2^{a}	39.2^{a}	2.4^{cd}	53.5^{def}
Vita 7	25.9^{cde}	5.0^{a}	43.6^{a}	1.7^{de}	60.3^{cde}
Vuli-1	28.4^{cd}	4.4^{a}	42.2^{a}	1.7^{de}	60.2^{cde}
F-statistics	31**	1.3^{ns}	0.95^{ns}	84.1^{**}	25.8^{***}
C.V [%]	10	16	9	38	12

Table 4. Dry matter yield, N and C concentrations, and symbiotic N in cowpea leaves sampled at 72 days after planting at Manga, Ghana, in 2006. Values with *different letters* in a column are significantly different at $p \leq 0.01^{**}$

Genotype	Leaf dry mass [mg per leaf]	N [%]	C [%]	δ^{15}N [‰]	Ndfa [%]
Apagbaala	53.8d^{ef}	4.1^{a}	44.3^{a}	1.6^{b}	55.4^{de}
Botswana White	59.6^{bc}	3.8^{a}	46.0^{a}	0.6^{d}	65.7^{bc}
Brown Eye	39.6^{i}	3.6^{a}	43.2^{a}	1.3^{bc}	63.8^{bc}
CH14	90.2^{a}	4.4^{a}	44.5^{a}	1.4^{b}	55.9^{de}
Glenda	51.6^{efg}	4.0^{a}	43.2^{a}	2.3^{a}	47.4^{f}
IT82D-889	62.6^{c}	3.8^{a}	43.4^{a}	1.5^{b}	56.0^{de}
IT84S-2246	41.6^{hi}	4.5^{a}	44.4^{a}	1.6^{b}	53.2^{ef}
IT90K-59	54.8^{def}	3.5^{a}	43.9^{a}	1.6^{b}	56.5^{de}
IT93K-2045-29	70.0^{b}	4.4^{a}	46.2^{a}	0.2^{e}	75.6^{a}
IT97K-499-39	50.4^{fg}	4.0^{a}	42.9^{a}	0.9^{cd}	66.3^{b}
ITH98-46	48.1^{fgh}	4.0^{a}	44.5^{a}	1.5^{b}	59.8^{de}
Sanzie	58.0^{bcd}	4.2^{a}	42.6^{a}	1.8^{b}	53.8^{de}
Soronko	26.7^{j}	3.8^{a}	43.0^{a}	1.5^{b}	54.9^{de}
TVu11424	47.2^{gh}	4.1^{a}	43.0^{a}	1.5^{b}	63.2^{cd}
Vuli-1	36.7^{i}	4.1^{a}	44.2^{a}	0.5^{d}	66.7^{b}
F-statistics	39.2^{**}	1.7^{ns}	0.94^{ns}	5.4^{**}	12^{**}
C.V [%]	8	16	18	46	6

The data for 2006 showed a similar pattern for leaf growth, N and C concentrations, as well as for $\delta^{15}N$ and %Ndfa (Table 4). The dry matter yield of cowpea leaves used for photosynthetic measurements was greater in CH14, followed by IT93K-2045-29 and IT82D-889, and the lowest in Soronko and Vuli-1 (Table 4). The N concentrations in leaves were again similar for 15 cowpea genotypes in 2006. However, $\delta^{15}N$ and %Ndfa differed markedly among genotypes. For example, Glenda and Sanzie exhibited the highest $\delta^{15}N$ values (2.3 ‰ and 1.8 ‰, respectively) and therefore the lowest N derived from fixation (47% and 53%, respectively), IT93K-2045-29, Vuli-1, Botswana White, and IT97K-499-39 recorded the lowest $\delta^{15}N$ values (+0.2, +0.5, +0.6, and + 0.9 ‰, respectively) and hence the highest N obtained from fixation (76, 67, 66, and 66%, respectively).

Amounts of total N and fixed-N in cowpea leaves: There were marked variations in leaf total N and leaf N-fixed among the 30 cowpea genotypes tested at Manga in 2005. Leaf total N was the highest in IT84S-2246, followed by Fahari, Ngonji, IT90K-59, and IT93K-452-1, and the lowest in Pan 311 and ITH98-20, followed by Bensogla, IT94D-437-1, and Omondaw (Table 5). The remaining genotypes had intermediate leaf N concentrations. The amount of N-fixed in photosynthetic leaves was greater in IT93K-2045-29, followed by IT84S-2246, IT90K-59, Ngonji, Fahari, and TVu11424 (Table 5). In contrast, symbiotic N in photosynthetic leaves was the lowest in IT94D-437-1, Apagbaala, Botswana White, ITH98-20, Pan 311, and TVu1509 (Table 5). In this study, leaf total N was the highest in CH14, followed by IT93K-2045-29, IT82D-889, Sanzie, Botswana White, and Apagbaala, while Soronko showed the lowest leaf total N, followed by Brown Eye and IT90K-59 (Table 6). The amount of N-fixed in cowpea leaves used for gas-exchange studies was also much greater in CH14 and IT93K-2045-29, followed by Botswana White, while Soronko again showed the lowest amount of N-fixed, followed by IT90K-59 and Brown Eye.

Table 5. Leaf total N, leaf N-fixed, Rubisco N, leaf C, and photosynthetic N-use efficiency (PNUE) in leaves of field-grown cowpea genotypes sampled at 46 days after planting at Manga, Ghana, in 2005. Means with *different letters* in a column are significant at $p \leq 0.01^{**}$.

	Leaf [mg per leaf]				Leaf PNUE [mg mg^{-1}]		
Cowpea genotypes	Total N	N-fixed	Rubisco N	C	C/total N	C/N-fixed	C-fixed/ Rubisco N
Apagbaala	0.9^{def}	0.2^{hi}	0.7^{def}	9.3^{ef}	10.1^{a}	40.6^{c}	12.6^{bc}
Bensogla	0.7^{ef}	0.5^{fg}	0.5^{f}	6.4^{f}	10.6^{a}	12.2^{ghi}	12.4^{bc}
Botswana White	1.5^{bcd}	0.2^{hi}	1.2^{cd}	16.8^{bc}	11.0^{a}	84.0^{a}	14.0^{ab}
Brown Eye	1.2^{cd}	0.8^{de}	0.9^{de}	11.4^{de}	8.6^{a}	15.7^{fg}	18.4^{a}
CH14	1.3^{cd}	0.7^{ef}	1.1^{cde}	13.1^{cd}	9.5^{a}	19.6^{ef}	12.3^{bc}
Fahari	2.1^{ab}	1.0^{bc}	1.7^{ab}	16.3^{bc}	7.9^{a}	16.3^{efg}	9.7^{e}
Glenda	1.3^{cd}	0.8^{de}	1.0^{cde}	12.0^{cde}	9.1^{a}	15.0^{fg}	11.4^{cd}
Iron Grey	1.0^{de}	0.4^{fgh}	0.8^{def}	10.1^{def}	9.6^{a}	24.1^{de}	12.4^{bc}
IT82D-889	1.3^{cd}	0.8^{de}	1.0^{cde}	11.8^{de}	9.2^{a}	15.0^{fg}	11.6^{cd}
IT84S-2246	2.4^{a}	1.3^{ab}	1.9^{a}	22.7^{a}	9.5^{a}	17.5^{efg}	12.0^{bcd}
IT90K-59	1.8^{abc}	1.2^{ab}	1.4^{bcd}	16.4^{bc}	9.3^{a}	14.3^{fgh}	11.6^{cd}
IT90K-76	1.2^{cd}	0.4^{fgh}	1.0^{cde}	10.9^{cde}	8.9^{a}	28.6^{de}	11.1^{cd}
IT93K-2045-29	1.7^{bc}	1.4^{a}	1.4^{bcd}	16.7^{bc}	9.4^{a}	12.1^{ghi}	12.5^{bc}
IT93K-452-1	1.8^{abc}	0.6^{efg}	1.5^{bc}	14.6^{bcd}	8.1^{a}	26.1^{de}	10.2^{cde}
IT94D-437-1	0.7^{ef}	0.1^{i}	0.5^{f}	6.9^{f}	10.7^{a}	69.0^{ab}	13.9^{abc}
IT97K-499-39	1.1^{cde}	0.7^{ef}	0.9^{de}	9.9^{ef}	8.1^{a}	9.0^{i}	11.9^{cd}
ITH98-20	0.6^{f}	0.3^{gh}	0.5^{f}	6.0^{f}	9.0^{a}	10.0^{hi}	11.7^{cd}
ITH98-46	1.2^{cd}	0.6^{efg}	1.0^{cde}	11.4^{de}	9.0^{a}	19.8^{ef}	12.1^{bc}
Line 2020	1.3^{cd}	0.6^{efg}	1.1^{cde}	12.1^{cde}	9.0^{a}	20.8^{def}	11.3^{cd}
Mamlaka	1.2^{cd}	0.5^{fg}	1.0^{cde}	11.2^{de}	9.4^{a}	21.0^{def}	12.8^{bc}
Mchanganyiko	1.2^{cd}	0.8^{de}	1.0^{cde}	11.0^{de}	9.0^{a}	14.3^{fgh}	11.2^{cd}
Ngonji	1.9^{abc}	1.1^{bc}	1.5^{bc}	15.5^{bcd}	8.2^{a}	14.5^{fgh}	11.2^{cd}
Omondaw	0.8^{ef}	0.6^{efg}	0.6^{ef}	6.9^{f}	9.0^{a}	10.7^{hi}	11.3^{cd}
Pan 311	0.6^{f}	0.3^{gh}	0.5^{f}	6.6^{f}	10.7^{a}	22.0^{def}	13.2^{abc}
Sanzie	1.2^{cd}	0.9^{cd}	1.0^{cde}	11.8^{de}	9.6^{a}	13.0^{gh}	12.4^{bc}
TVu11424	1.5^{bcd}	1.0^{bc}	1.2^{cd}	13.5^{cd}	11.1^{a}	13.8^{gh}	11.8^{cd}
TVu1509	1.1^{cde}	0.3^{gh}	0.9^{de}	9.4^{ef}	8.6^{a}	35.2^{cd}	10.7^{cde}
TVx3236	0.9^{def}	0.5^{fg}	0.7^{def}	6.9^{f}	7.5^{a}	13.8^{gh}	9.1^{e}
Vita 7	1.3^{cd}	0.8^{de}	1.0^{cde}	11.3^{de}	8.9^{a}	14.1^{fgh}	11.4^{cd}
Vuli-1	1.2^{cd}	0.8^{de}	1.0^{cde}	11.9^{de}	9.0^{a}	16.1^{efg}	12.0^{bcd}
F-statistics	13.4^{**}	19.2^{**}	13.4^{**}	43.3^{**}	7.2^{ns}	25^{**}	2.1^{***}
C.V. [%]	15	18	16	15	9	24	12

Table 6. Leaf total N, leaf N-fixed Rubisco N, leaf C, and photosynthetic N-use efficiency (PNUE) in leaves of field-grown cowpea genotypes sampled at 72 days after planting at Manga, Ghana, in 2006. Means with *different letters* in a column are significant at $p \leq 0.01^{**}$ or $p \leq 0.001^{***}$.

Cowpea genotypes	Leaf N and C [mg per leaf]				Leaf PNUE [mg mg^{-1}]		
	total N	N-fixed	Rubisco-N	C	C/total N	C/N-fixed	C/Rubisco N
Apagbaala	2.2^{c}	1.2^{bcd}	1.8^{c}	23.9^{bcd}	10.8^{a}	18.6^{cd}	13.5cd
Botswana White	2.3^{c}	1.5^{b}	1.8^{c}	27.5^{bc}	12.3^{a}	16.2^{def}	13.4d
Brown Eye	1.4^{ef}	0.9^{def}	1.1^{ef}	17.6^{def}	12.3^{a}	16.8^{def}	15.4bc
CH14	4.0^{a}	2.2^{a}	3.2^{a}	44.1^{a}	13.1^{a}	16.4^{def}	13.5cd
Glenda	2.1^{cd}	1.0^{cde}	1.7^{cd}	26.4^{bc}	14.7^{a}	32.2^{a}	18.4a
IT82D-889	2.4^{c}	1.3^{bc}	1.9^{c}	23.2^{cde}	11.9^{a}	20.0^{bc}	15.7b
IT84S-2246	1.9^{cde}	1.0^{cde}	1.5^{cde}	15.1^{ef}	13.1^{a}	18.1^{cd}	13.9bcd
IT90K-59	1.4^{ef}	0.8^{def}	1.1^{ef}	24.4^{bcd}	12.6^{a}	21.1^{b}	15.5bc
IT93K-2045-29	3.1^{b}	2.3^{a}	2.5^{b}	31.2^{b}	10.8^{a}	13.4^{g}	12.7d
IT97K-499-39	2.0^{cde}	1.3^{bc}	1.6^{cde}	22.3^{cde}	11.2^{a}	16.9^{def}	14.0bcd
ITH98-46	1.9^{cde}	1.1^{bcd}	1.5^{cde}	20.5^{cde}	11.5^{a}	18.1^{cd}	13.6cd
Sanzie	2.4^{c}	1.3^{bc}	1.9^{c}	21.1^{cde}	12.3^{a}	18.9^{cd}	13.5cd
Soronko	1.0^{f}	0.6^{f}	0.8^{f}	11.5^{f}	11.5^{a}	19.4^{bcd}	14.4bcd
TVu11424	1.9^{cde}	1.2^{bcd}	1.5^{cde}	21.7^{cde}	11.3^{a}	17.9^{def}	14.1bcd
Vuli-1	1.5^{def}	1.0^{cde}	1.2^{def}	15.8^{ef}	10.5^{a}	15.8^{f}	13.1d
F-statistics	12.4***	14.0***	12.4**	7.5**	1.4^{ns}	35.0***	4.2**
C.V. [%]	17	17	17	21	13	7	8

C content and photosynthetic N-use efficiency in cowpea leaves: The amount of photosynthetic leaf C differed significantly between and among the 30 cowpea genotypes. For example, IT84S-2246 recorded the highest C content in photosynthetic leaves, followed by Botswana White, IT93K-2045-29, IT90K-59, Fahari, Ngonji, and IT93K-452-1, with amounts ranging from 14.6 to 22.7 mg(C) per leaf (Table 5). The genotype with the lowest C in photosynthetic leaves was ITH98-20, followed by Bensogla, Pan 311, IT94D-437-1, Omondaw, and TVx 3236, with C contents ranging from 6.0 to 6.9 mg per leaf (Table 5).

Photosynthetic N-use efficiency (measured as leaf C per leaf total N, and/or C per N-fixed) also varied markedly with genotype (Table 5). Although the C per leaf N was the same for all genotypes, C per leaf N-fixed differed significantly among the 30 cowpea genotypes. Botswana White, which was among the genotypes with high C and low N-fixed, showed the highest C per N-fixed in photosynthetic leaves, followed by IT94D-437-1, Apagbaala, and TVu1509 (Table 5). IT97K-499-39 exhibited the lowest value, followed by ITH98-20, Omondaw, IT93K-2045-29, Bensogla, Sanzie, TVu11424, and TVx 3236 (Table 5).

The amount of photosynthetic C in leaves and C per unit N-fixed also differed in the cowpea genotypes retested in 2006. Photosynthetic C accumulation was much higher in CH14, followed by IT93K-2045-29, Botswana White, and Glenda (Table 6). On the other hand, Soronko showed the lowest amount of C, followed by IT84S-2246, Vuli-1, and Brown Eye, with values ranging from 11.5 to 17.6 mg per leaf (Table 6). Although the C per unit of leaf N was similar for the 15 cowpea genotypes, photosynthetic C formed per unit of N-fixed differed significantly among the genotypes in 2006 (Table 6). Genotype Glenda recorded the highest photosynthetic C accumulated per unit of symbiotic N in leaves, followed by IT90K-59 and IT82D-889. In contrast, IT93K-2045-29 showed the lowest amount of C per unit of N-fixed, followed by Vuli-1, Botswana White, CH14, Brown Eye, and IT97K-499-39, with values ranging from 13.4 to 16.9 mg(C) per unit of N-fixed (Table 6).

Discussion

In this study, the data from gas-exchange studies of cowpea leaves showed significant differences in photosynthetic activity of 30 cowpea genotypes planted in the field in the Sudano-Sahelian savanna of Ghana. The results showed increased P_{N} in cowpea genotypes, such as IT84S-2246, Mchanganyiko, IT93K-452-1, Brown Eye, IT90K-59, and Ngonji, and very low levels in Apagbaala and TVu1509 in 2005 (Table 1). Some genotypes performed poorly in the first year but better in the second year, and *vice versa*. For example, IT84S-2246 showed the highest rate of photosynthesis in 2005 but dropped to tenth in 2006, while Apagbaala, which ranked fourteenth out of 30 genotypes in 2005 (Table 1), was third out of 15 genotypes in 2006 (Table 2). These inconsistencies in genotypic performance of cowpea are difficult to explain, but would be most likely due to microsite variation in soil nutrient supply and the symbiotic efficacy of rhizobia nodulating cowpea genotypes. We have shown elsewhere

Table 7. Correlation of symbiotic parameters, C-fixed and leaf Rubisco N in young leaves of field-grown cowpea genotypes sampled at 46 days after planting (DAP) at Manga, in 2005 in Ghana. * – $p<0.05$, ** – $p< 0.01$, *** – $p< 0.001$. P_N – photosynthetic rate, g_s – stomatal conductance, E – transpiration rate, WUE – plant transpiration efficiency, %Ndfa = percentage N derived from fixation.

	Leaf DM	%N	%Ndfa	Leaf ^{15}N	Leaf total N	Leaf N-fixed	Rubisco N	C/total leaf N	C/Rubisco N	Grain yield	P_N	g_s	E
Leaf DM													
%N	0.02												
%Ndfa	0.03	0.10											
Leaf ^{15}N	−0.06	−0.08	−0.95***										
Leaf total N	0.68**	0.28**	0.68**	−0.68**									
Leaf N-fixed	0.47**	0.15	0.82**	−0.80	0.94***								
Rubisco N	0.68**	0.28	0.68**	−0.68	1.00***	0.94***							
Leaf C-fixed	0.95***	0.12	0.03	−0.04	0.66	0.46	0.66						
C/total leaf N	−0.09	−0.35**	−0.82**	0.83**	−0.66**	−0.65**	−0.66**						
C/Rubisco N	0.95**	−0.35***	−0.82***	0.83***	−0.66***	−0.65**	−0.66**	1.00***					
C/ leaf N-fixed	−0.07	−0.28**	−0.73**	0.76**	−0.58**	−0.56**	−0.58**	0.98***	0.98***				
P_N	0.22***	0.05	0.07	−0.06	0.08	0.07	0.08	−0.07	−0.07	−0.25**			
g_s	−0.12	0.03	0.17*	−0.24**	−0.03	−0.01	−0.03	−0.04	−0.04	0.08	0.39**		
E	−0.12	0.08	0.01	0.01	−0.05	−0.04	−0.05	0.04	0.04	−0.03	0.36**	0.56**	
WUE	−0.08	0.09	−0.01	−0.02	−0.22**	−0.23**	−0.22**	0.00	0.00	−0.05	−0.25**	0.18	0.30**

from studies of nodule occupancy that Apagbaala was the second best fixer of six genotypes, when nodulated by only one strain IGS type II, and the least fixer of nine genotypes, when it nodulated promiscuously by four IGS types II, V, VIII, XVIII (Pule-Meulenberg *et al.* 2010). These subtleties in host plant performance at the level of nodule occupancy could account for the differences in photosynthetic rates between genotypes in time and space.

As to be expected, the genotypes with high P_N exhibited much greater g_s and water loss *via* leaf transpiration, while those with reduced photosynthetic activity showed very low g_s and leaf E (Table 1). There were, however, no significant differences in WUE in 2005. The results for 2006 also revealed increased P_N, g_s, and leaf E in CH14, IT93K-2045-29, Apagbaala, and Soronko, in contrast to Vuli-1, which showed low P_N, g_s, and WUE (Table 2). It, however, remains to be determined whether these variations in gas-exchange were due to differences in leaf stomatal density. Whatever the case, similar gas-exchange data were obtained by Anyia and Herzog (2004) for cowpea.

Leaf dry matter yield, but not N or C concentrations, also differed among the cowpea genotypes (Table 3). Furthermore, the leaf δ^{15}N values of Omondaw, IT93K-2045-29, Sanzie, and Bensogla were very low, resulting in significantly higher percentage N derived from fixation (83.9%, 82.9%, 76.3%, and 83.1%, respectively), and greater amounts of N-fixed. In contrast, the leaf δ^{15}N values of Botswana White, IT94D-437-1, TVu1509, Apagbaala, Pan 311, IT90K-76, and IT93K-452-1 were markedly high and resulted in lower percentage N derived from fixation, and significantly decreased amounts of N-fixed (Table 5). Interestingly, the genotypes that showed greater leaf N-fixed (*e.g.* IT84S-2246, Ngonji, Fahari, Sanzie, and Mchanganyiko) also exhibited higher symbiotic N yield at whole-plant level, while those with lower leaf N-fixed closely mirrored the small amounts of N-fixed by whole cowpea plants (Belane and Dakora 2009). Furthermore, the percentage N derived from fixation in 2005 and 2006 were generally low (15% – 83.9% in 2005, and 47.4% – 75.6% in 2006). A comparison of total leaf N with leaf N-fixed in 2005 (Table 3) identified ten genotypes (*i.e.* Apagbaala, Botswana White, Fahari, Iron Grey, IT90K-76, IT93K-452-1, IT94D-437-1, Line 2020, Mamlaka, and TVu1509) whose leaf N-fixed was less than half of the total leaf N; this could suggest suppression of N_2 fixation by endogenous soil N (Streeter and Wong 1988, Ayisi *et al.* 2000). Under the same soil conditions, however, genotypes Bensogla, IT93K-2045-29, Omondaw, and Sanzie showed much higher percentage N derived from fixation and significantly greater amounts of N-fixed. This could suggest tolerance of these genotypes to soil mineral N (Ayisi *et al.* 2000, Dakora *et al.* 1992, Dakora 1998).

In some instances, we found a link between leaf photosynthetic rates, leaf N-fixed, and whole-plant symbiotic N contribution. Genotypes IT84S-2246, IT93K-452-1, Mchanganyiko, and Ngonji, for example, showed relatively higher leaf P_N (Table 1), greater leaf N-fixed (Table 5), and higher symbiotic N yield at whole-plant level in 2005 (Belane and Dakora 2009). In contrast, genotypes Apagbaala and TVu1509 exhibited very low leaf P_N (Table 1), produced lower leaf N-fixed (Table 5), and yielded only small amounts of symbiotic N in 2005 (Belane and Dakora 2009). These data could be interpreted to mean that higher photosynthate supply increased N_2 fixation, and *vice versa*. However, a close scrutiny of the data showed that genotypes with high values of total leaf N and leaf N-fixed generally accumulated more C in their leaves than those with low N. This could suggest that N_2 fixation stimulated photosynthetic rates in the cowpea genotypes; such that the more the symbiotic N supplied to leaves, the higher was the photosynthetic stimulation. This view was supported by data on PNUE in some cowpea genotypes. The leaves of Botswana White, IT94D-437-1, Apagbaala, and TVu1509, for example, showed significantly high C per unit of N-fixed, indicating sink stimulation of photosynthesis (Kaschuk *et al.* 2009). In other words, the symbiotic N supplied to leaves from root nodules was a major determinant of the rate of photosynthesis.

Because photosynthetic CO_2 fixation accounts for a large part of plant biomass (Zelitch 1982, Khan *et al.* 2008), leaf C correlated with leaf dry mass (Table 7), just as leaf dry matter also positively correlated with photosynthesis. Leaf total N and leaf N-fixed also showed positive correlation with leaf dry matter because of the N needed for Rubisco biosynthesis. Furthermore, photosynthesis correlated with stomatal conductance and transpiration, in the same way that transpiration correlated with water-use efficiency (Table 7). Taken together, these correlation analyses show that the gas-exchange parameters are functionally interlinked, just as leaf C accumulation from photosynthesis is linked to leaf N content, be it leaf total N or leaf N-fixed.

A close scrutiny of the C/N, C/N-fixed, and C/Rubisco-N ratios of cowpea shoots revealed an interesting pattern. The C/N and C/Rubisco-N ratios were generally similar in magnitude when compared to C/N-fixed ratio. This is possibly due to the fact that Rubisco accounts for over 90% of photosynthetic leaf N, irrespective of whether it is formed from soil N or symbiotic N. But more importantly, cowpea genotypes that relied heavily on soil N for their N nutrition exhibited much higher C/N-fixed ratios, while conversely those that depended more on symbiosis for meeting their N demands showed markedly lower C/N-fixed ratios. Thus, in nodulated legumes, greater C/N-fixed ratios seem to indicate poor symbiosis, while much lower C/N-fixed values represent effective high-fixing symbiosis. For example, genotypes Omondaw, Bensogla, IT93K-2045-29, and Sanzie, which respectively derived 83.9, 83.1, 82.9, and 76.3% N from fixation in 2005 recorded lower C/N-fixed ratios of 10.7, 12.2, 12.1, and 13.0 mg mg^{-1} in that order (Tables 4, 5). By contrast, genotypes Botswana White, IT94D-437-1, TVu1509, and Apagbaala, which obtained a low 14.8, 15.0, 26.4, and 26.0% N from fixation, recorded the high C/N-fixed values of 84.0, 69.0, 35.2, and 40.6 mg mg^{-1}, respectively, in 2005 (Tables 4, 5). This clearly indicates that genotypes that obtained less N from symbiosis and more N from soil revealed very high C/N-fixed values. This argument is reinforced by the negative correlations obtained between the three ratios (*i.e.* C/N, C/Rubisco-N, and C/N-fixed) and leaf N concentration, %Ndfa, total N content, N-fixed, and Rubisco N (Table 7).

References

Ainsworth E.A., Rogers A., Blum H. *et al.*: Variation in acclimation of photosynthesis in *Trifolium repens* after eight years of exposure to free-air CO_2 enrichment (FACE). – J. Exp. Bot. **54**: 2769-2774, 2003.

Ainsworth E.A., Rogers A., Nelson R., Long S.P.: Testing the "source-sink" hypothesis of down-regulation of photosynthesis in elevated [CO_2] in the field with single gene substitutions in *Glycine max*. – Agr. Forest. Meteorol. **122**: 85-94, 2004.

Anyia A.O., Herzog H.: Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. – Eur. J. Agron. **20**: 327-339, 2004.

Ayisi K.K., Nkgapele R.J., Dakora F.D.: 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, 2000.

Belane A.K., Dakora F.D.: 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. – Symbiosis **48**: 47-56, 2009.

Cheng L., Fuchigami L.H.: Rubisco activation state decreases with increasing nitrogen content in apple leaves. – J. Exp. Bot. **51**: 1687-1694, 2000.

Dakora F.D., Atkins C.A., Pate J.S.: Effect of nitrate on nitrogen 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 Soil **140**: 255-262, 1992.

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

Dordas C.A., Sioulas C.: Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilization under rainfed conditions. – Indust. Crops Prod. **27**: 75-85, 2008.

Drake B.G., Gonzàlez-Meler M.A., Long S.P.: More efficient plants: a consequence of rising atmospheric CO_2? – Annu. Rev. Plant Phys. **48**: 609-639, 1997.

Driessen P., Deckers J., Spaargaren O.: FAO: World Soil Resources Reports 60. FAO-UNESCO Soil Map of the World-Revised Legend. Pp. 146. ISRIC, Wageningen 1990.

Elowad H.O.A., Hall A.E.: Influences of early and late nitrogen fertilization on yield and nitrogen fixation of cowpea under well-watered and dry field conditions. – Field Crop. Res. **15**:

229-244, 1987.

Evans J.R.: Photosynthesis and nitrogen relationships in leaves of C_3 plants. – Oecologia **78**: 9-19, 1989.

Field C., Mooney H.A.: The photosynthesis-nitrogen relationship in wild plants. – In: Givinish T.J. (ed.): On the Economy of Form and Function. Pp. 25-55. Cambridge University Press, Cambridge 1986.

Hamid A., Agata W., Kawamitsu Y.: Photosynthesis, transpiration and water-use efficiency in four cultivars of mungbean, *Vigna radiata* (L.) Wilczek. – Photosynthetica **24**: 96-101, 1990.

Heineke D., Kauder F., Frommer W. *et al.*: Application of transgenic plants in understanding responses to atmospheric change. – Plant Cell Environ. **22**: 623-628, 1999.

Hikosaka K., Terashima I.: A model of the acclimation of photosynthesis in the leaves of C_3 plants to sun and shade with respect to nitrogen use. – Plant Cell Environ. **18**: 605-618, 1995.

Isopp H., Frehner M., Almeida J.P.F. *et al.*: Nitrogen plays a major role in leaves when source–sink relations change: C and N metabolism in *Lolium perenne* growing under free air CO_2 enrichment. – Aust. J. Plant Physiol. **27**: 851-858, 2000.

Junk G., Svec H.: The absolute abundance of the nitrogen isotopes in the atmosphere and compressed gas from various sources. – Geochim. Cosmochim. Ac. **14**: 134-243, 1958.

Kaschuk G., Kuyper T.W., Leffelaar P.A. *et al.*: Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mychorrizal symbioses? – Soil Biol. Biochem. **41**: 1233-1244, 2009.

Khan W., Prithiviraj B., Smith D.L.: Nod factor [Nod Bj V (C18:1, MeFuc)] and lumichrome enhance photosynthesis and growth of corn and soybean. – J. Plant Physiol. **165**: 1342-1351, 2008.

Lawson T.: Guard cell photosynthesis and stomatal function. – New Phytol. **181**: 13-34, 2009.

Mächler F., Oberson A., Grub A. *et al.*: Regulation of photosynthesis in nitrogen deficient wheat seedlings. – Plant Physiol. **87**: 46-49, 1988.

Makoi J.H.J.R., Chimphango S.B.M., Dakora F.D.: Effect of legume density and mixed culture on symbiotic N_2 fixation in five cowpea (*Vigna unguiculata* L. Walp.) genotypes in South Africa. – Symbiosis **48**: 57-67, 2009.

Makoi J.H.J.R., Chimphango S.B.M., Dakora F.D.: Photosynthesis, water-use efficiency and ∂^{13}C of five cowpea (*Vigna unguiculata* L. Walp.) genotypes grown in mixed culture and at different densities with sorghum (*Sorghum bicolor* L.). – Photosynthetica **48**: 143-155, 2010.

Mariotti A., Germon J.C., Hubert P. *et al.*: Experimental determination of nitrogen kinetic isotope fraction: some principle; illustrations for the denitrification and nitrification processes. – Plant Soil **62**: 413-430, 1981.

Maskey S.L., Bhattarai S., Peoples M.B., Herridge D.F.: On-farm measurements of nitrogen fixation by winter and summer legumes in the Hill and Terai regions of Nepal. – Field Crop. Res. **70**: 209-221, 2001.

Moore B.D., Cheng S.H., Sims D. *et al.*: The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. – Plant Cell Environ. **22**: 567-582, 1999.

Morison J.I.L., Baker N.R., Mullineaux P.M. *et al.*: Improving water use in crop production. – Philos. T. R. Soc. B **363**: 639-658, 2007.

Pule-Meulenberg F., Belane A.K., Krasova-Wade T., Dakora F.D.: Symbiotic functioning and bradyrhizobial biodiversity of cowpea (*Vigna unguiculata* L. Walp.) in Africa. – BMC Microbiol **10**: 89, 2010.

Robertson M.J., Carberry P.S., Huth N.I. *et al.*: Simulation of growth and development of diverse legume species in APSIM. – Aust. J. Agric. Res. **53**: 429-446, 2002.

Rosenzweig C., Parry M.L.: Potential impact of climate change on world food supply. – Nature **367**: 133-8, 1994.

Shearer G., Kohl D.H.: N_2-fixation in field settings: Estimations based on natural ^{15}N abundance. – Aust. J. Plant Physiol. **13**: 699-756, 1986.

Streeter J., Wong P.P.: Inhibition of legume nodule formation and N_2 fixation by nitrate. – Crit. Rev. Plant Sci. **7**: 1-23, 1988.

Tubiello F.N., Rosenzweig C., Kimball B.A. *et al.*: Testing CERES wheat with free-air carbon dioxide enrichment (FACE) experimental data: CO_2 and water interactions. – Agron. J. **91**: 247-255, 1999.

Unkovich M.J., Herridge D., Peoples M. *et al.*: ^{15}N natural abundance method. – In: Unkovich M.J., Herridge D., Peoples M. *et al.* (ed.): Measuring plant-associated nitrogen fixation in agricultural systems. Pp. 131-132. Australian Centre for International Agricultural Research (ACIAR), Monograph No. 136, Canberra 2008.

Wang H., Ngwenyama N., Liu Y. *et al.*: Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in *Arabidopsis*. – Plant Cell **19**: 63-73, 2007.

Xu L.K., Hsiao T.C.: Predicted versus measured photosynthetic water-use efficiency of crop stands under dynamically changing field environments. – J. Exp. Bot. **55**: 2395-2411, 2004.

Zelitch I.,: The close relationship between net photosynthesis and crop yield. – BioScience **32**: 796-802, 1982.