Genotypic and within canopy variation in leaf carbon isotope discrimination and its relation to short-term leaf gas exchange characteristics in cassava grown under rain-fed conditions in the tropics

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Abstract

In a field rain-fed trial with 15 cassava cultivars, leaf gas exchanges and carbon isotope discrimination (Δ) of the same leaves were determined to evaluate genotypic and within-canopy variations in these parameters. From 3 to 7 months after planting leaf gas exchange was measured on attached leaves from upper, middle, and lower canopy layers. All gas exchange parameters varied significantly among cultivars as well as canopy layers. Net photosynthetic rate (P_N) decreased from top canopy to bottom indicating both shade and leaf age effects. The same trend, but in reverse, was found with respect to Δ, with the highest values in low canopy level and the lowest in upper canopy. There were very significant correlations, with moderate and low values, among almost all these parameters, with P_N negatively associated with intercellular CO_2 concentration (C_i), ratio of C_i to ambient CO_2 concentration C_i/C_a, and Δ. Across all measured leaves, Δ correlated negatively with leaf water use efficiency (WUE = photosynthesis/stomatal conductance, g_s) and with g_s, but positively with C_i and C_i/C_a. The later parameters negatively correlated with leaf WUE. Across cultivars, both P_N and Δ correlated positively with storage root yield. These results are in agreement with trends predicted by the carbon isotope discrimination model.

Additional key words: breeding; CO_2; cultivars; carboxylation enzymes; PEPC; RuBPCO; photosynthesis; rain-fed; stomata; stress; water use efficiency.

Introduction

Cassava (yuca, mandioca, or manioc: *Manihot esculenta* Crantz, Euphorbiaceae), has received attention from international research centers only few decades ago despite being one of six important food crops in tropical and subtropical Africa, Asia, and Latin America (El-Sharkawy 1993). This negligence was mainly due to the priority given to the main cereal crops such as wheat, rice, and maize by the few international donor agencies after the World War II, and partly to poor support for research on cassava by many of the developing countries where the crop is grown, with few exceptions. Recognition of the importance of cassava as a staple food, animal feed, and as a primary material for some industrial products has enhanced research on the many aspects of the crop. Advances in cassava research at the two international centres, the Institute of Tropical Agriculture (IITA) in

Nigeria and the Centro Internacional de Agricultura Tropical (CIAT) in Colombia have recently been reviewed (Hershey and Jennings 1992, Hillocks *et al.* 2002, Kawano 2003, El-Sharkawy 2004, 2006a).

At CIAT, germplasm collection has resulted in the assembly and conservation of a wide range of land races, cultivars, and breeding materials from the many countries in Latin America (providing the majority where cassava is native) as well as from Africa and Asia. Breeding programs adopted the strategy of improving yield with resistance to biotic and abiotic stresses encountered in the various eco-zones and ecosystems of the tropics and subtropics. The most notable among these stresses are prolonged water shortages due to erratic rainfall and occurrence of long dry periods (longer than three months) during cassava growing season (normally spanning from

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12 to 18 months, in low-land and high altitude zones, respectively) and low-fertility soils (Howeler and Cadavid 1990, Howeler 1991, 2002, El-Sharkawy 1993). Cassava physiology research was coordinated and linked with the breeding effort and focused on studying cassava responses to water/soil stresses under field conditions in order to elucidate underlying mechanisms of tolerance, productivity, and identifying useful plant traits for incorporation into breeding materials (El-Sharkawy 2006a). This research was aided by available modern techniques such as improved gas exchange and soil-plant-water measuring equipment, and some useful biochemical assays. Several physiological mechanisms inherent to cassava were uncovered including high photosynthetic capacity under favorable environments (El-Sharkawy et al. 1992a) and sustainable gas exchange rates under extended water shortages (El-Sharkawy et al. 1992b, El-Sharkawy 1993, 2006a, Cayón et al. 1997, de Tafur et al. 1997). Cassava stomatal apparatus exerts a tight control on gas exchanges, particularly under stressful atmospheric and soil-water conditions. Rapid close of cassava stomata in dry air and in response to soil-water deficit is a stress avoidance mechanism that obviates damages to leaves while the crop extracts slowly whatever available deep soil water (Connor and Palta 1981, Connor et al. 1981, El-Sharkawy and Cock 1984, El-Sharkawy et al. 1984, Cock et al. 1985, Guzman and El-Sharkawy 1989, El-Sharkawy et al. 1992b, Cayón et al. 1997, de Tafur et al. 1997, El-Sharkawy and Cadavid 2002, El-Sharkawy 2006a). These important findings laid a foundation for improving the genetic base of cassava and for enhancing selection and breeding for seasonally dry and semiarid eco-zones (Hershey and Jennings 1992, El-Sharkawy 1993).

As breeders are usually handling large breeding populations under field conditions that require time saving and simple methodology, most useful physiological research findings have not been applied to crop improvement as much as scientists have hoped for (Evans 1977, Kramer 1980, Shorter et al. 1991, Jackson et al. 1996, El-Sharkawy 2006b). Moreover, physiological research conducted under controlled conditions, though has contributed significantly to expand scientific knowledge frontiers, has its shortcomings unless verified under field conditions to avoid acclimation problems and to be of use in crop improvement (Bunce 2005, El-Sharkawy 2005, 2006a, Long et al. 2006). Also, in many cases, appropriate methodologies to be effectively used in breeding and selection were not available. We were able, however, to screen relatively large cassava accessions from CIAT core germplasm grown in representative environments including sub-humid, seasonally dry and semiarid, using portable infrared gas exchange analysers to assess photosynthetic capacity in relation to productivity. Beside identifying several useful plant traits such as leaf area duration, leaf longevity and retention, proper sink-source relationships, deep fine rooting systems, and

enhanced activities of key photosynthetic enzymes, many accessions with high single leaf photosynthetic rates that were correlated with yield across various environments have been identified for use as parental materials in crosses (El-Sharkawy and Cock 1990, El-Sharkawy et al. 1990, 1992a, 1993, Pellet and El-Sharkawy 1993, 1994, Cayón et al. 1997, de Tafur et al. 1997, El-Sharkawy 1993, 2004, 2006a). Selection for high photosynthesis coupled with high yield, high water use efficiency (WUE), particularly in seasonally dry and semiarid environments, is a priority in a crop like cassava that has to endure from three to seven months without effective rain. Short-term leaf gas exchange measurements can yield interesting information about inherent plant capacity to assimilate carbon as affected by climatic and soil conditions. However, by itself, the technique is neither sufficient nor adequate to address the long-term responses to drought in the field as atmospheric conditions are always changing during the life span of the crop.

The potential utility of some stable isotopes has been theoretically worked out and its use in assessing the long-term plant-soil-water relations and productivity were experimentally tested with several crops grown in controlled conditions as well as in the field. Among these new techniques is the use of the analysis of ($^{13}C/^{12}C$) isotope compositions in plant tissues, particularly in identifying genotypes efficient in water use and tolerant to the various abiotic stresses, and in ecophysiological research in general (Osmond et al. 1982, Farquhar and Richards 1984, Farquhar et al. 1989, Ehleringer et al. 1985, 1986, 1990, Ehleringer 1993, Martin and Thorstenson 1988, Condon et al. 1990, White 1993, White et al. 1994, de Water et al. 1994, Gutiérrez and Meinzer 1994, Ismail et al. 1994, Knight et al. 1994, Rao et al. 1994, Fischer et al. 1998, Impa et al. 2005). The theoretical basis of stable carbon isotope composition in plants is related to the overall abundance of the naturally occurring and heavier ^{13}C relative to ^{12}C. During the multiple steps in the photosynthetic process, discrimination against the isotope ^{13}C occurs (Farquhar et al. 1982). This process is first controlled by fractionation via air-leaf boundary layer and stomatal conductance (g_s) for gas diffusion, which is small in wide open stomata and increases with closing stomata. Once CO_2 enters leaf mesophyll, fractionation occurs mainly via the carboxylation characteristics of the key enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) in C_3 plants. The RuBPCO discriminates more against ^{13}C than to ^{12}C (in vitro discrimination is about −27 to −29 ‰, Wong et al. 1979), whereas the key C_4 enzyme phosphoenolpyruvate carboxylase (PEPC) in C_4 species apparently does not discriminate as much against ^{13}C (in vitro discrimination amounts to only −2 ‰, Benedict 1978). This differential behavior of RuBPCO and PEPC formed the basis for differentiating species with different photosynthetic pathways, i.e. CAM, C_3, and C_4. C_3, C_4, and CAM species have values of relative ^{13}C abundance (as

designated by the dimensionless notation $\delta^{13}C$ expressed in ‰) in the ranges of: –20 to –35, –6 to –17, and –9 to –25 ‰, respectively (Osmond *et al.* 1982).

The several uncultivated C_3-C_4 intermediate species known so far, irrespective of differences in leaf anatomy and CO_2 compensation concentration or levels of C_4 enzyme activities, such as those within the genera *Flaveria, Panicum, Mollugo, Moricandia,* or *Neurachne* have $\delta^{13}C$ values similar to those observed in C_3 species (Bauwe 1984, Holaday and Chollet 1984, Monson *et al.* 1984, Hattersley *et al.* 1986, Monson 1989, Apel *et al.* 1997). The only exception in this regard is *Flaveria brownii,* whose classification status is controversial. Holaday *et al.* (1985, 1988) and Araus *et al.* (1990, 1991) considered it C_4, then later C_4-like type, while Monson (1989) treated it as a highly advanced C_3-C_4 species, based on several anatomical, physiological, and biochemical characteristics. This species exhibits $\delta^{13}C$ values around –16 to –17, which are within the range observed in C_4 plants. It is clear from these discrepancies that classification of C_3-C_4 intermediate species based on carbon isotope composition characteristics is difficult as long as the key photosynthetic enzymes of both C_4 and C_3 cycles are not tightly separated and compartmentalised within specific leaf tissues. In none of the above mentioned C_3-C_4 intermediate species there are complete separation and compartmentalisation of the key C_3 and C_4 enzymes. Only, the enzyme glycine decarboxylase was reported to be confined to bundle-sheath cells (Hylton *et al.* 1988).

The "CO_2 leakage" from bundle sheath cells that is often observed in some C_4 species (*e.g.* the grain amaranth *Amaranthus edulis*), due to either effects of leaf age and/or environmental conditions such as irradiance during leaf development, can further complicate results of carbon isotope discrimination. El-Sharkawy *et al.* (1968) found no CO_2 release in a rapid stream of CO_2-free air during irradiation from fully-expanded young leaves of *A. edulis* grown in well-lit cabinets. On the other hand, old leaves from well-lit plants and young leaves of plants exposed to low irradiance during development released measurable amounts of CO_2 in CO_2-free air during irradiation. Moreover, C_4 plants have the necessary organelles and enzymes associated with the photorespiratory cycle, but they are able to recycle/re-assimilate all respiratory CO_2 either *via* PEPC in mesophyll cells and/or *via* RuBPCO in bundle sheath cells, thus showing no apparent CO_2 release in CO_2-free air during irradiation (El-Sharkawy and Hesketh 1965, El-Sharkawy *et al.* 1967, 1968, Tolbert *et al.* 1969, Volk and Jackson 1972, Dai *et al.* 1993). Some C_3-C_4 intermediate species might be capable of recycling, partially, their photorespiratory CO_2 *via* PEPC in mesophyll cells and/or *via* RuBPCO in the bundle-sheath cells where glycine decarboxylas is confined (Holaday and Chollet 1984, Monson *et al.* 1984, Holbrook *et al.* 1985, Hylton *et al.* 1988, Monson 1989, Monson and Moore 1989). This phenomenon should add another dimension to carbon isotope discrimination process.

Cassava is considered as C_3-C_4 intermediate on the basis of: (*a*) atypical C_3 leaf anatomy, where a pronounced bundle-sheath, but with thin-walled cells and not well-developed as in typical C_4 Kranz anatomy, exists with numerous large granal chloroplasts; (*b*) close physical association of chloroplasts with numerous mitochondria and peroxisomes in both bundle-sheath and mesophyll cells; (*c*) high percentage (30–60 %) of ^{14}C incorporation into C_4 acids within 5–10 s of exposure to $^{14}CO_2$ under irradiation; (*d*) low photorespiration [CO_2 compensation concentration (Γ) = 20–30 cm^3 m^{-3} CO_2 under high photon flux density and leaf temperature of 30 °C, and small CO_2 release, <10 % of P_N from irradiated whole leaves in CO_2-free air and high air flow rate]; (*e*) ability to recycle all respiratory CO_2 within the palisade cells of amphistomatous leaves at varying photon flux densities and temperatures, when the abaxial stomata are closed; (*f*) elevated PEPC activities (from 10 to 30 % of those in C_4 species such as maize and sorghum); (*g*) C_i/C_a ratio ($\approx$ 0.4), which is similar to values commonly observed in C_4 species and much smaller than values in C_3 species; and (*h*) immunological analysis and DNA hybridization of PEPC from cassava and wild *Manihot* species against antibodies and *ppc* probe from maize (Cock *et al.* 1987, El-Sharkawy and Cock 1987a, 1990, Riaño *et al.* 1987a,b, Bernal 1991, El-Sharkawy *et al.* 1992a, López *et al.* 1993, Aguilar 1995, El-Sharkawy 2004, 2006a). These characteristics, collectively, underpinned the high photosynthetic capacity of cassava shown under both favorable and stressful environments (El-Sharkawy 1993, 2004, 2006a). Cassava is the only C_3-C_4 intermediate species discovered so far among important cultivated crops. When cassava has to endure prolonged drought, longer than three months, coupled with hot-dry air in the tropics, its stomatal closure and elevated PEPC activities would enhance recycling internal CO_2. Recycling respiratory CO_2 may help in dissipating excess photon energy, thus avoiding photoinhibition of photosynthetic machinery and damage of leaf (Stuhlfauth *et al.* 1990, Ögren and Rosenqvist 1992). Under these extreme conditions, P_N values are still positive with sustained rates in the remaining few leaves [P_N ranged among cultivars grown in semiarid environment from 7 to 20 μmol(CO_2) m^{-2} s^{-1}, de Tafur *et al.* 1997]. Cassava oven-dried root yields ranging from 2 to 4 t per ha were obtained in semiarid environments with <500 mm of rain and 5 to 7 months of drought (El-Sharkawy 1993, de Tafur *et al.* 1997). No staple food crop, even among the most drought tolerant C_4 cereals such as millets and sorghums, can produce as much under these harsh environments (Blum and Sullivan 1986).

The objectives of the present studies were : (*1*) to test stable carbon isotope method, for the first time, on a limited number of cassava genotypes grown under rainfed conditions in conjunction with short-term leaf gas exchange measurements, (*2*) to determine genotypic and

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within canopy variations in carbon isotope discrimination and leaf gas exchanges, and (*3*) to determine possible associations between leaf gas exchange characteristics

and carbon isotope discrimination measured on the same population of leaves.

Materials and methods

A field trial with 15 cultivars (as shown in Table 1) was conducted under rain-fed conditions at Santander de Quilichao Experiment Station, Cauca, Colombia (altitude 990 m; 3°30'N, 76°31'W; mean annual temperature 24 °C). Woody stem cuttings (0.2 m) were planted on May 7, 1990, in ridges at 1×1 m distance in 5×5 m plot with four replications in a randomised complete-block design. All plots received 5 : 7.5 : 10 g m^{-2} N-P-K 30 d after planting and were mechanically kept weed-free. Total rainfall (1 148 mm) was slightly less than pan evaporation (1 304 mm); however, there were two dry periods (June–September and January–February) when rainfall was much less than evaporation, and one period with excess of water (October–December).

Measurements of leaf gas exchange (CO_2 uptake and H_2O loss) were made with *LCA-2* portable infrared gas analyzer (*Analytical Development Co.,* Hoddesdon, England) on several occasions from July 23 to November 26, 1990. Across all replications, 36 leaves were measured for each cultivar consisting of 12 leaves at the top, 12 at the middle, and 12 at the lower canopy level from different plants. All measurements were made from 08:00 to 11:00 h local time with a solar irradiance of 1 200–2 000 μmol m^{-2} s^{-1}. Normal air [325±10 cm^3 m^{-3}(CO_2)] was drawn from above canopy using a vertically mounted 4-m glass-fiber probe connected to a pump. A small leaf chamber (*Parkinson* Broad Leaf Model, *Analytical Development Co.,* Hoddesdon, England), connected to the

portable infrared gas analyzer, was clamped over the middle portion (6.25 cm^2 surface area) of the central lobe of the measured leaves, and was held toward the sun for 30–60 s to obtain steady-state gas exchange. Air temperature in the cuvette varied between 27 and 32 °C, depending on the time and date of measurement. Measurements were made during both low-and high-rainfall periods with the majority (80 %) made in the former.

Carbon isotope ratios ($\delta^{13}C$) were determined on the same individual leaves used for gas exchange measurements after it had been oven-dried at 70 °C for 3 d and ground to fine powder. The isotope composition was carried out using mass spectrometry techniques at the isotope analysis laboratory, Research School of Biological Sciences, Australian National University, Canberra. The carbon isotope ratio was calculated using the following equation as proposed by Farquhar *et al.* (1989):

$$\delta^{13}C \ [\permil] = [(^{13}C/^{12}C(\text{plant sample}) \div {}^{13}C/^{12}C(\text{PDB standard}) - 1] \ 1\ 000 \ [\permil]$$

The results were then expressed in the form of isotope discrimination (Δ) using the equation:

$$\Delta \ [\permil] = [\delta^{13}C(\text{air}) - \delta^{13}C(\text{plant sample})] \div [1 + \delta^{13}C(\text{plant sample})]$$

The $\delta^{13}C$ air was assumed to be –8 ‰. Data were analyzed using the Statistical Analysis Software (SAS).

Results and discussion

Genotypic and within canopy variations in leaf gas exchange characteristics and carbon isotope discrimination: Table 1 presents data on P_N, C_i, and Δ as determined in the three canopy levels. These parameters significantly varied among cultivars and within all canopy levels with the smallest P_N and Δ and the largest C_i values occurring in cv. CG 927-12. The cvs. M Bra 191 and CG 996-6 had the largest P_N and smallest C_i. Across cultivars, mean P_N values were significantly higher in upper and middle canopy leaves as compared to the lower canopy ones. On the other hand, C_i values were significantly smaller in upper and middle than in lower canopy. The three canopy layers also differed significantly in mean Δ with the smallest value in upper (18.5) and the largest in lower (19.9), while a medium value occurred in middle canopy (19.3). Peak cassava canopy depths (age 4–7 months) ranged from 1.5 to 2.0 m among this group of cultivars that might imply negligible variation in air carbon isotope compositions in this short distance between near soil surface and the top of the

canopy. Therefore the observed higher discrimination in the lower canopy leaves was mainly due probably to leaf anatomical, physiological, and biochemical features rather than to recycling lighter air (*i.e.* less enriched with the heavier ^{13}C) from soil respiration and decayed fallen leaves. Farquhar *et al.* (1989) in their review discussed several studies on variations in plant carbon isotope compositions within forests varying in type of vegetation and canopy depths. All studies showed increasing carbon isotope discrimination with moving from top to bottom of canopies. But the interpretation, and hence the underlying causes, were controversial. Earlier reports emphasized the role of recycling lighter air that results from decayed plant matter, and perhaps soil respiration at forest floors, but lacked measurement of isotope composition of air within canopy profile. Later studies, however, did not find measurable corresponding decreases in ^{13}C level in air with canopy depth. In coffee (*Coffea arabica*), using a short and compact variety in dense hedge rows, Gutiérrez and Meinzer (1994) found Δ values increasing from

Table 1. Leaf photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], intercellular CO_2 concentration, C_i [μmol(CO_2) mol^{-1}], and carbon isotopic discrimination, Δ [^{13}C ‰] determined in upper (U), middle (M), and lower (L) canopy leaves of field-grown cassava cultivars, at Santander de Quilichao, Cauca Dept., Colombia, 1990–1991. Twelve leaves for each canopy level were measured for gas exchange and carbon isotopic discrimination 3–7 months after planting. Values with the same letter are not significantly different at $p<0.05$. Means ($n = 12$).

Cultivar	P_N			C_i			Δ		
	U	M	L	U	M	L	U	M	L
CG 927-12	29.8	29.0	21.4	172	170	206	17.7	18.8	19.3
CG 996-6	39.1	39.6	31.4	153	144	180	19.1	19.3	20.1
CM 3456-3	35.8	35.4	28.2	157	156	181	18.1	19.3	20.0
CM 4145-4	35.7	36.1	27.1	158	156	192	18.3	19.3	19.9
CM 4575-1	35.4	37.0	30.1	155	145	170	18.5	19.1	19.6
CM 4617-1	35.5	33.8	26.4	156	160	192	18.9	19.7	20.3
CM 4701-1	34.9	35.9	27.3	159	152	183	18.6	19.3	20.0
CM 4711-2	34.0	33.9	28.7	156	155	179	18.6	18.8	19.7
CM 4716-1	36.9	35.5	25.1	147	152	192	18.6	19.4	20.0
CM 4864-1	38.3	39.4	30.2	145	137	179	18.5	19.6	19.8
CM 507-37	35.8	31.1	23.9	155	169	211	19.2	19.9	20.9
CM 523-7	35.9	33.2	24.8	156	163	195	18.4	19.8	19.8
M Bra 191	38.8	40.1	30.7	149	141	175	18.4	19.7	19.9
M Col 1468	34.0	34.5	21.9	161	155	199	18.1	18.9	18.9
M Col 1684	35.8	33.9	27.3	160	165	185	18.6	19.4	20.2
LSD at $p<0.05$	1.8	1.9	1.7	31	28	30	0.19	0.19	0.20
Mean	35.7a	35.2a	27.0b	156a	155a	188b	18.5a	19.3b	19.9c

Table 2*A*. Overall means of stomatal conductance, g_s [mmol m^{-2} s^{-1}], intercellular CO_2 concentration, C_i [μmol(CO_2) mol^{-1}], ratio of C_i and ambient CO_2 concentration, C_i/C_a, water use eficiency, WUE [μmol mol^{-1}], net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], and carbon isotope discrimination, Δ [‰]. Leaf gas exchange rates and carbon isotope discrimination were determined on the same leaves. Twelve attached leaves for each upper, middle, and lower canopy of field-grown cassava were measured. Measurements were made 3–7 months after planting at Santander de Quilichao, Cauca Dept., Colombia, 1990–1991. $n = 6$ (columns 2 and 3), $n = 30$ (columns 4 to 9). Maximum P_N and corresponding C_i/C_a values were determined during rainy period.

Cultivar	Max. P_N	C_i/C_a	g_s	C_i	C_i/C_a	WUE	P_N	Δ
CG 927-12	39.3	0.43	686	176	0.53	38.9	26.7	18.6
CG 996-6	49.7	0.37	895	160	0.48	41.0	36.7	19.5
CM 3456-3	43.7	0.43	791	164	0.50	41.8	33.1	19.1
CM 4145-4	43.9	0.40	778	178	0.54	42.4	33.0	19.2
CM 4575-1	42.8	0.39	775	159	0.48	44.1	34.2	19.1
CM 4617-1	42.8	0.46	805	169	0.51	39.6	31.9	19.6
CM 4701-1	42.2	0.45	768	159	0.48	42.6	32.7	19.3
CM 4711-2	41.3	0.45	725	158	0.48	44.4	32.2	19.0
CM 4716-1	43.6	0.42	716	158	0.48	45.4	32.5	19.3
CM 4864-1	45.1	0.39	760	158	0.48	47.4	36.0	19.3
CM 507-37	43.7	0.38	731	174	0.53	41.2	30.3	20.0
CM 523-7	42.3	0.45	771	174	0.53	40.6	31.3	19.3
M Bra 191	47.4	0.37	807	149	0.45	45.4	36.6	19.3
M Col 1468	42.3	0.44	695	160	0.48	43.3	30.1	18.6
M Col 1684	43.0	0.42	775	164	0.50	41.7	32.3	19.4
Mean	43.5	0.42	765	164	0.50	42.7	32.6	19.2
LSD 5 % for cultivars	1.7	0.08	91	23	0.06	5.8	1.6	0.17

upper and sun exposed leaves down to sun exposed middle canopy leaves. Also shaded upper and middle canopy leaves exhibited higher discrimination. Using a mass balance model based on leaf gas exchange characteristics and measured leaf Δ values, they found about 50 % of the carbon originally fixed during leaf development in the sun probably have been turned over in the shade.

Effects of variation in incident irradiance within cassava canopy and leaf acclimation/adaptation to shade and age are probably the factors underlying the higher carbon isotope discrimination at the bottom layer. In lower canopy leaves, mean P_N was smaller, C_i and C_i/C_a

were higher, and leaf WUE (= P_N/g_s) was smaller, irrespective of lower g_s, as compared to middle and upper canopy ones (Table 2*B*). In this case, other factors related to mesophyll characteristics such as changes in conductance to gas diffusion and in RuBPCO kinetics might be implicated. Probable decreases in RuBPCO amounts and activities might have occurred in cassava lower canopy leaves due to age and shade effects, as shown in other species such as wheat and rice (Makino *et al.* 1983, Fleck *et al.* 1986). Less quantity and activity of RuBPCO should have resulted in lesser discrimination as compared to younger upper canopy leaves, assumingly richer in soluble protein and exposed to higher irradiances. An indirect evidence for reduction in RuBPCO activity (*i.e.* the demand side for CO_2), can be derived from the higher C_i and C_i/C_a in lower canopy leaves despite their lower conductance to gas diffusion (the supply side for CO_2). The C_i/C_a ratio represents the balance between the demand for and supply of CO_2 in the photosynthetic process. Thus, in view of these trends in C_i, as measured with short-term leaf gas exchanges, it may be concluded that possible increases in carbon isotope fractionation caused by decreased physical conductances to CO_2 diffusion within the mesophyll (*via* the intercellular air spaces beyond stomatal cavities and the liquid path from outer mesophyll cell wall into the stroma of chloroplasts where RuBPCO is located) had occurred over the long life span (~6–9 weeks) of cassava leaves at the lower canopy. In this case, neither stomatal nor RuBPCO effects were the likely major causes underlying the higher discrimination observed in this trial. Irradiances to which leaves were exposed and acclimated during their development and leaf age affect leaf conductance to gas diffusion (Körner *et al.* 1979, Solárová and Pospíšilová 1983), as much as leaf anatomy, biochemistry, and sub-cellular finer structures such as membrane biophysical properties (Nobel 1980, Nobel and Hartsock 1981, Šesták 1985, Bunce 1986).

Table 2*B*. Overall means of cultivar net photosynthetic rate, P_N; carbon isotopic discrimination, Δ; leaf water use efficiency, WUE (P_N/g_s); intercellular CO_2 concentration, C_i, atmospheric CO_2 concentration, C_a; and stomatal conductance for water vapor, g_s.

| Parameter | Mean ($n = 180$) for each canopy level | | |
	upper	middle	lower
P_N	35.7a	35.2a	27.0b
Δ	18.5a	19.3b	19.9c
WUE	45a	46a	38b
C_i/C_a	0.47a	0.47a	0.56b
C_i	156a	155a	188b
g_s	808a	773a	714b

The present studies with cassava may point to the need for reconsidering and emphasizing the effects of changes in mesophyll physical conductance to CO_2

diffusion, due to both climatic changes and leaf developmental stages and age, in relation to carbon isotope fractionation steps. They have further implication for the utility of the isotope method when used as screening tools in crops with long growth season subjected to changing environment, such as prolonged water, temperature, and nutrient stresses that may alter leaf function and structure. Gutiérrez and Meinzer (1994), in their studies on coffee, cautioned against restricting leaf sampling to a certain type of leaves for carbon isotope analysis as well as using limited amount of current gas exchange data for assessing mechanisms of adjustment in Δ, and suggested that long-term measurements spanning through complete phenological cycles are needed.

Table 2*A* presents data of overall cultivar means, as averaged across canopy levels, for leaf gas exchange parameters and Δ. The maximum P_N values were recorded in upper canopy leaves when measured during rainy period with wide open stomata due to low leaf-to-air water vapor deficits. Cassava stomata close rapidly upon exposure to dry air both under controlled conditions and in the field, regardless of soil and leaf water status (Connor and Palta 1981, El-Sharkawy and Cock 1984, El-Sharkawy *et al.* 1984, Cock *et al.* 1985, El-Sharkawy 1990, de Tafur *et al.* 1997). There were significant cultivar differences with rates varying from 39 to 50 μmol(CO_2) m^{-2} s^{-1} for cvs. CG 927-12 and CG 996-6, respectively. These differences in maximum P_N coincided with differences in C_i and consequently with varying C_i/C_a ratio that ranged from 0.37 to 0.46 with overall mean of 0.42. These values are similar or approaching those in C_4 species and are much lower than those in typical C_3 plants, thus indicating the high photosynthetic capacity of cassava when grown in favorable environments. Recently, Bunce (2005) measured P_N at midday in the field for three weedy C_4 species and grain sorghum (C_4) at different nitrogen levels and found values of C_i ranging from 183 to 212 μmol(CO_2) mol^{-1} and C_i/C_a ratio of ~0.55, which were closer to those in C_3 species and much higher than values previously estimated for indoor-grown C_4 plants. These findings support the importance of using appropriately grown plants in natural conditions (El-Sharkawy *et al.* 2005, 2006a,b, Long *et al.* 2006). The mean P_N values of cassava, when most measurements were made in dry period, were significantly smaller in all cultivars [overall cultivar means $n = 540$: P_N 32.6 μmol(CO_2) m^{-2} s^{-1}, C_i 164 μmol(CO_2) mol^{-1}, C_i/C_a 0.5], but still comparable favorably with rates of C_4 plants, taking into account that cassava was rain-fed and experienced shortages in water for months. Other mean values of gas exchange parameters significantly varied among cultivars with cv. CG 927-12 again having lower P_N and higher C_i and C_i/C_a ratio than the overall means of the trial. This cultivar also had the lowest mean g_s to water vapor, WUE, and Δ compared to the overall means of the trial.

Assuming that short-term leaf gas exchange

measurements may relate to and could be used as indicators for changes in integrated long-term plant WUE and Δ, the trends observed in CG 927-12 seemed contrary to those predicted by the theoretical model for isotope fractionation (Farquhar *et al.* 1989). The model predicts lesser discrimination associated with lower values in g_s, C_i, and C_i/C_a. These apparent discrepancies are difficult to explain and may indicate that relating short-term gas exchanges to long-term carbon assimilation characteristics are inappropriate approach. On the other hand, assessing the overall performance at the whole crop level might shed some light on these discrepancies. This genotype had the smallest total dry biomass at final harvest (1.6 kg m^{-2}) and the smallest dry storage root yield (1.2 kg m^{-2}) compared to the overall trial means of 2.3 and 1.7 kg m^{-2} for total biomass and root yield, respectively (El-Sharkawy *et al.* 1993). Furthermore, in this trial seasonal average P_N was positively and significantly correlated with both total biomass ($r = 0.64$, $p<0.01$) and root yield ($r = 0.56$, $p<0.05$). Root yield was also significantly negatively correlated with C_i (El-Sharkawy 2006a). These findings might indicate that in cv. CG 927-12 there was, probably, a sink limitation for receiving assimilates that feed-backed on leaf photosynthesis. As a result, C_i increased. In this case, one might speculate on whether the lower g_s was also a consequence of a photosynthetic feedback inhibition. This might have been the case since C_i affects stomatal movements (Meidner 1962, Meidner and Mansfield 1968). If this line of analysis turns out to be correct, then the smaller values of carbon isotope discrimination, that were consistently observed at all canopy levels in this genotype (cv. CG 927-12), might had been the result of non-stomatal effects. This possibility deserves testing gas exchanges concurrently with carbon isotope determination in plants where sink-source relation is manipulated.

Martin and Thorstenson (1988) studied the relationship of plant WUE and carbon isotope compositions in a drought-tolerant wild tomato (*Lycopersicon pennellii*), a common domestic tomato (*Lycopersicon esculentum,* cv. UC82B), and their F_1 hybrid grown in 7 500 cm^3 containers in a greenhouse with varying watering regimes. They found strong negative correlations in all sets of plants between total plant dry mass and $\delta^{13}C$, indicating that Δ decreased with smaller plant mass. While there were significant positive correlations between season-long WUE (dry matter/water used) and leaf carbon isotope composition (*i.e.* less negative $\delta^{13}C$), instantaneous WUE based on leaf gas exchanges (*i.e.* CO_2 uptake/H_2O loss) had no relation to season-long WUE. Gutiérrez and Meinzer (1994) reported negative associations between coffee leaf area index and both Δ, as determined on sun leaves from upper canopy layer, and instantaneous crop WUE (photosynthesis/transpiration), which conflicted with prediction of the carbon isotope model. These authors concluded that scaling from instantaneous short-term measurements of WUE into long-term plant behavior may not be a realistic approach.

Correlations among leaf gas exchange parameters and carbon isotope discrimination (Table 3) were very significant, although with moderate and low values, among all parameters measured. The highest correlations between Δ and leaf gas exchange parameters were -0.36 ($p<0.0001$) with P_N and 0.34 ($p<0.0001$) with C_i. The positive correlations of Δ with C_i and C_i/C_a and the negative association with WUE are in agreement with the carbon isotope model and confirm its theoretical predictions in this case (Farquhar *et al.* 1982, 1989). Normally, the relation between Δ and the long-term estimates of integrated crop WUE (based on estimates of water use per dry matter production) is a better index in evaluating crop responses to environments as well as in identifying more water use efficient genotypes, particularly targeted to drier environments (Farquhar and Richards 1984, Condon *et al.* 1990, Ismail *et al.* 1994).

Table 3. Correlation coefficients for leaf gas exchange parameters and ^{13}C isotopic discrimination (Δ) in cassava leaves measured at three canopy levels of 15 cultivars at 3–7 months after planting. Santander de Quilichao, Cauca Dep., Colombia 1990–1991. Means ($n = 540$) of net photosynthetic rate, P_N 32.6 µmol(CO_2) m^{-2} s^{-1}; stomatal conductance, g_s 765 mmol m^{-2} s^{-1}; intercellular CO_2 concentration, C_i 164 µmol mol^{-1}; water use efficiency, WUE 42.7 µmol mol^{-1}; C_i/C_a 0.50; Δ 19.2. [**,****]correlation coefficients significant at $p<0.01$ or 0.0001, respectively; + autocorrelation.

	Δ	C_i/C_a	WUE	C_i	g_s
P_N	-0.36[****]	-0.72[****]	0.44[****]	-0.84[****]	0.40[****]
g_s	-0.11[**]	0.36[****]	-0.72[****]	0.41[****]	–
C_i	0.34[****]	$+0.99$[****]	-0.84[****]	–	
WUE	-0.24[****]	-0.84[****]	–		
C_i/C_a	0.16[****]	–			

The negative trend in Δ association with g_s observed here is, however, contrary to the model predictions too. This might be attributed partly to the consistently lower g_s in bottom canopy leaves across all cultivars. In addition, the negative association of Δ with P_N and the higher C_i, C_i/C_a as well as the lower g_s and P_N in lower canopy leaves are perhaps indicative of possible feedback inhibition in photosynthesis. This trend was further detected within leaf populations of the same cultivar (Table 4, for cv. CM 507-37). In this cv., for example,

there were trends of greater Δ, higher C_i and C_i/C_a, and lower g_s and P_N than the means of the trial (Table 2A). It is also a leafy type that retains higher seasonal average leaf area indices in both well-watered and prolonged midseason water stress that underlies its high yields (El-Sharkawy and Cock 1987b, El-Sharkawy et al. 1992b). It seems, therefore, that there is a compensatory effect between P_N and total plant leaf area (i.e. the greater leaf area per plant the smaller would be P_N as a result of feedback effects on leaf photosynthesis). This conclusion is supported by the effects of soil phosphorus (P) levels on growth, leaf area index (LAI), and photosynthesis across 33 field-grown cassava cultivars. Compared to zero P fertiliser application, plants that received adequate P (75 kg ha^{-1}) had significantly greater seasonal LAI but significantly lower upper canopy leaf P_N [in zero P plants mean LAI was 2.0 and P_N was 31 μmol(CO_2) m^{-2} s^{-1}, as compared to values in P-fertilised plants of 3.1 for LAI and 27 μmol(CO_2) m^{-2} s^{-1} for P_N] (CIAT, 1992). It is noteworthy that in these P fertiliser trials the ratio of mean harvestable total bimass production [(i.e. 18.2 t ha^{-1} with P application/12.6 t ha^{-1} without P application =1.35) was close to the ratio of (LAI × P_N) between the two P treatments (i.e. 83.7 with P application/62 witout P application = 1.44)]. With storage root yield, the corresponding ratio (root yield with P/yield without P) was

1.25. The lesser ratio in root yield (1.25) than that in total biomass (1.35) was attributed mainly to the higher harvest index (HI = root yield/total biomass) in zero P plants (i.e. HI = 0.81) than in P fertilised plants (i.e. HI = 0.70). These findings indicate the close relationship between canopy photosynthesis and biological productivity in cassava. Thus, the product of single leaf P_N and LAI could be used as an approximation of canopy photosynthesis, which is more difficult to determine in field-grown large population of breeding materials. In this case, both P_N and LAI have to be measured and averaged over the growth cycle in both wet and dry periods. There are available modern portable infrared gas exchange analysers as well as leaf canopy area analysers for measuring P_N and LAI, respectively, in large accessions grown under field conditions.

Among leaf gas exchange parameters, the highest correlations were between P_N and C_i and C_i/C_a with negative signs, indicating a stronger role of photosynthetic capacity (the demand side for CO_2) as compared to stomatal control (the supply side for CO_2) (Tables 3 and 4). This is further substantiated by the greater and negative values of correlations between C_i, C_i/C_a, and WUE. Yet, the later parameter was also significantly and negatively correlated with g_s implying a strong stomatal control on water loss.

Table 4. Correlation coefficients for leaf gas exchange parameters and ^{13}C isotopic discrimination (Δ) in cassava leaves measured at three canopy levels of cultivar CM 507-37 at 3–7 months after planting. Santander de Quilichao, Cauca Dep., Colombia 1990–1991. Means (n = 36) of net photosynthetic rate, P_N 30.3 μmol(CO_2) m^{-2} s^{-1}; stomatal conductance, g_s 731 mmol m^{-2} s^{-1}; intercellular CO_2 concentration, C_i 174 μmol mol^{-1}; water use efficiency, WUE 41.2 μmol mol^{-1}; C_i/C_a 0.53; Δ 20.0. *, **, ***, **** correlation coefficients significant at $p<0.05$, 0.01, 0.001, or 0.0001, respectively; + autocorrelation; NS not significant at $p<0.05$.

	Δ	C_i/C_a	WUE	C_i	g_s
P_N	−0.61****	−0.87****	0.66****	−0.85****	NS
g_s	NS	0.36*	−0.64****	0.38*	−
C_i	0.50**	+0.99****	−0.93****	−	
WUE	−0.54***	−0.94****	−		
C_i/C_a	0.48**	−			

Among cultivars, overall mean values of P_N and g_s were significantly positively correlated (r = 0.746, $p<0.01$). On the other hand, C_i and WUE were significantly negatively correlated (r = −0.721, $p<0.01$). Final dry root yield was significantly positively correlated with mean values of Δ (r = 0.703, $p<0.01$) and with mean g_s (r = 0.535, $p<0.05$), while total biomass was not. Mean P_N was significantly positively correlated with both root yield (r = 0.641, $p<0.01$) and total biomass (r = 0.653, $p<0.01$). WUE was also significantly positively correlated with total biomass (r = 0.606, $p<0.05$). Since root yield was also negatively correlated with C_i in large accessions grown in different environments and years (El-Sharkawy et al. 1990, de Tafur et al. 1997, El-Sharkawy 2006a), searching for genotypic variation in photosynthetic enzymes activity is warranted. Thus, both P_N and Δ might be used in combination as criteria, along

with other yield determinants, for selection for high yield. In this case, leaf gas exchange must be measured and averaged over longer time-span during the crop cycle including both wet and dry periods. When crops experience prolonged drought, as in seasonally dry and semiarid ecozones, new leaves formed after recovery from stress should be measured as they usually have higher photosynthetic rates compared to those in unstressed crops (El-Sharkawy 1993, 2006a, Cayón et al. 1997).

The role of PEPC in cassava photosynthesis and its implication for carbon isotope studies: The role of C$_4$ photosynthetic pathway in the evolution of the C$_3$-C$_4$ intermediate plants and its function is controversial, mostly because of the lack of necessary enzyme compartmentations that exists in typical C$_4$ Kranz anatomy leaves and the uncertainty about the coordination with C$_3$ cycle

(Monson 1989). However, the elevated activities of the key C_4 PEPC in cassava, relative to C_3 species (Table 5A,B, El-Sharkawy and Cock 1990, Bernal 1991, Lopéz *et al.* 1993, El-Sharkawy 2004, 2006a) cannot be overlooked. Moreover, PEPC activity correlated with P_N values measured on the same leaves of several cultivars grown under prolonged water shortages in the field
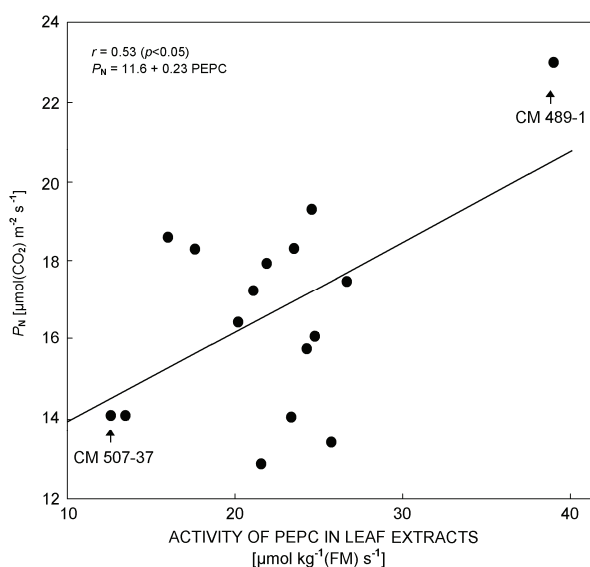

Fig. 1. Relationship between leaf net photosynthetic rate (P_N) and PEP carboxylase (PEPC) activity of field-grown cassava cultivars under prolonged water stress at CIAT-Quilichao, 1988. Both P_N and PEPC activity were determined on the same leaves of 6-month old plants subjected to water stress at 3 months after planting. Values are means of 3 fully expanded upper canopy leaves per cultivar in two replications. Source: M.A. El-Sharkawy, L. Bernal and Y. Lopez (unpublished).

(Fig. 1). Root yield of large group of genotypes that were grown across diverse environments and years significantly and positively correlated with upper canopy P_N, photosynthetic nitrogen use efficiency, (PNUE = CO_2 uptake rate per unit total leaf nitrogen, El-Sharkawy, 2004), and negatively with C_i (El-Sharkawy and Cock 1990, El-Sharkawy *et al.* 1990, 1993, Pellet and El-Sharkawy 1993, de Tafur *et al.* 1997, El-Sharkawy 2006a) indicating the predominance of non-stomatal factors (*i.e.* anatomical and biochemical factors) in controlling P_N and productivity. Farquhar and Richards (1984) suggested making allowances in the carbon isotope discrimination model for fractionation of CO_2 fixed by PEPC which occurs in C_3 species. Moreover, PEPC is involved in formation of carbon skeletons for some amino acids in plant metabolism. Although the values of Δ observed in the small number of cultivars tested were in the range normally encountered in C_3 and C_3-C_4 intermediate species and much higher than those in C_4 plant, the possibility of partial discrimination in favor of ^{13}C by the elevated activity of PEPC should not be discarded. In this case, searching for wider genetic variation in Δ in both cultivated cassava and in wild *Manihot* is warranted. Cassava cultivation is currently expanding towards drier areas in many developing countries in Asia, Africa, and Latin America, and the demands for drought tolerant cultivars are increasing (El-Sharkawy 1993). With the advent of the observed/documented 'global climate changes' and the consequent occurrence of severe water shortages in most of the tropical regions, cassava cultivars with more tolerance to drought will have greater comparative advantage compared to many warm-climate grain crops such as maize, sorghum, and millet. In Sub-Saharan Africa, food shortages are common in many

Table 5A. Activities of the photosynthetic enzymes PEPC and RuBPCO per various units in field-grown cassava cultivars. Means ± SD.

Cultivar	PEPC [μmol s⁻¹] kg⁻¹(FM)	[mmol s⁻¹] kg⁻¹(Chl)	kg⁻¹(protein)	RuBPCO [μmol s⁻¹] kg⁻¹(FM)	[mmol s⁻¹] kg⁻¹(Chl)	kg⁻¹(protein)	PEPC/RuBPCO per Chl
CM 523-7	93.0±8.8	26.2±1.7	1.2±0.1	214±37	60.3±10.3	2.7±0.4	0.43
CM 507-37	49.3±2.7	31.8±1.7	0.8±0.1	177±17	114.0±11.0	2.8±0.3	0.28
M Col 1684	71.3±4.5	48.5±3.2	1.0±0.1	170±29	116.0±19.7	2.3±0.4	0.42
M Col 1468	72.2±6.5	51.2±4.5	0.8±0.1	203±31	136.0±11.8	2.5±3.7	0.38

Table 5B. Representative ranges of chlorophyll-based values [mmol kg⁻¹(Chl) s⁻¹] for PEPC and RuBPCO activities for species with C_4, C_3, and C_3-C_4 intermediate characteristics (from Ku *et al.* 1983, 1991, Bauwe 1984, Holaday and Chollet 1984, Holaday *et al.* 1985, Adams *et al.* 1986, Monson and Moore 1989, and Moore *et al.* 1989).

	PEPC	RuBPCO	PEPC/RuBPCO
C_4	1.33–583.00	33.3–133.3	1.70–5.00
C_3	0.08–25.00	250.0–500.0	0.05–0.10
C_3-C_4	0.50–83.30	66.6–166.6	0.25–0.50

countries mainly because of frequent severe drought and the failure of grain crops, conditions that make cassava an attractive alternative source for food and animal feed.

Conclusions: The high leaf P_N and the significant negative correlation between P_N and C_i indicates the importance of non-stomatal factors (*i.e.* anatomical and biochemical factors) in controlling carbon assimilation in cassava. Previous studies showed strong positive correlation between storage root yield and upper canopy P_N, and negative association with C_i, across wide range of geno-

types, years, and environments (El-Sharkawy *et al.* 1990, de Tafur *et al.* 1997, El-Sharkawy 2006a). The C_i, C_i/C_a, and Δ might be used in combination for evaluating breeding materials in the field. Leaf gas exchange measurements should be conducted repeatedly during most of the root-bulking period (*e.g.* in the low-land tropics from 3–7 months after planting) and when leaf canopy is nearly closed, so as both carbon sources and sinks are in their most active stages. The genotypic and within canopy variations observed in this study in both leaf gas exchange characteristics and carbon isotope discrimination point to the need for more research using larger germplasm accessions grown in diverse environments, including wild *Manihot*. Growing cassava in pots and/or in greenhouse should not be used in order to avoid acclimation problems and sink-source feedback effects. This endeavor requires interdisciplinary/inter-institution approach to ensure efficiency and to reduce costs of research.

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