

# 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 ( $\Delta$ ) 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  $\Delta$ , 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  $\text{CO}_2$  concentration ( $C_i$ ), ratio of  $C_i$  to ambient  $\text{CO}_2$  concentration  $C_i/C_a$ , and  $\Delta$ . Across all measured leaves,  $\Delta$  correlated negatively with leaf water use efficiency ( $\text{WUE} = \text{photosynthesis/stomatal conductance}, g_s$ ) 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  $\Delta$  correlated positively with storage root yield. These results are in agreement with trends predicted by the carbon isotope discrimination model.

*Additional key words:* breeding;  $\text{CO}_2$ ; 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 ( $^{13}\text{C}/^{12}\text{C}$ ) 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  $^{13}\text{C}$  relative to  $^{12}\text{C}$ . During the multiple steps in the photosynthetic process, discrimination against the isotope  $^{13}\text{C}$  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  $\text{CO}_2$  enters leaf mesophyll, fractionation occurs mainly *via* the carboxylation characteristics of the key enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) in  $\text{C}_3$  plants. The RuBPCO discriminates more against  $^{13}\text{C}$  than to  $^{12}\text{C}$  (*in vitro* discrimination is about  $-27$  to  $-29\%$ , Wong *et al.* 1979), whereas the key  $\text{C}_4$  enzyme phosphoenolpyruvate carboxylase (PEPC) in  $\text{C}_4$  species apparently does not discriminate as much against  $^{13}\text{C}$  (*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,  $\text{C}_3$ , and  $\text{C}_4$ .  $\text{C}_3$ ,  $\text{C}_4$ , and CAM species have values of relative  $^{13}\text{C}$  abundance (as

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

The several uncultivated  $\text{C}_3\text{-C}_4$  intermediate species known so far, irrespective of differences in leaf anatomy and  $\text{CO}_2$  compensation concentration or levels of  $\text{C}_4$  enzyme activities, such as those within the genera *Flaveria*, *Panicum*, *Mollugo*, *Moricandia*, or *Neurachne* have  $\delta^{13}\text{C}$  values similar to those observed in  $\text{C}_3$  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  $\text{C}_4$ , then later  $\text{C}_4$ -like type, while Monson (1989) treated it as a highly advanced  $\text{C}_3\text{-C}_4$  species, based on several anatomical, physiological, and biochemical characteristics. This species exhibits  $\delta^{13}\text{C}$  values around -16 to -17, which are within the range observed in  $\text{C}_4$  plants. It is clear from these discrepancies that classification of  $\text{C}_3\text{-C}_4$  intermediate species based on carbon isotope composition characteristics is difficult as long as the key photosynthetic enzymes of both  $\text{C}_4$  and  $\text{C}_3$  cycles are not tightly separated and compartmentalised within specific leaf tissues. In none of the above mentioned  $\text{C}_3\text{-C}_4$  intermediate species there are complete separation and compartmentalisation of the key  $\text{C}_3$  and  $\text{C}_4$  enzymes. Only, the enzyme glycine decarboxylase was reported to be confined to bundle-sheath cells (Hylton *et al.* 1988).

The “ $\text{CO}_2$  leakage” from bundle sheath cells that is often observed in some  $\text{C}_4$  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  $\text{CO}_2$  release in a rapid stream of  $\text{CO}_2$ -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  $\text{CO}_2$  in  $\text{CO}_2$ -free air during irradiation. Moreover,  $\text{C}_4$  plants have the necessary organelles and enzymes associated with the photorespiratory cycle, but they are able to recycle/re-assimilate all respiratory  $\text{CO}_2$  either *via* PEPC in mesophyll cells and/or *via* RuBPCO in bundle sheath cells, thus showing no apparent  $\text{CO}_2$  release in  $\text{CO}_2$ -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  $\text{C}_3\text{-C}_4$  intermediate species might be capable of recycling, partially, their photorespiratory  $\text{CO}_2$  *via* PEPC in mesophyll cells and/or *via* RuBPCO in the bundle-sheath cells where glycine decarboxylase 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  $\text{C}_3\text{-C}_4$  intermediate on the basis of: (a) atypical  $\text{C}_3$  leaf anatomy, where a pronounced bundle-sheath, but with thin-walled cells and not well-developed as in typical  $\text{C}_4$  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  $^{14}\text{C}$  incorporation into  $\text{C}_4$  acids within 5–10 s of exposure to  $^{14}\text{CO}_2$  under irradiation; (d) low photorespiration [ $\text{CO}_2$  compensation concentration ( $\Gamma$ ) = 20–30  $\text{cm}^3 \text{m}^{-3} \text{CO}_2$  under high photon flux density and leaf temperature of 30 °C, and small  $\text{CO}_2$  release, <10 % of  $P_N$  from irradiated whole leaves in  $\text{CO}_2$ -free air and high air flow rate]; (e) ability to recycle all respiratory  $\text{CO}_2$  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  $\text{C}_4$  species such as maize and sorghum); (g)  $\text{C}_i/\text{C}_a$  ratio ( $\approx 0.4$ ), which is similar to values commonly observed in  $\text{C}_4$  species and much smaller than values in  $\text{C}_3$  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  $\text{C}_3\text{-C}_4$  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  $\text{CO}_2$ . Recycling respiratory  $\text{CO}_2$  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  $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ , 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  $\text{C}_4$  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 rain-fed conditions in conjunction with short-term leaf gas exchange measurements, (2) to determine genotypic and

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

## 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<sup>-2</sup> 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<sub>2</sub> uptake and H<sub>2</sub>O 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<sup>-2</sup> s<sup>-1</sup>. Normal air [325±10 cm<sup>3</sup> m<sup>-3</sup>(CO<sub>2</sub>)] 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

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

portable infrared gas analyzer, was clamped over the middle portion (6.25 cm<sup>2</sup> 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 (δ<sup>13</sup>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):

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

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

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

The δ<sup>13</sup>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 <sup>13</sup>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 <sup>13</sup>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,  $P_N$  [ $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ ], intercellular  $\text{CO}_2$  concentration,  $C_i$  [ $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ ], and carbon isotopic discrimination,  $\Delta$  [ $^{13}\text{C} \text{ ‰}$ ] 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	$P_N$ U	M	L	$C_i$ U	M	L	$\Delta$ 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 2A. Overall means of stomatal conductance,  $g_s$  [ $\text{mmol m}^{-2} \text{ s}^{-1}$ ], intercellular  $\text{CO}_2$  concentration,  $C_i$  [ $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ ], ratio of  $C_i$  and ambient  $\text{CO}_2$  concentration,  $C_i/C_a$ , water use efficiency, WUE [ $\mu\text{mol mol}^{-1}$ ], net photosynthetic rate,  $P_N$  [ $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ ], and carbon isotope discrimination,  $\Delta$  [ $‰$ ]. 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	$P_N$	$\Delta$
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  $\Delta$  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 2B). 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  $\text{CO}_2$ ), 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  $\text{CO}_2$ ). The  $C_i/C_a$  ratio represents the balance between the demand for and supply of  $\text{CO}_2$  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  $\text{CO}_2$  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 2B. Overall means of cultivar net photosynthetic rate,  $P_N$ ; carbon isotopic discrimination,  $\Delta$ ; leaf water use efficiency, WUE ( $P_N/g_s$ ); intercellular  $\text{CO}_2$  concentration,  $C_i$ ; atmospheric  $\text{CO}_2$  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
$\Delta$	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  $\text{CO}_2$

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  $\Delta$ , and suggested that long-term measurements spanning through complete phenological cycles are needed.

Table 2A presents data of overall cultivar means, as averaged across canopy levels, for leaf gas exchange parameters and  $\Delta$ . 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  $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$  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  $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$  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  $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ ,  $C_i$  164  $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ ,  $C_i/C_a$  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  $\Delta$  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  $\Delta$ , 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 ( $1.6 \text{ kg m}^{-2}$ ) and the smallest dry storage root yield ( $1.2 \text{ kg m}^{-2}$ ) compared to the overall trial means of 2.3 and  $1.7 \text{ kg m}^{-2}$  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  $7500 \text{ cm}^3$  containers in a greenhouse with varying watering regimes. They found strong negative correlations in all sets of plants between total plant dry mass and  $\delta^{13}\text{C}$ , indicating that  $\Delta$  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  $\delta^{13}\text{C}$ ), instantaneous WUE based on leaf gas exchanges (*i.e.*  $\text{CO}_2$  uptake/ $\text{H}_2\text{O}$  loss) had no relation to season-long WUE. Gutiérrez and Meinzer (1994) reported negative associations between coffee leaf area index and both  $\Delta$ , 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  $\Delta$  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  $\Delta$  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  $\Delta$  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  $^{13}\text{C}$  isotopic discrimination ( $\Delta$ ) 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,  $P_N$   $32.6 \mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ ; stomatal conductance,  $g_s$   $765 \text{ mmol m}^{-2} \text{ s}^{-1}$ ; intercellular  $\text{CO}_2$  concentration,  $C_i$   $164 \mu\text{mol mol}^{-1}$ ; water use efficiency, WUE  $42.7 \mu\text{mol mol}^{-1}$ ;  $C_i/C_a$  0.50;  $\Delta$  19.2. \*\*\*\* correlation coefficients significant at  $p < 0.01$  or 0.0001, respectively; + autocorrelation.

	$\Delta$	$C_i/C_a$	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  $\Delta$  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  $\Delta$  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  $\Delta$ , 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<sup>-1</sup>) 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  $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ , as compared to values in P-fertilised plants of 3.1 for LAI and 27  $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$  for  $P_N$ ] (CIAT, 1992). It is noteworthy that in these P fertiliser trials the ratio of mean harvestable total biomass production [*i.e.* 18.2 t ha<sup>-1</sup> with P application/12.6 t ha<sup>-1</sup> without P application = 1.35] was close to the ratio of (LAI  $\times$   $P_N$ ) between the two P treatments (*i.e.* 83.7 with P application/62 without 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  $\text{CO}_2$ ) as compared to stomatal control (the supply side for  $\text{CO}_2$ ) (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 <sup>13</sup>C isotopic discrimination ( $\Delta$ ) 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  $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ ; stomatal conductance,  $g_s$  731  $\text{mmol m}^{-2} \text{ s}^{-1}$ ; intercellular  $\text{CO}_2$  concentration,  $C_i$  174  $\mu\text{mol mol}^{-1}$ ; water use efficiency, WUE 41.2  $\mu\text{mol mol}^{-1}$ ;  $C_i/C_a$  0.53;  $\Delta$  20.0. \*, \*\*, \*\*\*, \*\*\*\* correlation coefficients significant at  $p < 0.05$ , 0.01, 0.001, or 0.0001, respectively; + autocorrelation; NS not significant at  $p < 0.05$ .

	$\Delta$	$C_i/C_a$	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  $\Delta$  ( $r = 0.703$ ,  $p < 0.01$ ) 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  $\Delta$  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, López *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

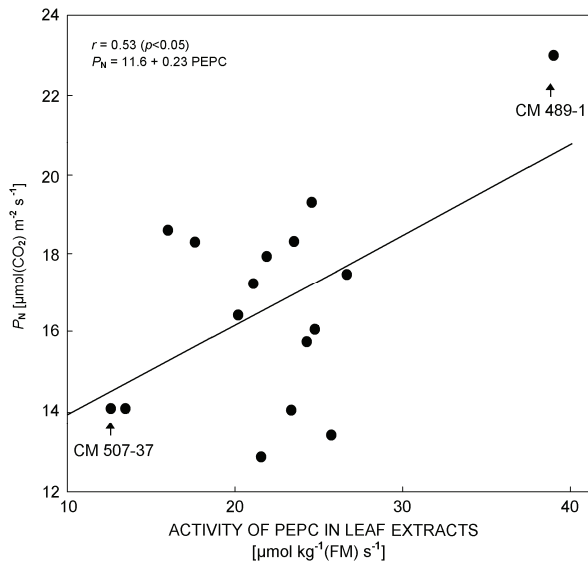


Fig. 1. Relationship between leaf net photosynthetic rate ( $P_N$ ) and PEP carboxylase (PEPC) activity of field-grown cassava cultivars under prolonged water stress at CIAT-Quilichao, 1988. Both  $P_N$  and PEPC activity were determined on the same leaves of 6-month old plants subjected to water stress 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  $\Delta$  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  $\Delta$  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/documentated '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  $\pm$  SD.

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

Table 5B. Representative ranges of chlorophyll-based values  $[\text{mmol kg}^{-1}(\text{Chl}) \text{s}^{-1}]$  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  $\Delta$  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 germ-plasm 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.

## References

- Adams, C.A., Leung, F., Sun, S.S.M.: Molecular properties of phosphoenolpyruvate carboxylase from  $C_3$ ,  $C_3$ - $C_4$  intermediate, and  $C_4$  *Flaveria* species. – *Planta* **167**: 218-225, 1986.
- Aguilar, L.P.: Ultraestructura foliar y fotosíntesis de yuca en diferentes cultivares *Manihot esculenta* (Crantz). [Leaf Ultrastructure and Photosynthesis of Cassava in Different Cultivars, (*Manihot esculenta* Crantz).] – BSc. Thesis. Universidad del Cauca, Popayan 1995. [In Spanish.]
- Apel, P., Horstmann, C., Pfeffer, M.: The *Moricondia* syndrome in species of the *Brassicaceae* – evolutionary aspects. – *Photosynthetica* **33**: 205-215, 1997.
- Araus, J.L., Brown, R.H., Byrd, G.T., Serret, M.D.: Comparative effects of growth irradiance on photosynthesis and leaf anatomy of *Flaveria brownii* ( $C_4$ -like), *Flaveria linearis* ( $C_3$ - $C_4$ ) and their  $F_1$  hybrid. – *Planta* **183**: 497-504, 1991.
- Araus, J.L., Brown, R.H., Bouton, J.H., Serret, M.D.: Leaf anatomical characteristics in *Flaveria trinervia* ( $C_4$ ), *Flaveria brownii* ( $C_4$ -like) and their  $F_1$  hybrid. – *Photosynth. Res.* **26**: 49-57, 1990.
- Bauwe, H.: Photosynthetic enzyme activities in  $C_3$  and  $C_3$ - $C_4$  intermediate species of *Moricondia* and *Panicum milioides*. – *Photosynthetica* **18**: 201-209, 1984.
- Benedict, C.R.: Nature of obligate photoautotrophy. – *Annu. Rev. Plant Physiol.* **29**: 67-93, 1978.
- Bernal, L.M.: Estudios sobre la actividad de fosfoenolpiruvato carboxilasa in cultivares de yuca (*Manihot esculenta* Crantz). [Studies on the Activity of Phosphoenolpyruvate Carboxylase in Cultivars of Cassava (*Manihot esculenta* Crantz).] – BSc. Thesis. Pontificia Universidad Javeriana, Bogota 1991. [In Spanish.]
- Blum, A., Sullivan, C.Y.: The comparative drought resistance of land races of sorghum and millet from dry and humid regions. – *Ann. Bot.* **57**: 835-846, 1986.
- Bunce, J.A.: Measurements and modeling of photosynthesis in field crops. – *CRC crit. Rev. Plant Sci.* **4**: 47-77, 1986.
- Bunce, J.A.: What is the usual internal carbon dioxide concentration in  $C_4$  species under midday field conditions? – *Photosynthetica* **43**: 603-608, 2005.
- Cayón, M.G., El-Sharkawy, M.A., Cadavid, L.F.: Leaf gas exchange of cassava as affected by quality of planting material and water stress. – *Photosynthetica* **34**: 409-418, 1997.
- CIAT: Cassava Program Annual Report for 1992. – Centro Internacional de Agricultura Tropical, Cali 1992.
- Cock, J.H., Porto, M.C.M., El-Sharkawy, M.A.: Water use efficiency of cassava: III. Influence of air humidity and water stress on gas exchange of field grown cassava. – *Crop Sci.* **25**: 265-272, 1985.
- Cock, J.H., Riaño, N.M., El-Sharkawy, M.A., Lopez, F.Y., Bastidas, G.:  $C_3$ - $C_4$  intermediate photosynthetic characteristics of cassava (*Manihot esculenta* Crantz). II. Initial products of  $^{14}CO_2$  fixation. – *Photosynth. Res.* **12**: 237-241, 1987.
- Condon, A.G., Farquhar, G.D., Richards, R.A.: Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. – *Aust. J. Plant Physiol.* **17**: 9-22, 1990.
- Connor, D.J., Cock, J.H., Parra, G.: Response of cassava to water shortage. I. Growth and yield. – *Field Crops Res.* **4**: 181-200, 1981.
- Connor, D.J., Palta, J.: Response of cassava to water shortage. III. Stomatal control of plant water status. – *Field Crops Res.* **4**: 297-311, 1981.
- Dai, Z., Ku, M.S.B., Edwards, G.E.:  $C_4$  photosynthesis. The  $CO_2$ -concentrating mechanism and photorespiration. – *Plant Physiol.* **103**: 83-90, 1993.
- De Tafur, S.M., El-Sharkawy, M.A., Calle, F.: Photosynthesis and yield performance of cassava in seasonally dry and semi-arid environments. – *Photosynthetica* **33**: 249-257, 1997.
- de Water, P.K.V., Leavitt, S.W., Betancourt, J.L.: Trends in stomatal density and  $^{13}C/^{12}C$  ratios of *Pinus flexilis* needles during last glacial-interglacial cycle. – *Science* **264**: 239-243, 1994.
- Ehleringer, J.R.: Variation in leaf carbon isotope discrimination in *Encelia farinosa*: implications for growth, competition, and drought survival. – *Oecologia* **95**: 340-346, 1993.
- Ehleringer, J.R., Rundel, P.W., Nagy, K.A.: Stable isotope in physiological ecology and food web research. – *Tree* **1**: 42-45, 1986.
- Ehleringer, J.R., Schulze, E.-D., Ziegler, H., Lange, O.L., Farquhar, G.D., Cowan, I.R.: Xylem-tapping mistletoes: Water or nutrient parasites? – *Science* **227**: 1479-1481, 1985.
- Ehleringer, J.R., White, J.W., Johnson, D.A., Brick, M.: Carbon isotope discrimination, photosynthetic gas exchange, and transpiration efficiency in beans and range grasses. – *Acta oecol.* **11**: 611-625, 1990.
- El-Sharkawy, M.A.: Effect of humidity and wind on leaf conductance of field grown cassava. – *Rev. bras. Fisiol. veget.* **2**: 17-22, 1990.
- El-Sharkawy, M.A.: Drought-tolerant cassava for Africa, Asia and Latin America. – *BioScience* **43**: 441-451, 1993.
- El-Sharkawy, M.A.: Cassava biology and physiology. – *Plant mol. Biol.* **56**: 481-501, 2004.
- El-Sharkawy, M.A.: How can calibrated research-based models be improved for use as a tool in identifying genes controlling crop tolerance to environmental stresses in the era of genomics—from an experimentalist's perspective. – *Photosynthetica* **43**: 161-176, 2005.
- El-Sharkawy, M.A.: International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics. – *Photosynthetica* **44**: 481-512, 2006a.

- El-Sharkawy, M.A.: Utility of basic research in plant/crop physiology in relation to crop improvement: a review and a personal account. – *Braz. J. Plant Physiol.* **18**: 419-446, 2006b.
- El-Sharkawy, M.A., Cadavid, L.F.: Response of cassava to prolonged water stress imposed at different stages of growth. – *Exp. Agr.* **38**: 333-350, 2002.
- El-Sharkawy, M.A., Cock, J.H.: Water use efficiency of cassava. I. Effects of air humidity and water stress on stomatal conductance and gas exchange. – *Crop Sci.* **24**: 497-502, 1984.
- El-Sharkawy, M.A., Cock, J.H.: C<sub>3</sub>-C<sub>4</sub> intermediate photosynthetic characteristics of cassava (*Manihot esculenta* Crantz). I. Gas exchange. – *Photosynth. Res.* **12**: 219-235, 1987a.
- El-Sharkawy, M.A., Cock, J.H.: Response of cassava to water stress. – *Plant Soil* **100**: 345-360, 1987b.
- El-Sharkawy, M.A., Cock, J.H.: Photosynthesis of cassava (*Manihot esculenta*). – *Exp. Agr.* **26**: 325-340, 1990.
- El-Sharkawy, M.A., Cock, J.H., Held, A.A.: Water use efficiency of cassava. II. Differing sensitivity of stomata to air humidity in cassava and other warm-climate species. – *Crop Sci.* **24**: 503-507, 1984.
- El-Sharkawy, M.A., Cock, J.H., Lynam, J.K., Hernández, A.d.P., Cadavid L, L.F.: Relationships between biomass, root-yield and single-leaf photosynthesis in field-grown cassava. – *Field Crops Res.* **25**: 183-201, 1990.
- El-Sharkawy, M.A., De Tafur, S.M., Cadavid, L.F.: Potential photosynthesis of cassava as affected by growth conditions. – *Crop Sci.* **32**: 1336-1342, 1992a.
- El-Sharkawy, M.A., De Tafur, S.M., Cadavid, L.F.: Photosynthesis of cassava and its relation to crop productivity. – *Photosynthetica* **28**: 431-438, 1993.
- El-Sharkawy, M.A., Hernández, A.D.P., Hershey, C.: Yield stability of cassava during prolonged mid-season water stress. – *Exp. Agr.* **28**: 165-174, 1992b.
- El-Sharkawy, M., Hesketh, J.: Photosynthesis among species in relation to characteristics of leaf anatomy and CO<sub>2</sub> diffusion resistances. – *Crop Sci.* **5**: 517-521, 1965.
- El-Sharkawy, M.A., Loomis, R.S., Williams, W.A.: Apparent reassimilation of respiratory carbon dioxide by different plant species. – *Physiol. Plant.* **20**: 171-186, 1967.
- El-Sharkawy, M.A., Loomis, R.S., Williams, W.A.: Photosynthetic and respiratory exchanges of carbon dioxide by leaves of the grain amaranth. – *J. appl. Ecol.* **5**: 243-251, 1968.
- Evans, L.T.: The plant physiologist as midwife. – *Search* **8**: 262-268, 1977.
- Farquhar, G.D., Ehleringer, J.R., Hubick, K.T.: Carbon isotope discrimination and photosynthesis. – *Annu. Rev. Plant Physiol. Plant mol. Biol.* **40**: 503-537, 1989.
- Farquhar, G.D., O'Leary, M.H., Berry, J.A.: On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. – *Aust. J. Plant Physiol.* **9**: 121-137, 1982.
- Farquhar, G.D., Richards, R.A.: Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. – *Aust. J. Plant Physiol.* **11**: 539-552, 1984.
- Fischer, R.A., Rees, D., Sayre, K.D., Lu, Z.-M., Condon, A.G., Saavedra, A.L.: Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. – *Crop Sci.* **38**: 1467-1475, 1998.
- Fleck, I., Fransi, A., Vidal, D.: Senescence patterns of several physiological and biochemical parameters of field grown flag leaves of *Triticum aestivum* L. cv. Kolibri. – *J. Plant Physiol.* **123**: 327-338, 1986.
- Gutiérrez, M.V., Meinzer, F.C.: Carbon isotope discrimination and photosynthetic gas exchange in coffee hedgerows during canopy development. – *Aust. J. Plant Physiol.* **21**: 207-219, 1994.
- Guzman, G., El-Sharkawy, M.A.: [Effect of soil water availability on leaf anatomy and gas exchange of two cassava cultivars (*Manihot esculenta* Crantz).] – *Rev. Comalfi* **16**: 11-18, 1989. [In Spanish.]
- Hattersley, P.W., Wong, S.-C., Perry, S., Roksandic, Z.: Comparative ultrastructure and gas exchange characteristics of the C<sub>3</sub>-C<sub>4</sub> intermediate *Neurachne minor* S. T. Blake (Poaceae). – *Plant Cell Environ.* **9**: 217-233, 1986.
- Hershey, C.H., Jennings, D.L.: Progress in breeding cassava for adaptation to stress. – *Plant Breed. Abstr.* **62**: 823-831, 1992.
- Hillocks, R.J., Thresh, J.M., Bellotti, A.C. (ed.): Cassava: Biology, Production and Utilization. – CABI, New York 2002.
- Holaday, A.S., Brown, R.H., Bartlett, J.M., Sandlin, E.A., Jackson, R.C.: Enzymic and photosynthetic characteristics of reciprocal F<sub>1</sub> hybrids of *Flaveria pringlei* (C<sub>3</sub>) and *Flaveria brownii* (C<sub>4</sub>-like species). – *Plant Physiol.* **87**: 484-490, 1988.
- Holaday, A.S., Chollet, R.: Photosynthetic/photorespiratory characteristics of C<sub>3</sub>-C<sub>4</sub> intermediate species. – *Photosynth. Res.* **5**: 307-323, 1984.
- Holaday, A.S., Talkmitt, S., Doohan, M.E.: Anatomical and enzymic studies of leaves of a C<sub>3</sub>×C<sub>4</sub> *Flaveria* F<sub>1</sub> hybrid exhibiting reduced photorespiration. – *Plant Sci.* **41**: 31-39, 1985.
- Holbrook, G.P., Jordan, D.B., Chollet, R.: Reduced apparent photorespiration by the C<sub>3</sub>-C<sub>4</sub> intermediate species, *Moricandia arvensis* and *Panicum milioides*. – *Plant Physiol.* **77**: 578-583, 1985.
- Howeler, R.H.: Identifying plants adaptable to low pH conditions. – In: Wrigh, J.R., Baligar, V.C., Murrmann, R.P. (ed.): Plant-Soil Interactions at Low pH. Pp. 885-904. Kluwer Academic Publ., Dordrecht 1991.
- Howeler, R.H.: Cassava mineral nutrition and fertilization. – In: Hillocks, R.J., Thresh, J.M., Bellotti, A.C. (ed.): Cassava: Biology, Production and Utilization. Pp. 115-147. CABI, New York 2002.
- Howeler, R.H., Cadavid, L.F.: Short-and long-term fertility trials in Colombia to determine the nutrient requirements of cassava. – *Fertilizer Res.* **26**: 61-80, 1990.
- Hylton, C.M., Rawsthorne, S., Smith, A.M., Jones, D.A., Woolhouse, H.W.: Glycine decarboxylase is confined to the bundle-sheath cells of leaves of C<sub>3</sub>-C<sub>4</sub> intermediate species. – *Planta* **175**: 452-459, 1988.
- Impa, S.M., Nadaradjan, S., Boominathan, P., Shashidhar, G., Bindumathava, H., Sheshshayee, M.S.: Carbon isotope accurately reflects variability in WUE measured at a whole level in rice. – *Crop Sci.* **45**: 2517-2522, 2005.
- Ismail, A.M., Hall, A.E., Bray, E.A.: Drought and pot size effects on transpiration efficiency and carbon isotope discrimination of cowpea accessions and hybrids. – *Aust. J. Plant Physiol.* **21**: 23-35, 1994.
- Jackson, P., Robertson, M., Cooper, M., Hammer, G.: The role of physiological understanding in plant breeding; from a breeding perspective. – *Field Crops Res.* **49**: 11-37, 1996.
- Kawano, K.: Thirty years of cassava breeding for productivity—biological and social factors for success. – *Crop Sci.* **43**: 1325-1335, 2003.
- Knight, J.D., Livingston, N.J., Kessel, C.: Carbon isotope discrimination and water-use efficiency of six crops grown under wet and dryland conditions. – *Plant Cell Environ.* **17**: 173-179, 1994.

- Körner, C., Scheel, J.A., Bauer, H.: Maximum leaf diffusive conductance in vascular plants. – *Photosynthetica* **13**: 45-82, 1979.
- Kramer, P.J.: The role of physiology in crop improvement. – In: Staples, R.C., Kuhr, R.J. (ed.): *Linking Research to Crop Production*. Pp. 51-62. Plenum Press, New York 1980.
- Ku, M.S.B., Monson, R.K., Littlejohn, R.O., Jr., Nakamoto, H., Fisher, D.B., Edwards, G.E.: Photosynthetic characteristics of C<sub>3</sub>-C<sub>4</sub> intermediate *Flaveria* species. 1. Leaf anatomy, photosynthetic responses of O<sub>2</sub> and CO<sub>2</sub>, and activities of key enzymes in the C<sub>3</sub> and C<sub>4</sub> pathways. – *Plant Physiol.* **71**: 944-948, 1983.
- Ku, M.S.B., Wu, J., Dai, Z., Scott, R.A., Chu, C., Edwards, G.E.: Photosynthetic and photorespiratory characteristics of *Flaveria* species. – *Plant Physiol.* **96**: 518-528, 1991.
- Long, S.P., Ainsworth, E.A., Leakey, A.D.B., Nösberger, J., Ort, D.R.: Food for thought: lower-than-expected crop yield stimulation with rising CO<sub>2</sub> concentration. – *Science* **312**: 1918-1921, 2006.
- López, Y., Vélez, W., El-Sharkawy, M.A., Mayer, J.E.: Biochemical characterization of PEPC from cassava: a preliminary report. – In: Roca, W.M., Thro, A.M. (ed.): *Proceeding of the First International Scientific Meeting of the Cassava Biotechnology Network*. Pp. 340-343. Centro Internacional de Agricultura Tropical, Cali 1993.
- Makino, A., Mae, T., Ohira, K.: Photosynthesis and ribulose 1,5-bisphosphate carboxylase in rice leaves. Changes in photosynthesis and enzymes involved in carbon assimilation from leaf development through senescence. – *Plant Physiol.* **73**: 1002-1007, 1983.
- Martin, B., Thorstenson, Y.R.: Stable carbon isotope composition ( $\delta^{13}\text{C}$ ), water use efficiency, and biomass productivity of *Lycopersicon esculentum*, *Lycopersicon pennellii*, and the F<sub>1</sub> hybrid. – *Plant Physiol.* **88**: 213-217, 1988.
- Meidner, H.: The minimum intercellular-space CO<sub>2</sub> concentration ( $\Gamma$ ) of maize leaves and its influence on stomatal movements. – *J. exp. Bot.* **13**: 284-293, 1962.
- Meidner, H., Mansfield, T.A.: *Physiology of Stomata*. – McGraw-Hill, London 1968.
- Monson, R.K.: On the evolutionary pathways resulting in C<sub>4</sub> photosynthesis and crassulacean acid metabolism (CAM). – *Adv. ecol. Res.* **19**: 57-110, 1989.
- Monson, R.K., Edwards, G.E., Ku, M.S.B.: C<sub>3</sub>-C<sub>4</sub> intermediate photosynthesis in plants. – *BioScience* **34**: 563-566, 571-574, 1984.
- Monson, R.K., Moore, B.d.: On the significance of C<sub>3</sub>-C<sub>4</sub> intermediate photosynthesis to the evolution of C<sub>4</sub> photosynthesis. – *Plant Cell Environ.* **12**: 689-699, 1989.
- Moore, B.d., Ku, M.S.B., Edwards, G.E.: Expression of C<sub>4</sub>-like photosynthesis in several species of *Flaveria*. – *Plant Cell Environ.* **12**: 541-549, 1989.
- Nobel, P.S.: Leaf anatomy and water use efficiency. – In: Turner, N.C., Kramer, P.J. (ed.): *Adaptation of Plants to Water and High Temperature Stress*. Pp. 43-55. J. Wiley & Sons, New York – Chichester – Brisbane – Toronto 1980.
- Nobel, P.S., Hartsock, T.L.: Development of leaf thickness for *Plectranthus parviflorus*. Influence of photosynthetically active radiation. – *Physiol. Plant.* **51**: 163-166, 1981.
- Ögren, E., Rosenqvist, E.: On the significance of photoinhibition of photosynthesis in the field and its generality among species. – *Photosynth. Res.* **33**: 63-71, 1992.
- Osmond, C.B., Winter, K., Ziegler, H.: Functional significance of different pathways of CO<sub>2</sub> fixation in photosynthesis. – In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (ed.): *Physiological Plant Ecology II*. Pp. 479-547. Springer-Verlag, Berlin – Heidelberg – New York 1982.
- Pellet, D., El-Sharkawy, M.A.: Cassava varietal response to phosphorus fertilization. I. Yield, biomass and gas exchange. – *Field Crops Res.* **35**: 1-11, 1993.
- Pellet, D., El-Sharkawy, M.A.: Sink-source relations in cassava: effects of reciprocal grafting on yield and leaf photosynthesis. – *Exp. Agr.* **30**: 359-367, 1994.
- Rao, I.M., Ayarza, M.A., Thomas, R.J.: The use of carbon isotope ratios to evaluate legume contribution to soil enhancement in tropical pastures. – *Plant Soil* **162**: 177-182, 1994.
- Riaño, M.A., Cock, J.H., López, Y.F., El-Sharkawy, M.: [Kranz anatomy, structure and distribution of chloroplasts in cassava leaves (*Manihot esculenta* Crantz).] – *Rev. Comalfi* **14**: 5-12, 1987a. [In Spanish.]
- Riaño, N.M., Cock, J.H., López, Y.F., El-Sharkawy, M., Bastidas, G.: [Photosynthetic characteristics of cassava (*Manihot esculenta* Crantz): initial products of <sup>14</sup>CO<sub>2</sub> fixation.] – *Rev. Comalfi* **14**: 13-17, 1987b. [In Spanish.]
- Šesták, Z. (ed.): *Photosynthesis During Leaf Development*. – Academia, Praha 1985; Dr W. Junk, Dordrecht – Boston – Lancaster 1985.
- Shorter, R., Law, R.J., Hammer, G.L.: Improving genotypic adaptation in crops – a role for breeders, physiologists, and modelers. – *Exp. Agr.* **27**: 155-175, 1991.
- Solárová, J., Pospíšilová, J.: Photosynthetic characteristics during ontogenesis of leaves. 8. Stomatal diffusive conductance and stomata reactivity. – *Photosynthetica* **17**: 101-151, 1983.
- Stuhlfauth, T., Scheuermann, R., Fock, H.P.: Light energy dissipation under water stress conditions. Contribution of re-assimilation and evidence for additional processes. – *Plant Physiol.* **92**: 1053-1061, 1990.
- Tolbert, N.E., Oeser, A., Yamazaki, R.K., Hageman, R.H., Kasaki, T.: A survey of plants for leaf peroxisomes. – *Plant Physiol.* **44**: 135-147, 1969.
- Volk, R.J., Jackson, W.A.: Photorespiratory phenomena in maize. Oxygen uptake, isotope discrimination, and carbon dioxide efflux. – *Plant Physiol.* **49**: 218-223, 1972.
- White, J.W.: Implications of carbon isotope discrimination studies for breeding common bean under water deficits. – In: *Stable Isotopes and Plant Carbon-Water Relations*. Pp. 387-398. Academic Press, New York 1993.
- White, J.W., Castillo, J.A., Ehleringer, J.R., Garcia-c, J.A., Singh, S.P.: Relations of carbon isotope discrimination and other physiological traits to yield in common bean (*Phaseolus vulgaris*) under rainfed conditions. – *J. agr. Sci.* **122**: 275-284, 1994.
- Wong, W.W., Benedict, C.R., Kohel, R.J.: Enzymic fractionation of the stable carbon isotopes of carbon dioxide by ribulose-1,5-bisphosphate carboxylase. – *Plant Physiol.* **63**: 852-856, 1979.