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Genotypic variations in activities of phospho*enol*pyruvate carboxylase and correlations with leaf photosynthetic characteristics and crop productivity of cassava grown in low-land seasonally-dry tropics

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Abstract

Field trials with a large group of cassava germplasm were conducted at the seasonally-dry and hot environments in southwest Colombia to investigate photosynthetic characteristics and production under drought conditions. Measurement of net photosynthetic rate (P_N), photosynthetic nitrogen use efficiency (PNUE), mesophyll conductance to CO_2 diffusion (g_m), and phospho*enol*pyruvate carboxylase (PEPC) activity of upper canopy leaves were made in the field. All photosynthetic characteristics were significantly correlated with final dry root yield (Yield). Correlations among the photosynthetic traits were also significant. PEPC activity was highly significantly correlated with P_N and PNUE, indicating the importance of the enzyme in cassava photosynthesis and productivity. Among a small selected group from the preliminary trial for yield performance, the second year Yield was highly significantly correlated with P_N measured on the first year crop. Thus variations in the measured photosynthetic traits are genetically controlled and underpin variations in yield. One short-stemmed cultivar M Col 2215 was selected for high root dry matter content, high harvest index, and tolerance to drought. It was tested under the semi-arid conditions of the west coast of Ecuador; participating farmers evaluated cultivar performance. This cultivar was adopted by farmers and officially released in 1992 under the name Portoviejo 650.

Additional key words: breeding; canopy; C_3-C_4; cultivar; drought; mesophyll conductance; photosynthesis; productivity; starch; stomata; water; yield.

Introduction

There is a growing consensus that conventional means of improving crop productivity based only on selection for yield *via* conventional breeding, at least in developed countries, are reaching their limits and that new means are required for future potential yield increases (*e.g.* Schrader 1980, Evans 1993, Mann 1997). The identification of—and selection for—beneficial morphological, structural, biochemical, and physiological traits in relation to yield may provide a greater potential for inreasing agricultural productivity aided with modern molecular biology tools in post-genomic era, particularly in stressful environments (Richards 2000, El-Sharkawy

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Abreviations: C_i – intercellular CO_2; DM – dry matter; g_m – mesophyll conductance to CO_2 diffusion; GO – glycolate oxidase; HI – harvest index; LAI – leaf area index; PEPC – phospho*enol*pyruvate carboxylase; PNUE – photosynthetic nitrogen use efficiency; PPFD – photosynthetic photon flux density; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; Γ – CO_2 compensation concentration.

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2005). These objectives will become even more important when considering recent trends in demands for renewable energy sources from crops such as maize (*Zea mays*), grain sorghum (*Sorghum bicolor*), sugarcane (*Saccharum officinarum*), and cassava (*Manihot esculenta*) (*e.g.* McLaren 2005, Hill *et al.* 2006). Partial substitution of fossil energy sources with biofuels will compete with demands for human food and animal feed. To deal with this dilemma, agricultural productivity has to be greatly enhanced. Because prime land and fresh water resources are limited in most regions, increasing crop productivity should depend more on innovative research to increase potential yields of cultivars. Actual yields in most developing countries are still far below what they should be (El-Sharkawy 2006b).

Compared to other staple food crops, scarce information existed on physiology of cassava before the creation in the late 1960's of the two research centres [*i.e.* the International Institute of Tropical Agriculture (IITA) in Nigeria and the Centro Internacional de Agricultura Tropical (CIAT) in Colombia, with regional and world mandates for cassava, respectively] (Hunt *et al.* 1977). Yet, this previously neglected crop is a major source for food and animal feed for more than 500 million people in tropical and subtropical Africa, Asia, and Latin America. Recent estimates of annual world production is approximately 160–170 million tons of fresh root (with 50 % or more in Africa, and Nigeria is currently the largest world producer with more than 35 million tons annually) (*e.g.* Hillocks *et al.* 2002, http://faostatclassic.fao.org).

Average world fresh root yield is low (≈ 10 t per ha) because the crop is mainly produced by resource-limited small farmers virtually without production inputs on low-fertility soils, and under rain fed conditions with frequent long periods of water shortages (El-Sharkawy 1993). To address these stressful conditions, the breeding strategy at CIAT shifted its focus towards selecting improved cultivars adaptable to a specific ecosystem/ecozone and with reasonable, but stable, productivity (Hershey and Jennings 1992). However, with the exception of Asia (*e.g.* Howeler 1995, Kawano 2003), yield is still low with little changes (for example, in Latin America and the Caribbean region, average yield increased from ≈11.7–12.1 t per ha between 1990 and 1999; for Sub-Saharan Africa yield stagnated below 10 t per ha – Hillocks *et al.* 2002). If the many socioeconomic constraints related to cassava production, utilization, and marketing were removed, farmers' yield could be greatly increased by adopting improved technology (Sarma and Kunchai 1991, El-Sharkawy 1993, Kawano 2003). Cassava physiological research has concentrated on both basic and applied aspects of the crop in relation to productivity, taking advantages of the available germplasm bank as well as the diverse environmental conditions in Colombia (*e.g.* Iglesias *et al.* 1995). Recent reviews summarised the findings on the many morphological, physiological, and biochemical traits associated with higher productivity under both favourable and stressful environments, as well as on mechanisms underlying tolerance of the crop to abiotic stresses such as prolonged drought and poor soils (*e.g.* Alves 2002, El-Sharkawy 2004, 2006a,b).

Based on several leaf anatomical, physiological, and biochemical characteristics, compared to typical C_3 and C_4 species, cassava is considered as a C_3-C_4 photosynthetically intermediate species (see El-Sharkawy and Cock 1987a, El-Sharkawy and De Tafur 2007). Notable among these traits is the elevated activity of phospho*enol*pyruvate carboxylase (PEPC) (in several cultivars, PEPC activity in leaf extracts ranged 10–30 % of activities in C_4 species such as maize and grain sorghum, El-Sharkawy and Cock 1990, Bernal 1991, López *et al.* 1993). Under prolonged water deficits in the field, PEPC activity correlated with net photosynthetic rate (P_N) of the same leaves (El-Sharkawy 2004). However, Kelly and Latzko (1991) in their review on photosynthesis concluded that "the concept of a relatively strict categorisation of plants into C_3, C_4, C_3-C_4, and CAM is being eroded as further studies have begun to reveal a spectrum of types between true C_3, on the one hand, and true C_4 or true CAM, on the other. A C_3-C_4 plant or tissue can now be thought of as any C_3 plant (or tissue) with a greater or lesser amount of C_4 metabolism". Nevertheless, subtle and cumulative changes in anatomical, physiological, and biochemical characteristics of the original C_3 system in green plant photosynthesis may lead to some heritable functional and structural properties fundamental to the evolutionary process toward the appearance of true CAM or true C_4 systems (for more information on the genetic basis underlying evolution of C_4 system from C_3 genes, see Engelmann *et al.* 2003, Westhoff and Gowik 2004, Gowik *et al.* 2006). The implications of these subtle changes, even in true C_3 system, for plant performance in general and for crop productivity in particular should not, however, be overlooked by photosynthetic researchers. Moreover, activities of PEPC, CO_2 compensation concentration (Γ), and dry matter production of the C_3 wheat (*Triticum aestivum* L.) cv. Sonalika, were found to be affected by the form of nitrogen fertiliser (Sharma and Sirohi 1988). Compared to NH_4-N grown plants, NO_3-N grown plants had statistically significant higher PEPC activities, lower Γ, and higher dry matter (DM) production. On the other hand, there were no significant differences due to form of N fertiliser in the activities of glycolate oxidase (GO) and ribulose 1,5-bisphosphate carboxylase (RuBPCO). Thus, the enhancement in dry matter production of NO_3-N grown wheat plants appears to be solely due to higher PEPC activities and lower photorespiratory carbon losses. Both RuBPCO and GO are associated with the C_2-photorespiratory cycle.

Because plant breeders require manageable and reliable screening methods for identifying parental materials as well as for evaluating large populations of breeding lines in the field with favourable physiological traits (El-Sharkawy 2006b), we have evaluated the utility of currently available methods and techniques. The infrared gas exchange analysis techniques, particularly those utilizing compact portable equipment associated with leaf chambers operating in both open-end and closed mode, were operationally practical and useful in this regard. Several genotypes with high P_N were identified in lowland and mid-altitude hot-humid climates, in high-altitude cool climates, as well as in seasonally dry and semi-arid hot climates (El-Sharkawy *et al.* 1990, CIAT 1992, 1994, De Tafur *et al.* 1997b, El-Sharkawy 2006a). Moreover, a large group of accessions were evaluated for leaf gas exchanges and productivity over several years under adequate phosphorus fertilisation (P) and low-P acidic soils (CIAT 1987–1994, Pellet and El-Sharkawy 1993a,b, El-Sharkawy 2004, 2006a). Numerous cultivars and breeding lines were identified with high tolerance to low-P soils (*i.e.* with high yield at both high and low P contents) and high P_N [which correlated positively with storage root yield and total biomass, with P tolerance, and with root number per plant (*i.e.* strong root sinks) (see CIAT 1990, El-Sharkawy 2004)]. Selection for high P_N in parental material when combined with other yield-related traits such as high harvest index (HI) (>0.5), optimal seasonal leaf area index (LAI) (with values larger than 2.5 for greater seasonal irradiance interceptions), better leaf retention and duration (*i.e.* longer leaf life), more storage roots per plant, as well as high tolerance/resistances to pests and diseases may lead to higher productivity (El-Sharkawy and Cock 1987b, Cock and El-Sharkawy 1988, CIAT 1990, 1995, Hershey and Jennings 1992, El-Sharkawy *et al.* 1992a,b, Pellet and El-Sharkawy 1993a, El-Sharkawy 1993, 2006a, Iglesias *et al.* 1995*,* Lenis *et al.* 2006).

In addition to leaf gas exchange measurements, analysis of leaf carbon isotope composition (*i.e.* $\delta^{13}C$) was also tested for assessing genotypic variations in relation to yield (El-Sharkawy and De Tafur 2007). Moreover, we applied biochemical assays for measuring activities of some photosynthetic enzymes in limited number of field-grown cassava cultivars that have led to the identification of few genotypes with enhanced activities of PEPC and RuBPCO (El-Sharkawy and Cock 1990, Bernal 1991, López *et al.* 1993, El-Sharkawy 2004, 2006a). The choice of any one or a combination of these methods by breeders will depend not only on their reliability and time requirement but also on the relative costs of equipment and operations involved. The objectives of this paper were (*1*) to evaluate a larger group of cultivars for activities of leaf PEPC in the field; (*2*) to determine the interrelationships among leaf PEPC activity, P_N, and other gas exchange characteristics; and (*3*) to assess, for the first time, whether there is any relationship between PEPC activity and root yield.

Materials and methods

To determine the relationship between productivity and leaf P_N, the initial trials included 127 genotypes from CIAT cassava core germplasm representing high, medium, and low top biomass types (for more information and experimental details see El-Sharkawy *et al.* 1990). In addition to the choice of genotypes, there was also the choice of relevant environmental stresses so that genetic variation in photosynthetic efficiency is expressed and can be assessed in relation to yield. The experiment was conducted on a private farm located in the Patia Valley, Cauca Dept., Southwest Colombia (altitude 600 m, latitude 2°09′N, longitude 77°04′W, mean annual temperature 28 °C with little seasonal fluctuation). The Patia Valley lies between the occidental and central Andean mountains and is characterised by high solar radiation (22 MJ m^{-2} d^{-1}), and high atmospheric humidity (70 %). The region represents seasonally-dry ecosystems with mean bimodal annual rainfall less than 1 000 mm, which is much less than potential evapotranspiration of ≈5–6 mm d^{-1}, and with two dry periods of 2–3 months each. During the trial periods in 1986–7 and 1988–9, annual precipitation was less than 1 000 mm (see Table 1 for the pattern of rainfall distribution in 1986–7 season, and also El-Sharkawy *et al.*1990).

Measurements of upper canopy single-leaf gas exchange rates (CO_2 uptake and H_2O loss) were made with a *LI-6000* portable gas analyser (*LI-COR*, Lincoln, NE, USA) from 09:00 to 13:00 h when solar irradiance exceeded 1 000 µmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD). Gas exchange rates were made during the dry period on several occasions spanning from late February to mid June with total of 10–12 leaves per cultivar (for more details on gas exchange measurements, see El-Sharkawy *et al.* 1990). A lateral lobe was cut from the measured leaves prior to enclosing the chamber on the central largest lobe, and the bulk leaf water potential (ψ_L) was determined with the standard Scholander pressure chamber technique. The ψ_L values ranged from –1.0 to –1.5 MPa with an overall average of –1.20±0.12 SD, across cultivars and measurement dates, indicating the tight stomatal control on water loss (Connor and Palta 1981, El-Sharkawy and Cock 1984, Cock *et al.* 1985, El-Sharkawy 1990). P_N values were averaged for each cultivar and used for calculating correlations with final root yield and PEPC activity.

At 160 d after planting, an independent sample of five upper-canopy leaves was harvested per cultivar for N, P, and K analysis. Photosynthetic nitrogen use efficiency (PNUE) was determined by expressing CO_2 exchange rate on the basis of leaf total nitrogen.

For the determination of PEPC activity, a group of 18 cultivars (Table 1) was selected representing the three different top biomass types. The biochemical assays were conducted following methods used by Hatch and Oliver

(1980) and O'Leary (1982). The necessary equipment and reagents were brought and housed in a cold room at the farm during measurements. For each cultivar, 4–5 leaves were harvested from the top canopy layer of five-month old plants between 09:00 and 11:00 h local time and leaf extracts were made immediately. Equal sets of leaves were frozen in liquid nitrogen and taken to CIAT Headquarters at Palmira, Valle Dept. (the distance between the farm and CIAT is ≈4–5 h drive) for further assays in the biochemistry laboratory.

A local cv. of grain sorghum (*Sorghum bicolor*) was also planted and used as a C_4 check species for assaying PEPC.

On 26–27 August 1987 (309–310 d after planting), the six central plants per plot for each cultivar were harvested for the determination of yield. Five kg of fresh roots per cultivar were cut into small pieces and oven-dried at 75 °C for 75 h for the determination of dry root yield (Yield). The mean root dry matter content across the 127 cultivars was 309±45 g kg^{-1}.

Selection, farmer-adoption, and release of a drought-tolerant cultivar (cv. Portoviejo 650) for the semiarid west coast of Ecuador: The low-top biomass cultivar M Col 2215 (a short-stemmed and with >0.70 HI) (Table 1) had the highest DM content of 405 g kg^{-1}. As an outcome of this trial, cv. M Col 2215 was selected by the cassava physiology/breeding sections and introduced in 1987 to the semi-arid west coast of Ecuador where it was further tested under the local environmental conditions of less than 600 mm annual rainfall with 4–7 months of dry period. It was officially released in August 1992 under the name Portoviejo 650 by the Instituto Nacional de Investigación Agropecuaria (INIAP), Portoviejo, Manabi Province, Ecuador (see CIAT 1993). Cultivar Portoviejo 650 or M Col 2215 was tested for yield and root quality trials in three locations at the west Coast of Ecuador, along with a check Tres Meses as well as several other CIAT introductions (unpublished). It demonstrated the most uniform growth and stable yields and the highest average DM content in storage roots, and a greater tolerance to drought in both regional and on-farm trials involving farmers evaluation of breeding materials. Before it was officially released, farmers started multiplying their own planting materials, thus indicating how is important for researchers to involve farmers in the selection process early on during the evaluation of improved cultivars (CIAT 1993). It is currently cultivated in a large portion of cassava area for fresh consumption, production of dried chips, and processed high-quality flour and starch low in fibre. Short-stemmed cultivars have similar yield as tall ones but with greater nutrient use efficiency in terms of storage root production because of their lower top biomass (El-Sharkawy *et al.* 1998a). Moreover, under prolonged water stress at early and mid-season stages of growth, aerial parts (stems and leaves) were reduced more than root yield in cassava, resulting in significant nutrient use efficiency at final harvests (El-Sharkawy *et al.* 1998b). This example illustrates the utility and efficiency of the integrated physiological-breeding efforts in selecting adaptable high yielding cultivars as well as in speeding up diffusion and adoption of improved technology across countries (*e.g.* Henry 1991, Iglesias *et al.* 1995).

Sixteen cultivars, including 14 out of the 18 that had been assayed for PEPC in the 1986–7 season, were selected from the preliminary trial on the basis of their yield performance and planted for a second season in 1988–9 to test whether the relation between their 1986–7 P_N and yield held up. The annual precipitation in the second season was ≈950 mm in 308 d; this resulted in higher root yield, compared to the first season.

Results and discussion

Yield and photosynthetic characteristics: Large and significant differences among cassava cultivars in Yield, P_N, PNUE, PEPC, and g_m were found (Table 1). Yield ranged from 1.03 to 1.83 kg m^{-2} with an overall mean of 1.36; P_N ranged from 21.0 to 30.6 μmol(CO_2) m^{-2} s^{-1} with an overall mean of 25.1; PNUE ranged from 9.4 to 16.2 mmol(CO_2) kg^{-1}(N) s^{-1} with an overall mean of 12.1; PEPC activity ranged from 6.30 to 14.00 μmol kg^{-1}(FM) s^{-1} with an overall mean of 9.65; and g_m ranged from 93 to 126 mmol m^{-2} s^{-1} with an overall mean of 103. PEPC activity in grain sorghum was 58 μmol kg^{-1}(FM) s^{-1}, which lies within the range normally observed in C_4 plants under similar environmental conditions (El-Sharkawy and De Tafur 2007) [but see also Chandra and Sirohi (1983) for the increased sorghum Γ under water stress at different stages of growth that indicated higher photorespiration in this case]. Compared to sorghum, the overall mean of cassava PEPC activity was 17 %, with a cultivar range from 11 to 24 %. These PEPC percent activities of cassava, relative to C_4 system, under the prolonged water deficits in this trial, compare well with activities obtained under sub-humid and wet conditions (El-Sharkawy and Cock 1990, Bernal 1991). Moreover, these findings indicate that PEPC in cassava remains active under prolonged water deficits in the field. Before determining PEPC activity on February 1987, the crop received insufficient rain from December to the time of measurements (a total of 73 mm in two months, which is 21 % of potential evapotranspiration) (Table 1, see also El-Sharkawy *et al.*1990). In another trial under prolonged water stress in sub-humid environment, RuBPCO activity was reduced more than activity of PEPC (El-Sharkawy 2004, 2006a). This points to the importance of PEPC for cassava photosynthesis and hence to productivity in seasonally dry and semi-arid ecosystems. It also supports the long-advocated strategy of breeding for longer leaf

Table 1. Dry root yield (Yield) [kg m^{-2}], net photosynthetic rate (P_N) [μmol(CO$_2$) m^{-2} s^{-1}], leaf photosynthetic nitrogen use efficiency (PNUE) [mmol(CO$_2$) kg^{-1}(N) s^{-1}], activity of phospho*enol*pyruvate carboxylase (PEPC) [μmol kg^{-1}(FM) s^{-1}], and mesophyll conductance to CO$_2$ diffusion (g_m) [mmol m^{-2} s^{-1}] of some cultivars. A large group of cassava accessions (127 cvs.) representing high, medium, and low-top biomass were rainfed-grown at Patia, Cauca Department, Colombia in 1986–7. P_N was determined with a portable gas analyser on upper canopy leaves (10–12 per cultivar) of 6–8-month-old-plants (El-Sharkawy *et al.* 1990). PEPC activity was determined spectrophotometrically in the field using extracts of upper canopy leaves (4–5 per cv.) on 5-month-old plants. The measurements were made during the dry period (total rainfall ≈750 mm, 257 mm in the first two months, October and November, no rainfall from June to 26 August). Daily pan evaporation >5 mm. $^+$Because of its high root dry matter (405 g kg^{-1}), its stable yield, and high tolerance to drought, this low-top biomass cv. was introduced and tested under the semi-arid (annual mean rainfall <600 mm, 4–7 months of water shortage) environment in the west coast of Ecuador, Manabi Province. It is commercially produced under the name Portoviejo 650.

Cv.	Yield	P_N	PNUE	PEPC	g_m
M Bra 12	1.15	27.4	14.7	11.86	111
M Col 1468	1.03	24.4	11.9	10.35	100
M Col 2215$^+$	1.22	23.7	9.5	8.70	95
CM 305-41	1.34	26.2	13.2	12.60	106
CM 308-197	1.55	23.4	11.8	10.60	99
CM 342-170	1.71	24.2	12.9	11.00	102
CM 523-7	1.00	21.0	9.5	6.77	96
CM 847-11	1.17	23.1	10.6	8.15	97
CM 922-2	1.44	24.9	10.8	7.00	102
CM 976-15	1.15	21.1	9.4	6.30	93
CM 975-5	1.40	21.7	11.1	8.70	94
CM 1015-42	1.31	25.4	11.5	7.77	102
CM 1016-34	1.83	29.1	14.6	14.00	113
CM 1022-34	1.79	28.1	13.4	11.15	112
CM 1297-9	1.56	27.5	13.5	13.50	110
CM 1299-2	1.40	30.6	16.2	8.62	126
CM 1335-4	1.06	25.2	11.9	8.85	95
CM1797-8	1.34	24.7	10.4	7.72	96
Mean (*n* = 18)	1.36	25.1	12.1	9.65	103
LSD 5 %	0.27	1.9	1.0	2.87	12
C$_4$ check sp. *Sorghum bicolor*				58.00	
Cassava/sorghum [%]				17.00	
Range [%]				11–24	

life and better leaf retention (El-Sharkawy and Cock 1987b, Cock and El-Sharkawy 1988, El-Sharkawy 1993, 2005, 2006a, Lenis *et al.* 2006) so that greater portion of photoassimilates could be diverted towards storage roots. Another possible role of the high activity of PEPC in cassava under water stress is that PEPC can recycle photorespiratory CO$_2$ when stomata close in hot dry climates, and hence dissipate excess photon energy and mitigate photosynthetic photoinhibition (Osmond *et al.* 1980, Stuhlfauth *et al.* 1990, Ögren and Rosenqvist 1992, Osmond and Grace 1995, Biehler *et al.* 1996).

It seems that the partial closure of cassava stomata in dry air and in water-deficient soils that was observed under laboratory controlled conditions and in the field (Connor and Palta 1981, Porto 1983, El-Sharkawy and Cock 1984, Cock *et al.* 1985, Guzman 1989, El-Sharkawy 1990, De Tafur *et al.* 1997b) is a defensive reaction, *i.e.* stress avoidance mechanism, that protects leaves from severe dehydration while they remain photosynthetically active for long periods under water stress. Another trait that may underlie tolerance of cassava to prolonged soil-water deficits is the fine roots that penetrate deeper into wetter soil layers (≈2 m soil depth) under stress and their ability to extract slowly available water (Connor *et al.* 1981, Porto 1983, El-Sharkawy and Cock 1987b, El-Sharkawy *et al.* 1992b, Caicedo 1993, De Tafur *et al.* 1997a, Cadavid *et al.* 1998, El-Sharkawy 2006a). These combined beneficial leaf and fine root traits would lead not only to the survival of cassava but also to optimizing water use efficiency over a long productive period. In regions suffering from prolonged drought such as in Sub-Saharan Africa, pacific coast of Ecuador and Peru, north coast of Colombia, north-eastern Brazil, as well as many drought-prone areas in Asia, cassava represents a food security crop when other staple food grain crops fail to produce (see Romanoff and Lynam 1992, El-Sharkawy 1993, Howeler 1995, Hillocks *et al.* 2002). For example, the short-stemmed cultivar M Col 2215 (Table 1) (later named Portoviejo 650 by INIAP, Ecuador 1992) that was selected from the preliminary screening trial, was rapidly accepted by farmers in Ecuador for its high tolerance to

Table 2. Correlation coefficients and regression equations for various plant trait combinations. *, **level of significance at 5 and 1 %, respectively. $n = 18$.

Trait combination x	 y	Correlation coeff. r	Regression equation y = a + bx
P_N	Yield	0.500^{*}	Yield = 0.178 + 0.047 P_N
PNUE	Yield	0.481^{*}	Yield = 0.605 + 0.062 PNUE
PEPC	Yield	0.547^{*}	Yield = 0.804 + 0.057 PEPC
g_m	Yield	0.479^{*}	Yield = –0.066 + 0.014 g_m
PEPC	P_N	0.597^{**}	P_N = 18.43 + 0.69 PEPC
PEPC	g_m	0.532^{*}	g_m = 83.5 + 2.0 PEPC
PEPC	PNUE	0.698^{**}	PNUE = 6.42 + 0.58 PEPC

local prolonged drought, high DM content in storage roots, and stable yield across years and locations (CIAT 1993).

Under favourable field conditions, maximum P_N in normal air ranges among cultivars of 40–50 µmol(CO_2) m^{-2} s^{-1}, with a corresponding ratio of the intercellular [CO_2] divided by atmospheric [CO_2], $C_i/C_a \approx 0.42$, indicating the high photosynthetic potential of cassava that can approach levels in typical C_4 plants (El-Sharkawy *et al.* 1992a, 1993, El-Sharkawy 2006a). Furthermore, in new leaves formed after release from water stress, P_N exceeded values in well-watered plants (CIAT 1990, El-Sharkawy 1993, Cayón *et al.* 1997). This photosynthetic capacity explains how crops water-stressed for >2–3 months at different stages of growth can quickly recover losses in dry matter production with yields approaching those in unstressed crops (Connor *et al.* 1981, Porto 1983, El-Sharkawy and Cock 1987b, El-Sharkawy *et al.* 1992b, Caicedo 1993, El-Sharkawy and Cadavid 2002, El-Sharkawy 1993, 2006a).

The preserved cassava leaf samples placed in liquid-N and assayed at the CIAT Headquarter's biochemistry laboratory had ≈30 % less PEPC activity, compared to activities in the freshly-assayed leaves at the experimental site (overall mean PEPC activity was 6.49 µmol kg^{-1}(FM) s^{-1} with cultivar ranging from 5.22 to 12.17). Sorghum PEPC activity was greatly reduced with an average activity ≈20 % of values of freshly assayed leaves. It is apparent that the enzyme was partially inactivated in preserved leaf tissues (see Hatch and Oliver 1980). These findings are of paramount importance and have implications for the viability/utility of enzyme analysis methods, and on whether they can be used in field trials located far away from analytical laboratories facilities.

Correlations of yield with photosynthetic traits: Table 2 presents correlation coefficients and regression equations for various traits. There were statistically significant ($p<0.05$) to highly significant ($p<0.01$) correlations among all traits studied. However, values were low to moderate for Yield in relation to all photosynthetic traits. This same relationship was observed across all 127 accessions, and became even stronger in medium and high top biomass cultivars. Because photon interception was much reduced in low-top cultivars, the relationship was lost (El-Sharkawy and Cock 1990, El-Sharkawy *et al.* 1990). Thus, with LAI near optimal, P_N of single upper canopy leaves could be a good indicator for yield, provided measurements are extended over a long period during crop growth including both wet and dry periods. Similar relationships were observed in sub-humid and semi-arid environments (El-Sharkawy *et al.* 1993, De Tafur *et al.* 1997b, El-Sharkawy 2006a). It appears, therefore, that the use of portable infrared gas analysis is effective in screening single-leaf P_N in field-grown large populations of germplasm and breeding lines. If possible, it should be coupled with determinations of canopy photon interception and LAI by using non-destructive methods (CIAT 1995). The product of seasonal P_N and seasonal mean LAI may be used to estimate canopy photosynthesis over a growth cycle (El-Sharkawy and De Tafur 2007).

The statistically significant correlation of Yield with PEPC activity is reported here for the first time in cassava, thus indicating the possibility of using the enzyme as a selectable trait at least in parental materials. The same correlation was also observed in leaves preserved in liquid N ($r = 0.564$, $p<0.05$, Yield = 0.86 + 0.075 PEPC). Although PEPC activity was 30 % less, compared to fresh tissue extracts, the assay may be used on preserved samples when laboratory facilities are not available at the experimental site. The importance of PEPC as a criterion in evaluating cassava germplasm in relation to productivity is further substantiated by the highly significant correlation with both P_N and PNUE (Table 2). The photosynthetic carboxylation efficiency, as roughly approximated by the calculated g_m from gas exchange measurements, was also correlated with both PEPC and Yield. Furthermore, at the level of the whole accessions, Yield was highly significantly correlated with PNUE when photon interception was not a limiting factor for canopy photosynthesis in medium and high top biomass cultivars (Fig. 1*A,B*, El-Sharkawy 2004). In two other field trials with 10 and 14 cultivars grown under rain-fed conditions in the seasonally dry and semi-arid environments of the north coast of Colombia, Yield was highly negatively correlated with intercellular CO_2 concentration (C_i) (Fig. 2, De Tafur *et al.* 1997b, El-Sharkawy 2004, 2006a). These findings clearly illustrate the importance of photosynthetic biochemical factors such as enzyme amount and activity in controlling both the P_N and Yield of cassava. Since RuBPCO constitutes a major portion (≈40–50 %) of the soluble leaf protein in C_3 and C_3-C_4 plants, it seems advantageous to evaluate cassava germplasm as well as wild *Manihot* species for variations in percent protein invested in PEPC relative to RuBCO. Based on the observed direct relationship of PEPC activity with P_N and PNUE, it is anticipated that a higher percent protein invested in cassava PEPC might enhance further crop productivity. Two wild species, *M. grahami* and *M. rubricaulis*, had high P_N , high PEPC activity, and a second palisade layer at the lower side of leaves, thus they may be used in further research along with other wild species (El-Sharkawy 2004, 2006a).

There was a close relationship between P_N measured in the 1986–7 crop and Yield in the 1988–9 crop for a group of cultivars selected from the preliminary screening trial on the basis of their yield performance, including 14 cultivars used in the PEPC assays (Fig. 3, El-Sharkawy *et al.* 1990). Moreover, P_N measured in the 1986–7 crop using the infrared gas analyser in a closed mode (*LI-6000*) over an extended crop growth period was significantly correlated with P_N measured only once in the 1988–9 crop with an open system (*LCA-2*, *Analytical Development Co.*, Hoddeson, Great Britain) ($r = 0.580$, $p<0.05$). These findings confirm the genetic basis of the variations observed in leaf photosynthetic capacity and its direct relation with yield in cassava.

From the research presented here and in previous related published reports on cassava photosynthesis in relation to productivity, it is reasonable to conclude that breeding strategy must include P_N as a fundamental criterion in evaluating the potential productivity of a crop

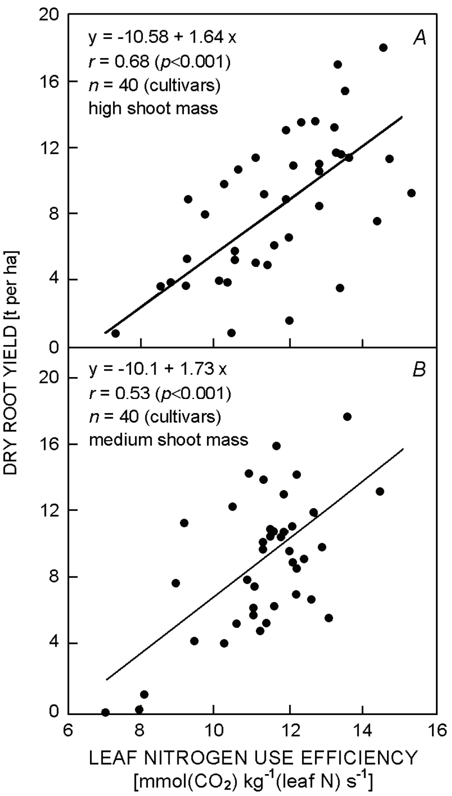

Fig. 1. Relationship between dry root yield and leaf photosynthetic nitrogen use efficiency (PNUE) in field-grown cassava. PNUE values were calculated from leaf CO_2 exchange measurements and total leaf N contents. Cultivars with high (*A*) or medium (*B*) shoot mass and large LAI. Figure constructed from data of El-Sharkawy *et al.* (1990) – see El-Sharkawy (2004).

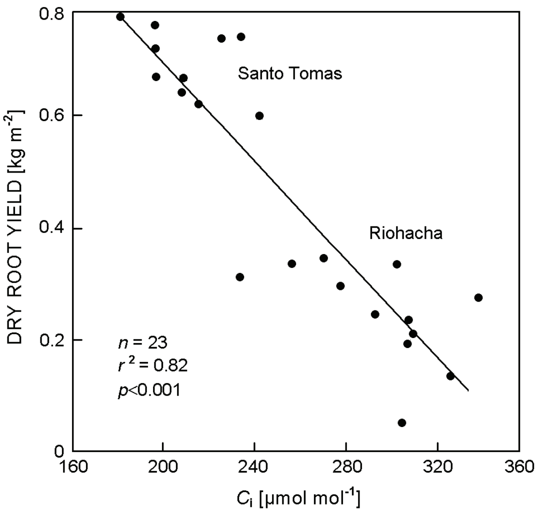

Fig. 2. Relationship between dry root yield and intercellular CO_2 concentration (C_i) for two groups of cassava cultivars grown under rain-fed conditions at Riohacha (semi-arid) and Santo Tomas (seasonally dry). The C_i values were calculated from leaf gas fluxes *via* standard Gaastra equations: the higher photosynthetic rates, the lower C_i values. Regression equation: Yield = 1.520 – 0.004 C_i; $r^2 = 0.82$ ($p<0.001$). (From De Tafur *et al.* 1997b.)

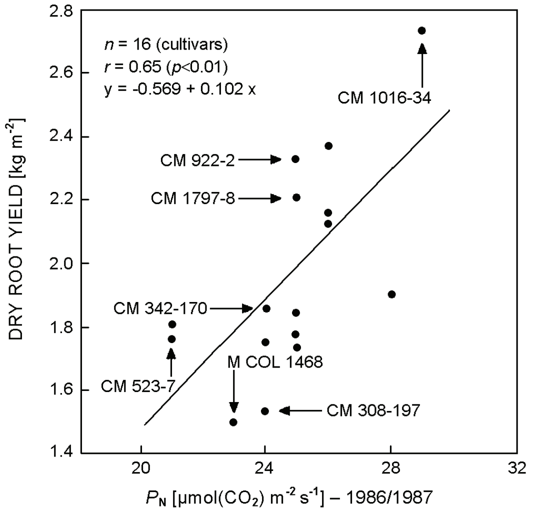

Fig. 3. Relationship between the 1988/1989 dry root yield and the single-leaf photosynthesis measured in the 1986/1987 season. Arrows indicate cultivars listed in Table 1. (From El-Sharkawy *et al.* 1990.)

under a range of diverse environments where the crop is normally cultivated. The three methods applied in evaluating such relations are: (*1*) the infrared gas analysis using portable equipments in the field was reliable and manageable for evaluating large breeding materials; (*2*) the biochemical assay for photosynthetic enzymes such as PEPC and RuBPCO is viable for detecting genotypic variations related to leaf photosynthetic efficiency as well as to yield. Its application, however, is more complicated in the field where nearby biochemical laboratories are not available. Preserved leaf samples might be used if they are calibrated against fresh samples; (*3*) the analysis of leaf carbon isotope composition ($\delta^{13}C$) is a powerful technique for studying long-term crop-water relations, and perhaps for detecting genotypic differences in long-term crop water use efficiency. A combination of this with leaf gas exchange measurements in the field is recommended (El-Sharkawy and De Tafur 2007).

A final conclusion directed to young scientists, donors, and research managers is that, "crop physiology has proven its utility, at least in cassava research done with limited financial resources from donors and under antagonistic and counterproductive short-sighted decisions from 'research managers' " (see El-Sharkawy 2005, 2006a,b). Physiological research at CIAT, in a relatively short time, has led to many important discoveries related to crop productivity and water relations in the field. These discoveries have significant implications for solving the so many problems encountered by the 'resource-poor' cassava farmers across tropical and subtropical countries in Africa, Asia, and Latin America".

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X.F. Yi, Z.B. Zhang: Influence of insect-infested cotyledons on early seedling growth of Mongolian oak, *Quercus mongolica* − Photosynthetica 46: 139-142, 2008.

Please, replace *Acknowledgements* on p. 139 with the following correct version:

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Y. Kitahashi, T. Ichie, Y. Maruyama, T. Kenzo, S. Kitaoka, S. Matsuki, L. Chong, T. Nakashizuka, T. Koike: Photosynthetic water use efficiency in tree crowns of *Shorea beccariana* and *Dryobalanops aromatica* in a tropical rain forest in Sarawak, East Malaysia. − Photosynthetica 46: 151-155, 2008.

Please, replace Table 1 on p. 152 with the following correct version:

Table 1. Species differences, differences between upper and lower parts of the crown, and diurnal differences in photosynthetic water use efficiency, PWUE [μmol mmol^{-1}], stomatal conductance, g_s [kmol m^{-2} s^{-1}], xylem water potential, Ψ_{xylem} [MPa], specific leaf area, SLA [m^2 kg^{-1}], leaf nitrogen content [g kg^{-1}], leaf area [cm^2], and leaf thickness [mm]. Means±SD. Statistical differences between morning and midday values of PWUE and g_s were significant at $p<0.5$ with the exception of lower PWUE of *Dryobalanops*. At the same p, only midday Ψ_{xylem} differences of upper and lower crowns were significant. No other differences of characteristics within the crown were significantly different.

	Shorea		*Dryobalanops*	
	upper	lower	upper	lower
PWUE morning	40.80±2.16	47.06±3.26	42.60±2.67	49.26±3.53
PWUE midday	57.76±4.84	52.68±2.74	55.61±2.70	50.19±5.19
g_s morning	2.98±0.40	2.65±0.15	2.17±0.12	1.81±0.11
g_s midday	1.12±0.10	1.78±0.07	1.10±0.15	1.52±0.14
Ψ_{xylem} predawn	–0.357±0.063	–0.325±0.082	–0.491±0.057	–0.464±0.074
Ψ_{xylem} midday	–1.331±0.144	–0.958±0.152	–1.109±0.112	–0.701±0.078
SLA	43.1±4.4	42.4±4.9	58.9±7.5	55.2±5.4
N content	13.48±1.02	11.67±1.06	11.36±1.42	11.04±1.15
Leaf area	40.18±3.20	41.80±3.80	6.16±0.40	6.32±0.50
Leaf thickness	0.457±0.050	0.452±0.050	0.303±0.020	0.299±0.020

The publisher and authors apologize for this errors and for any inconvenience it may have caused.