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REVIEW

International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics

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Abstract

The review sums up research conducted at CIAT within a multidiscipline effort revolving around a strategy for developing improved technologies to increase and sustain cassava productivity, as well as conserving natural resources in the various eco-edaphic zones where the crop is grown, with emphasis on stressful environments. Field research has elucidated several physiological plant mechanisms underlying potentially high productivity under favourable hot-humid environments in the tropics. Most notable is cassava inherent high capacity to assimilate carbon in near optimum environments that correlates with both biological productivity and root yield across a wide range of germplasm grown in diverse environments. Cassava leaves possess elevated activities of the C_4 phospho*enol*pyruvate carboxylase (PEPC) that also correlate with leaf net photosynthetic rate (P_N) in field-grown plants, indicating the importance of selection for high P_N. Under certain conditions such leaves exhibit an interesting photosynthetic C_3-C_4 intermediate behaviour which may have important implications in future selection efforts. In addition to leaf P_N, yield is correlated with seasonal mean leaf area index (*i.e.* leaf area duration, LAD). Under prolonged water shortages in seasonally dry and semiarid zones, the crop, once established, tolerates stress and produces reasonably well compared to other food crops (*e.g.* in semiarid environments with less than 700 mm of annual rain, improved cultivars can yield over 3 t ha^{-1} oven-dried storage roots). The underlying mechanisms for such tolerance include stomatal sensitivity to atmospheric and edaphic water deficits, coupled with deep rooting capacities that prevent severe leaf dehydration, *i.e.* stress avoidance mechanisms, and reduced leaf canopy with reasonable photosynthesis over the leaf life span. Another stress-mitigating plant trait is the capacity to recover from stress, once water is available, by forming new leaves with even higher P_N, compared to those in non-stressed crops. Under extended stress, reductions are larger in shoot biomass than in storage root, resulting in higher harvest indices. Cassava conserves water by slowly depleting available water from deep soil layers, leading to higher seasonal crop water-use and nutrient-use efficiencies. In dry environments LAD and resistance to pests and diseases are critical for sustainable yields. In semiarid zones the crop survives but requires a second wet cycle to achieve high yields and high dry matter contents in storage roots. Selection and breeding for early bulking and for medium/short-stemmed cultivars is advantageous under semiarid conditions. When grown in cooler zones such as in tropical high altitudes and in low-land sub-tropics, leaf P_N is greatly reduced and growth is slower. Thus, the crop requires longer period for a reasonable productivity. There is a need to select and breed for more cold-tolerant genotypes. Selection of parental materials for tolerance to water stress and infertile soils has resulted in breeding improved germplasm adapted to both favourable and stressful environments.

Additional key words: breeding; canopy; enzymes; growth; leaf; *Manihot*; nutrients; photosynthesis; productivity; root; soil; stress; water; yield.

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Introduction

Cassava (manioc, yuca, or mandioca: *Manihot esculenta* Crantz, Euphorbiaceae), is widely grown for its starchy roots as a staple food and animal feed in countries of tropical and sub-tropical Africa, Asia, and Latin America, between latitudes 30 N and S and from sea level to just above 2 000 m a.s.l. The production areas for cassava are almost entirely confined to developing tropical and sub-tropical countries with over 40 % of world production occurring in sub-Saharan Africa. It is grown mostly by small, resource-limited farmers on marginal and highly eroded low-fertility acidic soils, and virtually without applications of agrochemicals (El-Sharkawy 1993, 2004, Ruppenthal *et al.* 1997). Because of its inherent tolerance to various edapho-climatic stresses, the crop has recently been expanding into more marginal lands, particularly in sub-Saharan Africa (Romanoff and Lynam 1992), where other staple food crops are failing to produce reasonably because of the increasing degradation of these marginal African ecosystems (El-Sharkawy 1993, De Tafur *et al.* 1997b, Cadavid *et al.* 1998, Flörchinger *et al.* 2000). Cassava storage roots are used as a source of saccharides (protein is less than 3 % in dry roots) mainly for human consumption, whether prepared fresh (in the case of sweet cultivars low in cyanogenic glycosides) or after processing into dry products such as flour, starch, and animal feed (in the case of bitter cultivars high in cyanogenic glycosides, see Durfour 1988, Essers 1995, Balagopalan 2002, Westby 2002). Because of perishability after harvest (van Oirschot *et al.* 2000), the roots have to be used immediately or processed into dry products, though pruning of tops three weeks before harvest reduces the rate of deterioration. Irrespective of these attributes and potential productivity, the crop had received little attention prior to about 1970 from policy makers as well as researchers in the developing countries where the crop is widely grown. Early attention to the crop was some limited work by western colonial communities in parts of Africa, Asia, and Latin America during the first half of the 20th century (Verteuil 1917, 1918, Nijholt 1935, Cours 1951, James 1959, Hunt *et al*. 1977, Cock 1985).

Although it is the most important food source for saccharides after rice, sugarcane, and maize for over 500 million people in the developing countries within the tropical and subtropical belt, the crop was sidestepped by the so-called 'Green Revolution', as a result of the International Agricultural Research Centres effort in the 1960s, which focused on improvement of the main cereal crops such as wheat, rice, and maize, with the help of international agricultural development and research agencies supported by a few western donors. For that reason, the Consultative Group on International Agricultural Research (CGIAR) that was established in 1971 under the sponsorship of the World Bank, UNDP, and FAO (Wortman 1981), gave high priority to research on other crops including cassava, as well as on production ecosystems in the humid tropics of Africa (*via* the International Institute of Tropical Agriculture, IITA, located in Nigeria) and in South America (*via* the Centro Internacional de Agricultura Tropical, CIAT, located in Colombia). Given the financial support needed, core teams of international multidisciplinary scientists were able, for the first time, to conduct extensive research on cassava, in collaboration with the already existing few national research programs, through improvements in germplasm collection and characterization, breeding, agronomic, and cropping system management practices, control of pests and diseases, and utilization of the crop, based upon a better understanding of the physiological processes involved. Recent publications (Hillocks *et al*. 2002, Kawano 2003) review results on the many aspects of cassava research in the last three decades in Africa, Asia, and Latin America.

I review here research, both published and unpublished, conducted at CIAT over more than 15 years on cassava photosynthesis, productivity, physiology, and eco-physiology in response to the environmental stresses normally encountered in the tropics. The review addresses a need for assembling and integrating this dispersed information for interest of scientists in general and those concerned with cassava in particular. Focuses are on the strategy adopted for the improvement of the crop, taking into account the conditions faced by the cassava growers lacking high production input resources, in contrast to the capital-intensive practices used in the Green Revolution crops. Original results have been regularly documented and reported in progress annual reports that were exchanged across countries (*e.g*. CIAT 1983–1998), some of which have been published in peer-reviewed technical journals, reviews, student theses, proceedings, and books (*e.g*. Porto 1983, El-Sharkawy 1990, 1993, 2004, 2005, El-Sharkawy and Cock 1984, 1986, 1987a,b, 1990, El-Sharkawy *et al.* 1984a,b,c,d, 1985, 1990, 1992a,b, 1993, 1998a,b, Cock *et al.* 1985, 1987, Guzman 1989, Cock and El-Sharkawy 1988a,b, Bernal 1991, Hershey and Jennings 1992, Caicedo 1993, López *et al.* 1993, Pellet and El-Sharkawy 1993a,b, 1994, 1997, Tenjo *et al*. 1993, Tscherning *et al*. 1995, Cayón *et al*. 1997, De Tafur *et al*. 1997a,b, Cadavid *et al*. 1998, Flörchinger *et al*. 2000, El-Sharkawy and Cadavid 2000, 2002).

Cassava research strategy at CIAT

The multidisciplinary cassava program established in the early 1970s with a global mandate for cassava within the CGIAR system, centred its research strategy on collecting, conserving, and characterizing the world-wide available germplasm which came mainly from Latin America, and on selecting and breeding more broadly-adapted germplasm for the various environments prevailing in the tropics and sub-tropics in both Latin America and Asia. At an early stage, breeding objectives were directed toward developing high yielding cultivars under favourable conditions in absence of biotic and abiotic stresses (Kawano *et al.* 1978, Cock *et al.* 1979). This strategy was based mainly on the selection for high yield per unit land area (in contrast to traditional vigorous cultivars/landraces suited for intercropping), high dry matter content (*i.e.* high starch content) in the storage roots and harvest indices (HI) (HI = root yield/total plant biomass) higher than the 0.5 or less found in most of the low-yielding traditional cultivars and land races (Kawano 1990, 2003). This early work showed that cassava germplasm is genetically diverse with a high potential for productivity under near optimal environments.

Because most cassava production occurs in environments having various degrees of stress and with little or no production inputs by resource-limited small farmers, goals of the more recent breeding strategy have centred on selecting and developing cultivars with reasonable and stable yields and the ability to adapt to a wide range of biotic and abiotic stresses (Kawano *et al.* 1978, Hershey 1984, Hershey *et al.* 1988, Hershey and Jennings 1992, Jennings and Iglesias 2002, Kawano 2003). This strategy was further stimulated by the inherent capacity of cassava to tolerate adverse environments, particularly where other main staple food crops such as cereals and grain legumes would fail to produce, and by avoiding or reducing the negative consequences on the environment caused by adopting high-input agro-chemically-aided production systems (El-Sharkawy 1993). The strategy took advantage of the wide genetic diversity within the more than 5 000 accessions conserved at CIAT headquarters in the Cauca Valley, mostly of Latin American origin, as well as the diversity of edapho-climatic conditions with high pressures of pests and diseases that exist in various eco-zones throughout Colombia and are representative of most cassava production ecosystems in the tropics and sub-tropics (Hershey and Jennings 1992, El-Sharkawy 1993). In light of this environmentally sound breeding strategy, research on cassava physiology has focused on investigating both basic and applied aspects of the crop under the prevailing environments, mainly in the field, to understand and elucidate better the characteristics and mechanisms underlying productivity and tolerance to stresses (Cock and El-Sharkawy 1988a,b, El-Sharkawy 1993, 2004). The objectives included: (*1*) characterization of the core germplasm for tolerance to extended water shortages, either natural or imposed, and to low-fertility soils, (*2*) investigation of leaf photosynthetic potential in relation to productivity under various edapho-climatic conditions, and (*3*) identification of plant traits that might be of use in breeding programs. Moreover, the multidisciplinary research approach we adopted was pivotal in achieving these objectives.

Responses of cassava leaf gas exchanges to environment

Air humidity and water stress: Under controlled laboratory conditions it was observed that when leaves of both well-watered and water-stressed cassava plants, grown outdoors, were exposed to high air humidity in leaf chambers and then to a short period of low humidity, net photosynthetic rates (P_N) measured with an infra-red gas exchange system at saturating photosynthetic photon flux density (PPFD) and at ambient [CO_2] sharply declined and the response was more pronounced in stressed plants (Fig. 1, El-Sharkawy and Cock 1984). This effect of short term exposure to dry air was completely reversible, a reaction that was also observed in several woody species (Davies and Kozlowski 1974), but was only partially reversible after much longer exposure to dry air, resulting in an about 80 % reduction in leaf photosynthesis. The terminal leaf water potential (Ψ_L) of the tested lobes (as measured with a pressure chamber and compared with that of free lobes on the same leaf) in both plants remained unchanged during the several hours of gas exchange monitoring, thus indicating a direct response of cassava stomata to changes in air humidity. This response was previously termed a feed-forward reaction (see Cowan 1977, Farquhar 1978) that differs from the feedback response to changes in bulk Ψ_L. However, since we did not determine abscisic acid (ABA) contents in leaves in this case, it was not clear if ABA played a role in stomatal closure during this short exposure to dry air without changes in leaf Ψ_L (see Henson 1984a,b).

When leaves were exposed to stepwise rises in leaf-to-air vapour pressure deficits (VPD), steady P_N remained stable in the range of 1.0–1.5 kPa, then rapidly declined above that range (Fig. 2*A*). Transpiration rate (*E*) initially increased with rising VPD up to 2 kPa, then levelled off or declined with further increases in VPD (Fig. 2*B*, and Berg *et al.* 1986, El-Sharkawy 1990). Leaf conductance (g_s) also declined sharply at VPD higher than 2 kPa (Fig. 2*C*). These observations showed that cassava was sensitive to changes in atmospheric humidity irrespective of both plant and soil water status. Furthermore, compared to several woody and herbaceous species, cassava

was more sensitive to changes in air humidity (El-Sharkawy *et al.* 1984d, 1985, El-Sharkawy and Cock 1986, Cock and El-Sharkawy 1988b), and the response was related to stomatal density and maximum g_s (El-Sharkawy *et al.* 1984d, 1985, El-Sharkawy and Cock 1986). Cassava leaves possess large number of stomata on the abaxial epidermis (>400 stomata mm^{-2}, see Pereira 1977, Connor and Palta 1981, El-Sharkawy *et al.* 1984a, Guzman 1989) that may underlie its strong response to humidity (El-Sharkawy *et al.* 1985, El-Sharkawy and Cock 1986).

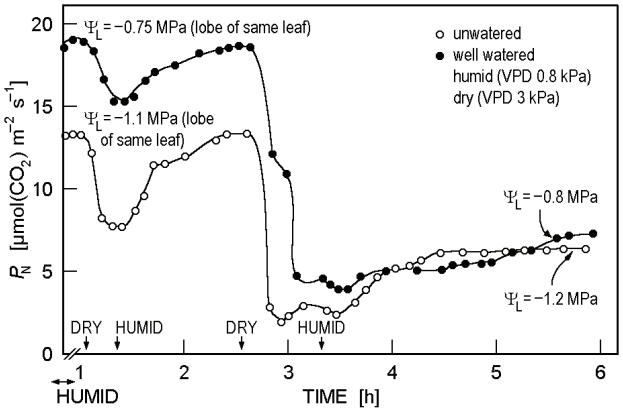

Fig. 1. Response of net leaf photosynthetic rate (P_N) to changes in air humidity. Plants of cv. M Col 88 grown in 40 000 cm^3 pots were left in the open throughout the growing period; the well-watered plants were regularly irrigated whenever needed. Pots of un-watered plants were covered at soil level with plastic covers 33 d after planting to exclude rain water. P_N was measured on fully expanded, young, attached leaves under controlled laboratory conditions at saturating photon flux density and normal air using differential multi-channel open end infra-red gas analyzer (El-Sharkawy and Cock 1984).

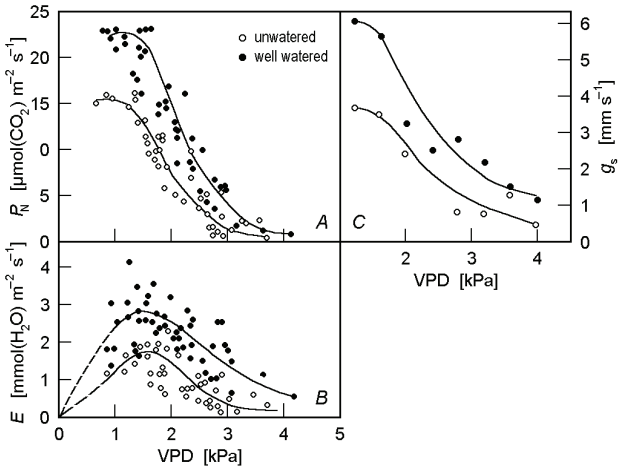

Fig. 2. Responses of leaf net photosynthetic rate, P_N (*A*), transpiration rate, *E* (*B*), and leaf conductance to water vapour, g_s (*C*) to stepwise increases in leaf-to-air vapour pressure deficit (VPD) in cv. M Col 90. Growth conditions and gas measurements as in Fig. 1 (El-Sharkawy and Cock 1984).

The phenomenon of direct stomatal response to low air humidity (irrespective of water supply to the root system or leaf Ψ_L) was observed as early as the late 19th and early 20th centuries by botanists (see Haberlandt 1914, Thoday 1938, El-Sharkawy and Cock 1986). Numerous more recent reports showed that several herbaceous and woody plant species tended to close their stomata in response to dry air whether within a plant community or in attached leaves or isolated epidermal strips (*e.g.* Hoffman and Rawlins 1971, Hoffman *et al.* 1971, Lange *et al.* 1971, Schulze *et al.* 1972, Aston 1976, Hall and Hoffman 1976, Rawson *et al.* 1977, Sheriff and Kaye 1977, Lösch 1977, 1979, Lösch and Schenk 1978, Tibbitts 1979, Ludlow and Ibaraki 1979, Lösch and Tenhunen 1981, Farquhar *et al.* 1980, Jarvis 1980, Tazaki *et al.* 1980, Hall and Schulze 1980, Bunce 1981, 1982, 1984, Leverenz 1981, Fanjul and Jones 1982, Meinzer 1982, Kaufmann 1982, Schulze and Hall 1982, Gollan *et al.* 1985, Körner 1985, Körner and Bannister 1985, Schulze 1986, Jarvis and McNaughton 1986, Ward and Bunce 1986, Bongi *et al.* 1987, Hirasawa *et al.* 1988, Pettigrew *et al.* 1990, Held 1991, Kappen and Haeger 1991, Tinoco-Ojanguren and Pearcy 1993). This apparently widespread phenomenon indicated the need for further detailed studies, and for its inclusion in plant community/environment ecosystem models (Jarvis and McNaughton 1986).

Mechanisms underlying stomatal response to changes in air humidity: The possible mechanisms underlying stomatal sensing of changes in air humidity and the role of the so-called 'peristomatal transpiration' (first hypothesized by Seybold 1961/1962), that is water loss from the cuticle of the guard cells and subsidiary cells and their adjacent epidermal cells, in the control of stomatal movement, was often reviewed (see Meidner and Mansfield 1968, Lange *et al.* 1971, Meidner 1976, Sheriff 1977, 1979, 1984, Lösch and Schenk 1978, Maier-Maercker 1979a,b, 1983, Tyree and Yianoulis 1980, Lösch and Tenhunen 1981, Zeiger 1983). Support for Seybold's hypothesis on the role of peristomatal transpiration was demonstrated *via* extensive research by the German workers using intact leaves and isolated epidermal strip systems without water stress (*e.g.* Lange *et al.* 1971, Maier-Maercker 1979a,b, 1983, Lösch and Tenhunen 1981). Others (Meidner and Mansfield 1968) argued that stomatal movements are unlikely to be affected by changes in atmospheric humidity, but primarily by water status of mesophyll tissue (feed-back reaction). Kramer (1983) cautioned against the proposed role of peristomatal transpiration until more information was available concerning the degree of cutinisation of mesophyll tissue (where the bulk of evaporation presumably takes place) and of the epidermal and inner/external walls of guard cells. Appleby and Davies (1983) demonstrated possible sites of evaporation in cuticle-free areas in the guard cell walls of oak (*Quercus robur*), poplar (*Populus*

nigra), and pine (*Pinus sylvestris*). These areas would be exposed on the outside of the leaf during stomatal closure in dry air. Also, Körner and Cochrane (1985) reported lesser degrees of cutinisation of the external walls of guard cells in *Eucalyptus pauciflora* that may underlie its stomatal sensitivity to changes in air humidity.

Sheriff (1977, 1979, 1984) suggested that the mechanism underlying direct stomatal response to low humidity involves both evaporation from the epidermis and lower hydraulic conductivity within the leaf that may accelerate water stress in the epidermis regardless of leaf water content. Tyree and Yanoulis (1980) used physical models of sub-stomatal cavities to calculate water vapour diffusion patterns and concluded that high evaporation from the guard cells could close stomata in direct response to low humidity. They suggested that localized water stress or dehydration in guard cells may take place as a result of a high leaf resistance to flow of liquid water from the minor leaf veins to the guard cells. The strong association between stomatal density (*i.e*. exposed epidermal areas) and the degree of sensitivity to changes in air humidity that was observed in well-watered plants across many herbaceous and woody species (El-Sharkawy *et al.* 1985) may indicate the occurrence of localized dehydration in the stomatal apparatus and adjacent exposed epidermal cells, hence supporting the role of peristomatal transpiration in controlling stomatal movement. Moreover, the poor physical connection between the numerous stomatal areas (where evaporation may take place) and the mesophyll tissue observed in cassava leaf (see El-Sharkawy and Cock 1986) could accelerate water stress in the epidermis and stomatal apparatus, thus leading to the striking sensitivity to changes in atmospheric humidity without noticeable decreases in bulk leaf water potential (Figs. 1–3, see also Connor and Palta 1981, Porto 1983, El-Sharkawy *et al.* 1984d, 1992b, El-Sharkawy and Cock 1986, El-Sharkawy 1990, Cayón *et al*. 1997, De Tafur *et al.* 1997a). This conclusion was further substantiated by the closure of stomata in field-grown cassava in response to high wind speed, despite wet soil and high bulk leaf water potential conditions (El-Sharkawy 1990). Bunce (1985) also reported larger water loss under high wind speed from the outer surface of the epidermis of herbaceous species, providing further evidence in support of peristomatal transpiration.

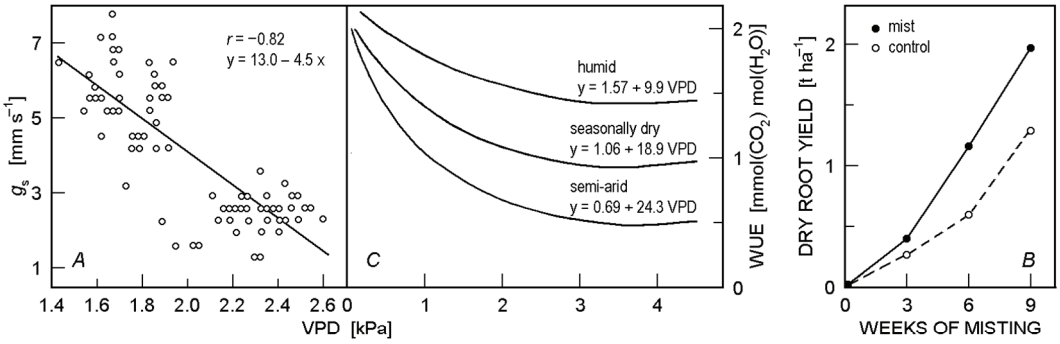

Fig. 3. (*A*) Response of stomatal conductance (g_s) to vapour pressure deficit (VPD) in field-grown cassava at the Carimagua experimental station (ICA-CIAT), Meta Dept., Colombia. Soil water was near field capacity (*i.e*. 32 % by volume). Data points represent single leaves, measured on the abaxial surface, with a diffusion porometer (*MKII* type *APII*, *Delta T Devices*, England). Measurements were made at 10:00–11:00 and 13:00–14:00 h local time. The bulk leaf water potential, as measured with the standard pressure chamber technique, varied between –1.3 and –1.4 MPa during the measurement period (El-Sharkawy 1990). (*B*) Oven-dried storage root yield (cv. M Col 1684) at periodic harvests after 3, 6, and 9 weeks of misting. Ages of plants at harvests were 65, 85, and 105 d, respectively. The differences in yield between the two crops (grown in large plots with total area >2 000 m^2) were significant at all harvests (p<0.01). Top biomass and leaf area index were not different between the two crops, while total biomass was significantly larger after 6 and 9 weeks of misting (Cock *et al.* 1985, El-Sharkawy and Cock 1986). (*C*) Response of leaf water use efficiency (WUE) to vapour pressure deficit (VPD) in field-grown cassava in mid-altitude warm sub-humid climate. Measurements of gas exchanges in upper canopy attached leaves were always made between 09:00 and 13:00 h local time, when PPFD always exceeded 1 000 µmol(photon) m^{-2} s^{-1} using portable infra-red gas analyzer. VPD progressively increased from morning to midday. Thirty three clones were evaluated and were grouped into humid, sub-humid/seasonally dry, and semiarid habitats. Sensitivity to VPD increased from humid to semiarid habitat. Differences between plant groups illustrate the genetic diversity within cassava germplasm in response to changes in atmospheric humidity (El-Sharkawy 2004, M.A. El-Sharkawy, M.C. Amézquita, H.F. Ramirez, and G. Lema, unpublished).

Responses of cassava to changes in air humidity in field-grown plants and its implication for breeding strategy: Stomatal sensitivity to changes in atmospheric humidity was also observed in field-grown cassava in wet soils at different locations (Cock *et al.* 1985, Berg *et al.* 1986, El-Sharkawy 1990, and Fig. 3*A*). Over a range of cultivars representing the core cassava germplasm from different habitats and grown at a mid-altitude location, significant differences in stomatal sensitivity to humidity were observed among cultivars (El-Sharkawy 2004, and Fig. 3*C*). Furthermore, total biomass and storage root yield were larger in high humidity environments

enhanced by misting and correlating with higher leaf photosynthetic rates (Cock *et al.* 1985, El-Sharkawy and Cock 1986, and Fig. 3*B*). These findings illustrate that stomatal sensitivity to changes in air humidity is translated into crop canopy, and hence to productivity, and have important practical implications for cassava breeding for different ecosystems and edapho-climatic zones. In wet/humid zones such as the Amazonian basin, equatorial west Africa, west Java in Indonesia, and in zones with short intermittent water deficits, less sensitive cultivars (with lower stomatal density on the abaxial surface of hypostomatous leaves and/or with amphistomatous leaves with equal leaf surface conductances) should be bred for to maximize productivity, as optimizing water use efficiency (WUE) is not of importance in this case (El-Sharkawy and Cock 1986, El-Sharkawy 2004). On the other hand, in sub-humid/seasonally dry and semi-arid zones where there are prolonged periods of water deficits (>3 months), it is advantageous to breed and select for more sensitive cultivars in order to conserve and deplete slowly the limited soil water, thus optimizing WUE rather than maximizing productivity, over a longer period during the growth cycle.

Since new leaf formation is very restricted under prolonged drought (Connor and Cock 1981, Porto 1983, El-Sharkawy and Cock 1987b, El-Sharkawy *et al.* 1992b), higher degrees of stomatal sensitivity should be combined with longer leaf retention (*i.e.* longer leaf life, El-Sharkawy 1993, 2004). Recently, cassava leaf retention was found to be positively correlated, over a wide range of cultivars and breeding lines, with productivity under naturally extended water deficits (Lenis *et al.* 2006). Moreover, leaves of plants subjected to imposed prolonged water stress (>2 months) in the field in sub-humid zones had 40 % of P_N in well-watered plants and were capable of completely recovering after termination of stress (CIAT 1987–1994, El-Sharkawy 1993). Selection for longer leaf life span is advantageous in saving dry matter already invested in leaf canopy formation (see Chabot and Hicks 1982), thus resulting in more assimilates diverted toward storage roots that would result in higher HI and harvestable yield (Cock and El-Sharkawy 1988a, El-Sharkawy 1993).

De Tafur *et al.* (1997b) reported a wide range of variations among rain-fed cassava in P_N, as measured in the field during the driest months. In the seasonally dry zone, P_N ranged from 27 to 31 μmol(CO_2) m^{-2} s^{-1}, with significant differences among cultivars, and in the semi-arid zone, P_N was 7–20 μmol(CO_2) m^{-2} s^{-1}, with significant difference among cultivars. This genetic variation should be exploited in breeding improved genotypes. In this case, host-plant tolerance/resistance for pests and diseases must be incorporated in cultivars targeted to seasonally dry and semi-arid zones in order to maintain as long as possible a functioning leaf canopy (Byrne *et al.* 1982, Hershey and Jennings 1992, Bellotti 2002, Calvert and Thresh 2002, Hillocks and Wydra 2002).

Screening for stomatal characteristics in large populations: Although the great majority of cassava cultivars possess large number of stomata only on the abaxial leaf surface, there is also a wide genetic diversity in stomatal density within cassava germplasm with a small percentage possessing amphistomatous leaves that may be used in breeding programs. Several accessions with significant number of stomata on the adaxial surface have been identified (less than 5 % of more than 1 500 land races and cultivars that were screened in the field using transient porometer techniques and microscopic observations on replicas of leaf surfaces made by spraying leaves with collodion solution) (El-Sharkawy *et al.* 1984a, 1985, Guzman 1989). Moreover, several wild *Manihot* species (*e.g. M. rubricaulis, M. grahami, M. guaranitica, M. crassisepala, M. chlorosticta* – El-Sharkawy 2004, C. Castillo, J. Mayer, M.A. El-Sharkawy, unpublished) were found to possess amphistomatous leaves. Both porometry (Kirkham 2005) and leaf surface replicas combined with microscopic observations are easy to use for screening large numbers of breeding progeny in the field for stomatal characterization, though the leaf replica method has some limitations in the case of hairy leaves and sunken stomata (see North 1956, Slavík 1971). The leaf surface replica method was effective on tissue-cultured young seedlings (El-Sharkawy *et al.* 1984a), and this may facilitate early screening of large populations. Zelitch (1962) described similar techniques using silicon rubber combined with cellulose acetate solution for obtaining stomatal impressions.

Responses to temperature: Potted cassava grown outdoors at high-altitude cool climate and at mid-altitude warm climate: Cassava requires a warm climate for both optimal growth and productivity, but it is also cultivated in cool climates at high altitudes in the tropics (>1 700 m elevations) and at low altitudes in the subtropics (Irikura *et al.* 1979). As the growth and productivity depend largely on leaf canopy capacity to intercept solar radiation during most of the growth cycle and on leaf photosynthetic potential and performance under prevailing field conditions (see Cock *et al.* 1979, El-Sharkawy *et al.* 1990, De Tafur *et al.* 1997a,b, El-Sharkawy 2004), it was warranted to study the effects of temperature during growth on leaf photosynthesis. To get at temperature differences under natural conditions we took advantage of the closeness of a high altitude site (elevation 2 000 m, 17 °C mean annual temperature, located at 18-km distance from CIAT Headquarters which is located at about 965 m a.s.l., mean annual temperature 23.8 °C). Several cultivars representing various habitats were grown in large pots (40 000 cm^3) filled with a mixture by mass of 40 % top soil, 33 % compost, 27 % sand, and were adequately fertilized. The plants were grown in the open and well-watered throughout their period of growth at the high altitude location where duration and solar irradiation were similar to the mid-

altitude site at CIAT Headquarter (see details in El-Sharkawy *et al.* 1992a, 1993).

Measurements of leaf gas exchanges were made under laboratory-controlled conditions using an infrared CO_2 analyzer, to test responses to leaf temperature at saturating PPFD (>1 800 µmol m^{-2} s^{-1}) and also responses to PPFD. Fig. 4*A–C* (for more information see El-Sharkawy and Cock 1990, El-Sharkawy *et al.* 1984c, 1992a, 1993) illustrates responses of leaves developed in the cool climate and then acclimated for seven days in the warm climate, and of leaves of the same plants that were later developed in the warm climate, in two cultivars from contrasting habitats. In both cultivars, leaf P_N was substantially lower in leaves that had developed in the cool high-altitude climate than in leaves developed in the mid-altitude warm climate (Fig. 4*A,B*). Leaves that developed in the cool climate and then acclimated for seven days in warm climate partially recovered their photosynthetic capacities but P_N remained much lower than that in leaves developed in the warm climate. In the hot-climate cultivar (M Bra 12, from lowland areas in Brazil) maximum P_N in all sets of leaves was higher than in the cool-climate cultivar (M Col 2059, from high altitude areas in Colombia). This trend was also observed at all leaf temperatures. A broad temperature optimum of 25–40 °C with peaks at 30–35 °C was observed in the hot-climate cultivar for all sets of leaves, while in the cool-climate cultivar there was an apparent upward shift in optimum temperature in both the acclimated and warm-climate leaves compared to a wide plateau in the non-acclimated, cool-climate leaves. In both cultivars and in all sets of leaves P_N declined rapidly at temperatures higher than 40 °C reaching zero at 50 °C.

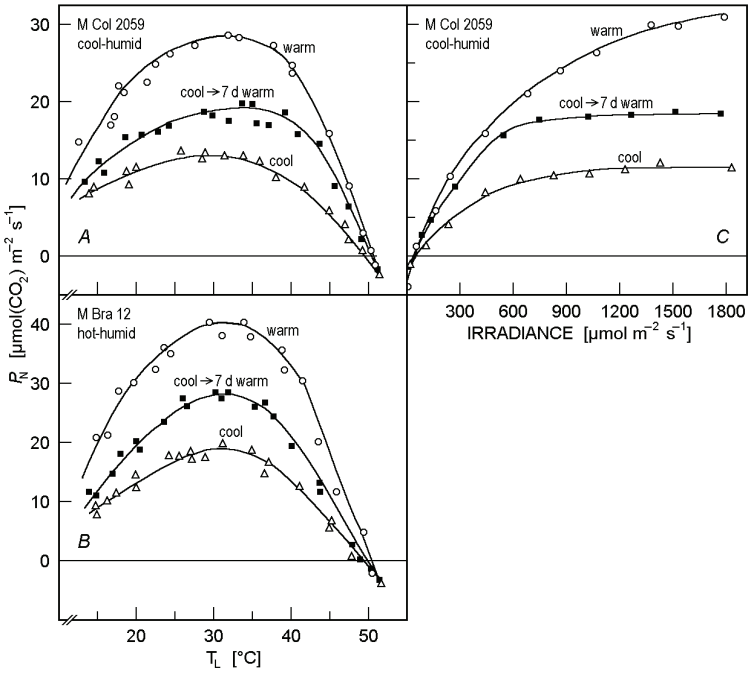

Fig. 4. Responses of net photosynthetic rate (P_N) to leaf temperature (T_L) in cv. M Col 2059 show the apparent upward shift in optimum temperature from cool to warm-acclimated and warm climate leaves (*A*), and the lack of photon saturation in warm climate leaves as compared to cool and warm-acclimated leaves (*C*). Higher maximum P_N was found in all sets of leaves of cv. M Bra 12 from hot-humid habitat (*B*) compared to the cool-climate cv. M Col 2059 (*A, C*) (CIAT 1992, El-Sharkawy *et al.* 1992a, 1993).

During seven days of acclimation in the warm climate, changes in non-stomatal components of photosynthesis (photosystems 1 and 2, and CO_2 fixation reactions) are more likely than changes in physical stomatal characteristics (see Berry and Björkman 1980). Moreover, P_N in the cool-climate leaves was much lower at all PPFD and had a lower saturation PPFD than both the acclimated and warm-climate leaves (Fig. 4*C*). The differences in PPFD-saturated P_N among these sets of leaves may be attributed mainly to differences in CO_2 fixation capacity (Björkman *et al.* 1980). Warm-climate leaves were not photon-saturated up to 1 800 µmol m^{-2} s^{-1}. The same phenomenon was observed in several field-grown cassava cultivars in a warm climate when leaf P_N was measured during the high rainfall period (Fig. 5 and El-Sharkawy and Cock 1990, El-Sharkawy *et al.* 1992a, 1993). Pereira (1977) also reported increases in cassava leaf P_N with rising PPFD up to 2 000 µmol m^{-2} s^{-1}. Maximum P_N of field-grown cassava over a range of cultivars was higher than 40 µmol(CO_2) m^{-2} s^{-1} with a mean C_i/C_a ratio of 0.42, which is comparable with that observed in C_4 species and much lower than in values of C_3 species (El-Sharkawy *et al.* 1992a). These data indicate that cassava possesses a large photosynthetic capacity that can be fully expressed only in hot-humid climates with high solar irradiation. Thus, when grown in environments that deviate from these fundamental climatic requirements, its photosynthetic capacity is not fully expressed.

Studies that showed much lower P_N [15–20 µmol(CO_2) m^{-2} s^{-1}), lower saturation PPFD, lower optimum temperatures, or lower photosynthetic enzyme activities in greenhouse or cabinet-grown plants (*e.g.* Aslam *et al.*

1977, Mahon *et al.* 1977a,b, Edwards *et al.* 1990, Angelov *et al.* 1993, Ueno and Agarie 1997) are of a limited value if such results are to be interpreted in relation to the real potential of cassava and to the underlying mechanisms controlling the overall photosynthetic process (El-Sharkawy and Cock 1987a, El-Sharkawy *et al.* 1992a, 1993, El-Sharkawy 2004). Lower P_N of potted cassava grown in cabinets or greenhouses were probably the result of lower activities of photosynthetic enzymes, as has long been observed in other plant species, and/or changes in leaf anatomy because of exposure to suboptimal PPFD and air temperature during leaf development, plus other consequences such as imbalances in source-sink relations in the whole plant system and possible feed-back inhibition of P_N resulting from restricted root sinks for assimilates (particularly when growing cassava in small pots) (see for example Nösberger and Humphries 1965, Humphries 1967, Neales and Incoll 1968, Moss and Musgrave 1971, Nobel 1976, 1980, Boardman 1977, Björkman *et al.* 1980, Herold 1980, Nobel and Hartsock 1981, Šesták 1985, Bunce 1986, Ho 1988, Evans 1993, Wardlaw 1990, Pellet and El-Sharkawy 1994).

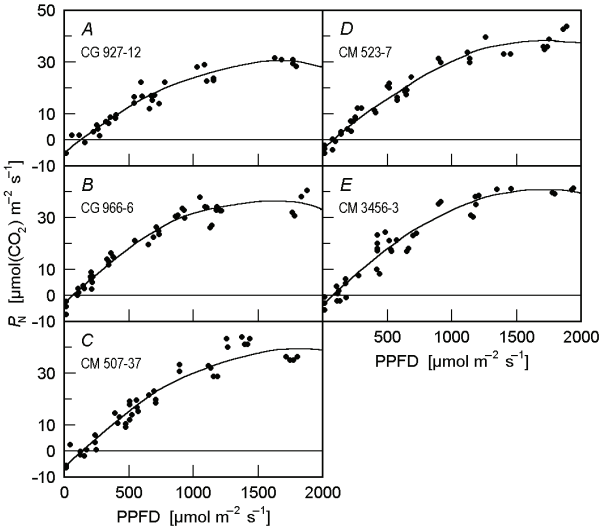

Fig. 5. Responses in cassava of leaf photosynthetic rate (P_N) to photosynthetic photon flux density (PPFD) in upper canopy leaves of field-grown cassava cultivars (*A*–*E*) during the rainy season. Measurements were made using portable infra-red gas analyzer. Crops were adequately fertilized without water stress. Several leaves from different plants were measured for each irradiance dependence curve. Note the lack of PPFD saturation at the maximum obtainable irradiances in field, and the maximum P_N in this group of cultivars that was above 40 μmol(CO_2) m^{-2} s^{-1} confirming the high photosynthetic potential of cassava in near optimum environment. Across 15 clones, the C_i/C_a ratio (*i.e.* intercellular CO_2/ambient CO_2) was about 0.42 which is comparable with values in C_4 species and much lower than values in C_3 species. In this group of clones, seasonal average upper canopy leaf P_N was significantly correlated with root yield (r = 0.56, p<0.05) and with total biomass (r = 0.64, p<0.01) (CIAT 1992, El-Sharkawy *et al.* 1992a, 1993).

El-Sharkawy (2005) recently reviewed and discussed the normally encountered problems of plant acclimation or adaptation to environments that have bewildered scientists in general and plant photosynthesis researchers in particular. In this paper, the limited value of data collected on plants inappropriately grown in non-representative environments was emphasized. The invalidity of such data particularly when used for crop modelling or when used for predicting responses in natural environments without the necessary calibration (*i.e.* using field-data collected under conditions in which plants or crops are normally grown) was highlighted. This conclusion was further substantiated by the recent findings from long-term free air CO_2-enriched field trials: both photosynthesis and productivity were much less than those previously observed with potted plants grown in greenhouses/cabinets or in field enclosures at higher than ambient CO_2 concentrations where perhaps elevated humidity and temperature occurred (Long *et al.* 2006). Using such data from unaturally grown plants, without the essential field calibration, for either crop modelling and/or for simulating the effects of the increasing atmospheric CO_2 and temperature (*i.e.* global climate changes) on productivity, as previously done, would lead to unrealistic predictions.

Field evaluations of cassava core germplasm for leaf photosynthesis performances in sub-humid high-altitude cool climate and in mid-altitude warm climate: Having pointed out the importance of field research and the need to assess cassava potential photosynthesis under representative environments, large populations of cassava genotypes that included cultivars, land races, and improved CIAT breeding materials were grown on three sites, with different climatic conditions, normally used by the cassava breeding program. The objective was to identify cultivars and lines with high photosynthetic potential in the field to be used by plant breeders as parent materials in crosses for improved productivity (El-Sharkawy 1993) in combination with the other main breeding objectives such as yield stability, broad-adaptation, and tolerance/resistance to edapho-climatic stresses and to pests and diseases (Hershey and Jennings 1992, Jennings and Iglesias 2002). This objective was warranted since our previous research in different locations in sub-humid, seasonally dry, and semiarid environments showed significant correlations between upper canopy leaf photosynthesis, as measured in the field with portable infrared gas analyzers across a wide range of core germplasm and edapho-climatic conditions, and total biomass and storage root yields (Fig. 6, see CIAT 1987–1995, El-Sharkawy and Cock 1990, El-Sharkawy *et al.* 1990, 1993, Pellet and El-Sharkawy 1993a, De Tafur *et al.* 1997b, El-Sharkawy 2004).

As an example, Table 1 (see also El-Sharkawy and Cock 1990, El-Sharkawy *et al.* 1990, 1992a, 1993, De Tafur *et al.* 1997b) presents data on upper canopy leaf

photosynthesis measured in the high-altitude cool climate (at Cajibio, Cauca Department, elevation about 1 800 m, mean annual temperature about 19 °C). P_N values for the mid-altitude warm climate [at CIAT-Quilichao experiment station, Cauca Department, and at CIAT Headquarters (CIAT-HQ) experiment station, Palmira, Valle Department, with elevation about 965–1 000 m, mean annual temperatures of 23.8 °C] were higher by 100 and 120 %, respectively (CIAT 1992, 1994). Crops were grown under rain-fed conditions with minimum fertilizer applications. Measurements were made, mainly during dry periods, on several occasions and then averaged. Chambers enclosing the central leaf lobe, or part of it (depending on the type of equipments and leaf chambers used) were always directed toward the sun and at PPFD higher than 1 000 µmol m^{-2} s^{-1} between 09:00 and 12:00 local time on 4–6 months' old plants when the leaf canopy was nearly closed (high leaf capacity source) and storage root bulking was at its highest rate (high root sink demand).

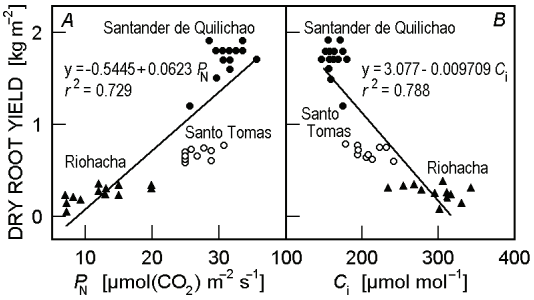

Fig. 6. Relationships between dry root yield and upper canopy leaf net photosynthetic rate, P_N (*A*) and the intercellular CO_2 concentration, C_i (*B*) for a group of cassava cultivars grown at three locations in Colombia: sub-humid (Santander de Quilchao, Cauca Dept.), seasonally dry (Santo Tomas, Atlantic Dept.), and semiarid (Riohacha, Guajira Dept.). P_N was made with portable infrared gas analyzers between 08:00 and 13:00 h local time on 4–6 months' old crops during the driest period in all locations. Crops were rain-fed and harvested at 10–12 months (El-Sharkawy *et al.* 1993, De Tafur *et al.* 1997b). Similar relations were previously observed between single-leaf photosynthesis, C_i and total biomass and yield across 127 clones grown in large field trials at a seasonally dry location in Patia Valle, Cauca Dept., Colombia (El-Sharkawy *et al.* 1990, El-Sharkawy and Cock 1990).

Table 1. Leaf net photosynthetic rate, P_N [µmol(CO_2) m^{-2} s^{-1}], stomatal conductance, g_s [mmol m^{-2} s^{-1}], and intercellular CO_2 concentration, C_i [cm^3 m^{-3}] for some cassava clones with high photosynthetic capacity. Plants were grown on a private farm in 1992/1993 at Cajibio (altitude 1 800 m), Cauca Department, Colombia (CIAT 1994).

Clone	P_N	g_s	C_i
SM 1961-1	17.3	196	98
SM 526-12	16.7	320	154
SM 1054-4	16.6	225	114
M Per 501	16.4	391	166
SM 1053-9	15.7	225	122
Mean (n = 107)	12.3	312	183
LSD 5 %	1.3	32	14

In all locations, average leaf P_N varied significantly among screened cultivars and land races with rates greatly reduced at the high-altitude, cool climate, thus confirming results and patterns observed with potted cassava grown at high-altitude, cool climate sites (see Table 1, Fig. 4). The accessions evaluated at the high-altitude cool climate site were all local traditional cultivars or land races from cool-climate regions collected from several countries, and included improved CIAT materials bred and selected for better adaptation to high-altitude cool climates. Compared to the overall mean P_N [12.3 µmol(CO_2) m^{-2} s^{-1}], the few higher ranking materials with rates from 15.7 to 17.3 were four CIAT improved clones and a Peruvian cultivar (M Per 501) (Table 1). This finding indicates the narrow genetic base for this ecosystem and also the relative effectiveness of the breeding strategy adopted by the cassava program at CIAT for specific edapho-climatic zones and ecosystems. The results also point to the importance of including leaf P_N as selection criteria in parental materials for enhancing productivity (El-Sharkawy and Cock 1990, El-Sharkawy *et al.* 1990, El-Sharkawy 2004). The enhanced P_N in these few clones could not be attributed to stomatal control as their average stomatal conductance (271 mmol m^{-2} s^{-1}) was significantly lower than the overall mean of accessions (Table 1). On the other hand, C_i was much reduced in these clones, thus indicating the possible control of non-stomatal factors such as leaf anatomy and biochemistry (*e.g.* activities of enzymes). Since the rate of leaf formation is much lower in high-altitude cool climates but leaf life is much longer, compared to warm climate conditions (Irikura *et al.* 1979), selection for enhanced photosynthesis and tolerance to low temperature becomes even more important in this case.

In the mid-altitude warm climate sites, average P_N was much higher than in cool climates, particularly at the CIAT-HQ location (100-120 % higher than in cool climate; CIAT 1992, 1994). The measurements were all made during the dry period, and thus were lower than the maximum P_N observed in wet conditions (see Fig. 5). The majority of the materials evaluated at CIAT-HQ were a collection of cultivars and land races from Brazil with eight accessions from Argentina and one accession each from Colombia (HMC 1) and Bolivia (M Bol 1). The mean P_N was significantly higher in the germplasm from Argentina [26 µmol(CO_2) m^{-2} s^{-1}] compared to the germplasm from Brazil, since many of the Brazilian accessions had rates lower than the overall mean of 22 µmol(CO_2) m^{-2} s^{-1}. Nevertheless, there were several Brazilian accessions with high P_N, particularly M Br 12 (Fig. 4*B*) and M Br 110 that could be used in crossing and breeding for use in warm climate ecosystems. Since

the accessions from Argentina are presumably better adapted to subtropical ecosystems, with some tolerance to low winter temperature than the warm climate germplasm from tropical ecosystems, they could be used in crossing and breeding for enhancing photosynthesis of high-altitude cool climate germplasm.

The accessions screened at Quilichao were a mix of cultivars and land races from Latin America, the majority, and from Asia. Again average P_N varied widely among cultivars with several high ranking accessions from Brazil, Colombia, and Malaysia. The highest ranking accession from Malaysia, M Mal 48, also had the highest dry root yield (15.6 t ha^{-1}) as compared to the overall mean of the trial (10.6 t ha^{-1}). This clone had already been used in the crossing and breeding processes at CIAT.

Evaluation of cassava core germplasm for leaf area duration, LAD (seasonal average leaf area index, LAI) and productivity at mid-altitude warm climate: To complement the joint physiology/breeding efforts in characterizing cassava core germplasm and in the identification of useful yield-determining traits, a field trial was conducted at the Quilichao mid-altitude, warm climate, CIAT experimental station, where 30 clones were evaluated for leaf duration over the growth cycle (seasonal average LAI as measured un-destructively with a plant canopy analyzer) (CIAT 1995). Comparison of clones as concerns yield, shoot and total biomass, seasonal average LAI, and root dry matter showed wide variations in standing shoot (top biomass excluding fallen leaves) and total biomass, yield, dry matter content of roots, and in seasonal LAI. Several accessions from Brazil were among the highest ranked in terms of yield, total biomass, and dry matter content in storage roots, thus highlighting the importance of the Brazilian germplasm. Breeding at CIAT, while diversifying the genetic base, has incorporated many of these accessions for their useful plant traits. Outstanding among these accessions is the clone M Br 12, with its high P_N in both potted cassava grown outdoors in mid-altitude warm climate and in field-grown crops (Fig. 4*B*), and its high yield coupled with resistance to mites (see Byrne *et al.* 1982). Other accessions of Brazilian origin, M Br 383 and M Br191, that ranked high in this group of clones, were also among the highest ranked clones (fourth and fifth, respectively, among 33 clones evaluated) for tolerance to soils low in phosphorus (see CIAT 1990, El-Sharkawy 2004). In this group of accessions, standing shoot biomass correlated with root yield ($r = 0.7$, $p<0.001$), thus confirming previous findings suggesting use of this trait as a proxy for leaf area formation and duration while evaluating large breeding populations (CIAT 1990, El-Sharkawy *et al.* 1990, El-Sharkawy 2004). Also, dry root yield correlated with seasonal LAI in this group of clones (Fig. 7, $r = 0.65$, $p<0.001$; CIAT 1995), further substantiating earlier reports (Pellet and El-Sharkawy 1993a) and supporting the concept of breeding for longer leaf life and optimum LAD for maximizing productivity under favourable conditions, as well as for sustainable yields under stressful environments (El-Sharkawy and Cock 1987b, Cock and El-Sharkawy 1988a,b, El-Sharkawy *et al.* 1992b, El-Sharkawy 1993, 2004, Lenis *et al.* 2006).

Although the use of leaf P_N as a selection criterion in cassava improvement programs might be difficult to handle in evaluating large breeding populations (but it is much easier compared to crop canopy photosynthesis that requires more complex techniques), it should be included, at least in the evaluation and selection processes of parental materials, in combination with other important yield-related traits, particularly relatively high HI (>0.5; Kawano 1990, 2003), large root sink (using root number per plant as a criterion) (Cock *et al.* 1979, Pellet and El-Sharkawy 1993a, 1994), longer leaf life (persistent leaf retention and duration over the growth cycle; El-Sharkawy and Cock 1987b, Cock and El-Sharkawy 1988a,b, El-Sharkawy *et al.* 1992b, El-Sharkawy 1993, 2004, Lenis *et al.* 2006). The recent advances in the area of molecular biology and in developing and manufacturing more precise techniques/methods and equipment must further enhance and speed up elucidation of the fundamental mechanisms underlying photosynthetic potential and associated beneficial traits and their controlling genes.

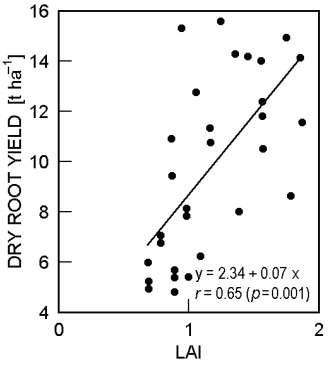

Fig. 7. Relationship between final dry root yield of 30 clones from the core cassava germplasm and seasonal average leaf area index (LAI). Crops were grown at CIAT-Quilichao station at mid-altitude warm climate. Leaf area was determined using leaf canopy analyzer throughout the growth period. The significant correlation indicates the importance of leaf area duration for yield formation. Because of the small leaf area of canopy in the first 1–3 months and in the final 8–12 months, the seasonal average canopy area still limiting yield and indicating the need to breed and select for higher and more sustainable canopy during most of the growth cycle combined with enhanced leaf photosynthesis, high harvest index, and strong root sink (larger storage root number/plant). Cv. M Bra 12 had high yield with LAI lower than the overall average of the trial indicating its high leaf photosynthetic potential (see also Fig. 4*B*) (CIAT 1995).

Responses to extended water shortages imposed at different stages of growth in the field: Other than during crop establishment, cassava has no specific water stress sensitive growth stages as compared to grain crops. The crop shows a high degree of tolerance in areas with low and erratic precipitation (<600 mm annually), coupled with dry air during a great part of the growth cycle, high air temperatures, high potential evapotranspiration, low fertility soils, and intense pest and disease pressures. These latter conditions are experienced in north eastern Brazil, northern coastal areas of Colombia, northern areas of the Peruvian coastal plain, in much of the production areas in sub-Saharan Africa, and in parts of Thailand (El-Sharkawy 1993). Although it presumably originated in hot humid climates along the northern Amazonian forest margins (see Allem 2002), the crop shows wide adaptation to adverse conditions. Under these conditions, other staple food crops such as grain cereals and legumes, rarely survive and produce. These inherent characteristics have motivated expansion of the crop into more marginal lands across many parts of Africa, Asia, and Latin America by resource-limited, poor farmers. We outlined above some inherent plant mechanisms that may underlie such tolerances, most notably the striking sensitivity of cassava to changes in both atmospheric humidity and soil water deficits (*via* partially closing its stomata and restricting water losses when exposed to dry air and/or dry soils, and thus protecting the leaf from severe dehydration) coupled with leaf capability to retain partially its photosynthetic capacities under prolonged water shortages.

Moreover, cassava, though having sparse fine root systems as compared to other crops such as cereals and tropical grasses (see Tscherning *et al.* 1995), is capable of penetrating deeper soil layers below 2 m, thus enabling plants to deplete slowly deeper stored water and to endure long periods of drought with the end result of higher seasonal crop WUE, although with reduced productivity (Connor *et al.* 1981, El-Sharkawy and Cock 1986, 1987b, El-Sharkawy *et al.* 1992b, El-Sharkawy 1993, 2004). Further research with a wider range of germplasm that was exposed at various stages of growth to long periods (3–6 months) of water shortages showed some interesting results. The plants were grown, either in large field drainage lysimeters, 30.0, 15.0, 2.3 m deep, at the CIAT station, Santander de Quilichao, which were excavated and refilled with the same soil layers (see for more details El-Sharkawy and Cock 1987b), or on adjacent undisturbed larger areas. Water stress was always initiated by covering the wet soil with calibre 6 white plastic sheets which were kept manually free of rain water and of ruptures/leaks during the stress treatments. Soil water was periodically monitored using soil-samples or with a calibrated neutron meter at 1.8–2.0 m depths; Ψ_L was assessed with the standard pressure chamber technique (see Kirkham 2005 for more thorough descriptions of equipment and methods); leaf gas exchange was measured with portable infrared gas analyzers and leaf area coverage/index was measured un-destructively with a solar-irradiance sensing analyzer (*LAI-2000* Plant Canopy Analyzer, *LI-COR*), and/or periodic harvests were made for determining yield and biomass (CIAT 1987–1995, El-Sharkawy and Cock 1987b, El-Sharkawy *et al.* 1992b, 1998b, Cayón *et al.* 1997, De Tafur *et al.* 1997a,b, El-Sharkawy and Cadavid 2002).

Evaluation of germplasm under mid-season water stress in field drainage lysimeters

Productivity and root cyanogenic potential hydrocyanic acid (HCN): Sixteen cultivars from the cassava core germplasm collection were evaluated over several years in the above field drainage lysimeters with a 3-month water stress initiated at 90–100 d after planting (mid-season stress). Fig. 8 illustrates dry root yield accumulation patterns for four representative accessions as affected by stress during the growth cycle. Water stress significantly reduced root yield in all accessions at the end of the stress treatment, as well as shoot biomass (data not shown, see CIAT 1991–1992, El-Sharkawy *et al.* 1992b). After recovery from stress, however, final root yields were equal to those of well-watered plants in some accessions, while less in others (see Table 2; El-Sharkawy 1993). There were also significant differences among cultivars in final root yield with the hybrid CM 489-1 having the highest yield under both stress (18 t ha^{-1} oven dry roots) and non-stress (19 t ha^{-1}) conditions. Cv. CM 489-1 also showed high activity of the C_4 enzyme phospho*enol*pyruvate carboxylase (PEPC) in leaf extracts under extended field water shortages and this was correlated with leaf P_N across a group of cultivars (see El-Sharkawy 2004). In separate field trials, CM 489-1 showed high values for yield, high leaf P_N, better nutrient use efficiencies in terms of root production, better radiation use efficiencies in terms of total biomass production, and a large number of harvestable storage roots per plant across a range of phosphorus fertilizer applications on acidic soils in the sub-humid warm-climate (see Pellet and El-Sharkawy 1993a,b, 1994, 1997). Across accessions, reductions were much larger in shoot biomass (28 %) compared to roots (9 %) with about 6 % increases in HI, indicating the potential of cassava to tolerate prolonged mid-season stress in sub-humid zones and also its ability to recover and compensate for possible losses in productivity, an advantage over other staple food crops (El-Sharkawy and Cock 1987b, CIAT 1991–1992, El-Sharkawy *et al.* 1992b). There is genetic variability in tolerances to stress that should be exploited in breeding and improvement of cassava germplasm for dry environments (CIAT 1991, El-Sharkawy *et al.* 1992b, 1995, Hershey and Jennings 1992, El-Sharkawy 1993).

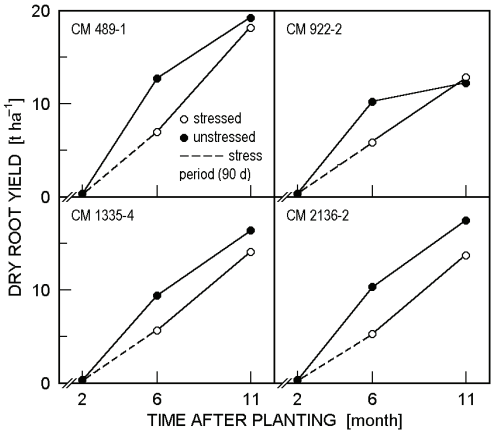

Fig. 8. Dry root yield in a group of clones as affected by a 3-month-water stress period initiated at 90 d after planting (midseason stress). The yield was significantly lower at the end of stress, but recovered rapidly with watering and the final yields were approaching those of the controls. There were differences among cultivars with cv. CM 489-1 having the highest yield in both water regimes (see also Table 2). Cv. CM 489-1 had high rate of leaf net photosynthesis with the least reduction under stress (see Fig. 15) and high PEPC activities in leaves of field-grown crops under extended water stress (CIAT 1992, El-Sharkawy 2004).

Most cassava cultivars show increases in HCN content (as an indicator of cyanogenic potential) in their storage roots when exposed to extended water deficits, and thus become less suitable for human consumption if not properly processed to remove most if not all HCN (see Dufour 1988, Rosling 1994, Essers 1995). Although some crop management practices such as applying moderate amounts of NPK fertilizers and/or plant residues as mulch to infertile sandy soils in zones with long dry periods (Cadavid *et al.* 1998), and applying K to clayey acidic soils low in K in sub-humid zones (El-Sharkawy and Cadavid 2000) greatly reduced HCN content in cassava roots, selection for low HCN cultivars remains a main objective in most breeding programs, particularly for germplasm targeted to stressful environments (El-Sharkawy 1993). In this case, the identification of less drought sensitive and low HCN genotypes as shown here (Table 2) offers a good genetic source for breeding sweet cultivars. Nassar (1986) also reported on some wild species with low HCN and high protein in roots.

Nevertheless, others (see Pereira 1977, Poulton 1990) argued that high contents of HCN in plants may play a role as a defensive mechanism in protecting crops against predators, herbivores, and rodents, or serve as a source of stored nitrogen, particularly in seeds. This presumably defensive role ascribed to HCN has not been observed in the case of cassava pests and diseases (Brekelbaum *et al.* 1978). Field observations in north-eastern Brazil and northern Colombia, where cassava crops experience several months of water shortages, showed higher mite infestations under water stress when

Table 2. Root and top dry biomasses [t ha^{-1}], and total hydrocyanic acid (HCN) content [mg kg^{-1}(dry root)] at final harvest (11 months) as affected by 3-month midseason water stress commencing 90–100 d after planting; average of 1987–1989 seasons (Cassava Physiology Section database; CIAT 1991, El-Sharkawy 1993).

Clone	Unstressed			Stressed		
	Roots	Tops	HCN	Roots	Tops	HCN
CM 489-1	19.1	7.2	214	18.0	7.1	401
CM 922-2	14.8	7.6	142	15.0	5.9	190
CM 1335-4	18.1	7.8	107	16.5	5.1	123
CM 2136-2	19.3	12.4	166	15.5	7.3	338
Mean	17.8	8.8	157	16.2	6.4	263
% change by stress				–9	–28	+68

leaf HCN is normally elevated in most cultivars, compared to lower degree of infestation in unstressed crops (unpublished). Thrips were found to feed on cassava regardless of HCN contents in leaves (van Schoonhoven 1978). Other pests with different feeding habits, whether on shoots or on roots, may have different responses. Recent work at CIAT (see Bellotti *et al.* 1988, Bellotti and Arias 1993, Bellotti and Riis 1994, Bellotti 2002) showed that the root-burrowing bug, *Cyrtomenus bergi*, preferred feeding on cassava roots low in HCN as compared to bitter cassava, particularly in wet soils; although several sweet cultivars were noted to have potential resistance/tolerance to the bug (Riis 1997). One mechanism that may deter/reduce the bug from feeding on sweet cassava is a high HCN content in the storage root peels, compared to the parenchyma tissue (Riis 1997). Since the feeding habit of the first two nymphal instars with short stylets is confined mainly to the root peels (Riis 1990, Riis *et al.* 1995), selection for sweet cultivars having high HCN in thicker root peels might be advantageous in this case. Some cultural practices such as intercropping cassava with the sunn-hemp, *Crotalaria* sp., which possesses natural insecticidal substances, reduces effectively the bug attack and damage to cassava roots, although cassava yield was reduced as a result of competition and land occupation by *Crotalaria* (Bellotti *et al.* 1988, Bellotti 2002).

In a recent social study conducted with the native Tukanoan Indians in the north-western Amazonian basin, Brazil, whose subsistence depends more on cultivating bitter cultivars high in HCN (perhaps because of its predominance rather than to inherent adaptive advantages, as sweet cultivars are less abundant there), no consistent relations/patterns were demonstrated between the social preferences of cultivating bitter cultivars *versus* sweet ones and resistance to predators, particularly pests and diseases (Wilson 2003). Wilson and Dufour (2002) reported that higher yield, often observed in bitter cultivars in that region, is the likely criterion for choosing high HCN cassava by the natives of the Amazonian basin. However, to our knowledge there is no conclusive

evidence based on sound research of an inherent superior potential productivity in bitter cultivars compared to sweet ones. Data of Table 2 and those reported in 14 other cultivars tested for five consecutive growth cycles under different rates of K-fertilizer on acidic clayey soils in sub-humid zone in Colombia (see El-Sharkawy and Cadavid 2000) indicated no consistent relation between productivity and HCN contents in roots. Moreover, several tested clones such as HMC-1, HMC-2, M Cub 74, M Pan 70, M Col 1505, CM 91-3, CM 523-7, CMC 40, CM 1585-13, as well as those shown in Table 2, have high yields and moderate to low HCN contents in the root parenchyma. Most of these clones are improved materials, thus indicating the compatibility of selection and breeding for both high yield and low HCN. More research is warranted, therefore, to uncover other possible reasons for choosing bitter cassava in the Amazonian region and elsewhere.

Photosynthesis and C_3-C_4 intermediate characteristics

Previous research on cassava photosynthesis demonstrated the importance of elevated activities of the C_4 PEPC enzyme [several cassava cultivars and wild species showed activities from 15 to 25 % of those in C_4 species such as maize and sorghum, with activities ranging from 8.3 to over 80.0 mmol kg^{-1}(Chl) s^{-1}] that may partially underlie the high photosynthetic capacity in cassava which was correlated with productivity across environments and genotypes (see Fig. 6, Table 3; Cock *et al.* 1987, El-Sharkawy and Cock 1987a, 1990, CIAT 1990–1994, El-Sharkawy *et al.* 1990, 1992a, 1993, Bernal 1991, López *et al.* 1993, Pellet and El-Sharkawy 1993a, De Tafur *et al.* 1997b, El-Sharkawy 2004). The PEPC activities observed in cassava and its wild relatives are much higher than those observed in C_3 species such as field beans and are comparable with activities found in several C_3-C_4 intermediate *Flaveria* species with limited functional C_4 cycle, and 2–3 times higher than those in the C_3-C_4 Kranz-like *Panicum milioides* (Ku *et al.* 1983, Brown and Bouton 1993).

Table 3. Activity of phospho*enol*pyruvate carboxylase expressed per fresh mass (FM) [µmol(NADH) kg^{-1}(FM) s^{-1}] or chlorophyll (Chl) [mmol(NADH) kg^{-1}(Chl) s^{-1}] content in leaf extracts of various plant species and cultivars. Means of four leaves ± standard deviation (El-Sharkawy and Cock 1990, Bernal 1991, M.A. El-Sharkawy and Y. López, unpublished).

Species	per FM	per Chl
Maize cv. CIMMYT 346	250±27	116±60
Common beans cv. Calima G4494	3±1	5±2
Cassava cv. M Mex 59	53±10	37±16
Cassava cv. M Nga 2	22±2	7±15
Manihot grahami	67±14	47±20
Manihot rubricaulis	97±9	57±22

The presence of C_4 PEPC in cassava was further determined immunologically (see Fig. 9*B,C*, CIAT 1991). The PEPC in cassava is of at least two different forms

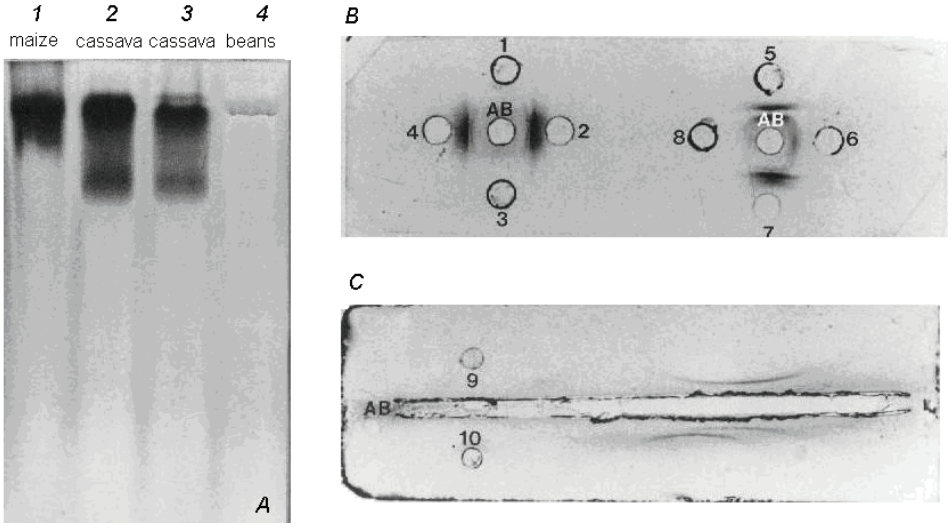

Fig. 9. (*A*) Simple PAGE patterns of phospho*enol*pyruvate carboxylase (PEPC) from leaf extracts. Lanes: *1* maize; *2* and *3* cassava cv. M Col 22; *4* beans. Extracts in lanes *1*, *2*, and *4* were obtained by using a 0.05 M Tris-HCl buffer, pH 8.3, whereas that for line *3* was obtained by using 0.05 M phosphate buffer, pH 7.6. Two molecular forms of PEPC were observed in cassava (lanes *2*, *3*), and a low-activity form was observed in beans (lane *4*), confirming findings with the immuno-electrophoresis patterns shown in *C*. (*B*) Immunological detection of PEPC. Double immuno-diffusion: wells *1*, *3*, and *8* for beans; *2*, *4*, and *5* for purified maize PEPC; *6* for cassava cv. M Col 22; and *7* for maize. AB = antiserum containing anti-PEPC. (*C*) Immuno-electrophoresis in 1.2 % agarose gel: wells *9* for purified maize PEPC and *10* for cassava. Note the apparent two forms of PEPC (isozymes) in cassava (CIAT 1991; Y. López, M.A. El-Sharkawy, J.H. Cock, and H. Ramírez, unpublished).

(isozymes) as compared with PEPC from maize. The presence of enzyme does not, however, in itself demonstrate that the enzyme is active. By using the stain Fast Violet BB, which is relatively specific for oxaloacetate, we demonstrated that the PEPC is, indeed, active in cassava (Fig. 9*A*, lanes *2* and *3* for cassava cv. M Col 22, lane *1* for maize, and lane *4* for beans), thus confirming the quantitatively assayed activities in centrifuged leaf extracts (see Table 3). More recent research showed that a maize PEPC specific antiserum (maize *ppc* probe from T. Nelson, Yale University, USA) cross-reacted with cassava PEPC, indicating homologous antigenic determinants (CIAT 1993, López *et al.* 1993). This was also shown at the DNA level in Southern blot hybridization studies with a maize *ppc* probe and total enzyme digested cassava genomic DNA (Fig. 10*A*, see

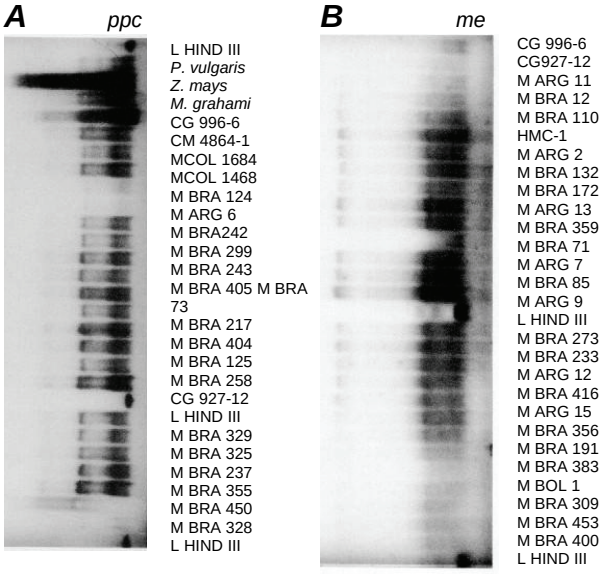

Fig. 10. Southern hybridization of BamHI digested cassava DNA hybridized with a maize *ppc* (*A*) or *me* (*B*) probe. Note the variable degrees of hybridization with maize *ppc* probe within cassava germplasm and wild *Manihot grahami* in *A* (CIAT 1993, 1994; J.E. Mayer, M.A. El-Sharkawy, C. Castillo, and F.A. Tenjo, unpublished).

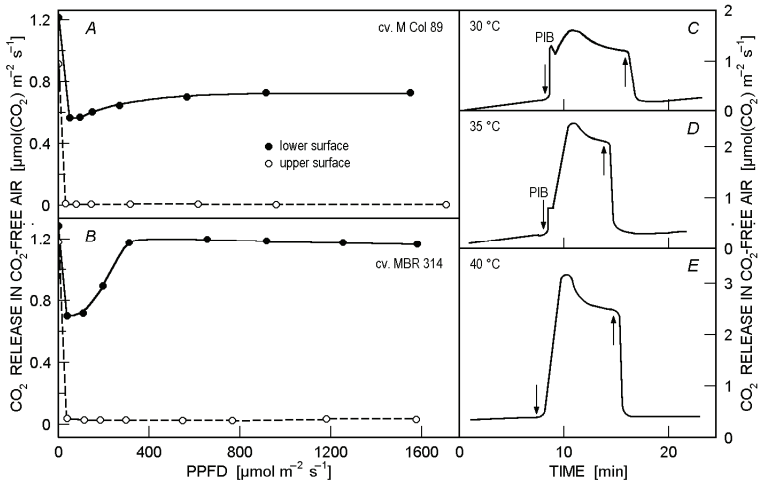

Fig. 11. Differential CO_2 releases in CO_2-free air from the upper and lower surfaces of amphistomatous cassava leaves of cv. M Col 89 (*A*) and cv. M Br 314 (*B*) as a function of photosynthetic photon flux density (PPFD) at a constant leaf temperature (27 °C). Note the consistent lack of CO_2 release from the upper surface of both cultivars, when the abaxial stomata were blocked, as compared with the release from lower surface indicating the complete refixation/recycling of respiratory carbon dioxide (both photorespiration and dark mitochondrial) within the long palisade layer (that occupies more than 60 % of leaf thickness). The spongy mesophyll in cassava is very thin, about two-cell thick layer with large air spaces. In these amphistomatous leaves rate of gas exchanges in normal air and saturating PPFD was substantial from both surfaces and in proportion to stomatal densities. (*C–E*) Recorder traces of CO_2 releases in CO_2-free air in light and dark from the upper surface of amphistomatous cassava leaves (cv. M Bra 314) at 30, 35, and 40 °C leaf temperatures. PPFD was 1 200 µmol m^{-2} s^{-1} (↓: light off, ↑: light on). PIB: post-illumination CO_2 burst. Note the lack of carbon dioxide release in light which was observed in several light-dark cycles over longer time (>one hour); the decrease in the magnitude of PIB with rising leaf temperature and eventually its disappearance at 40 °C; the pronounced surge of carbon dioxide within 3 min in darkness. The lack of carbon dioxide release in light was attributed, mainly, to an efficient refixation/recycling system in the palisade cells (El-Sharkawy and Cock 1987a, 1990).

also CIAT 1993, López *et al.* 1993, Tenjo *et al.* 1993). These studies were repeated with more accessions (about 60) including *me* (malic enzyme) and *mdh* (malate dehydrogenase) maize probes (Fig. 10*B*). No polymorphisms were found that might relate the elevated activities of cassava PEPC to higher copy number of the genes involved. Moreover, the corresponding gene sequences in cassava seem to be similar to the maize probes used, as shown by good hybridization signals at high stringency (CIAT 1993).

Preliminary studies on the compartmentalization of PEPC in cassava indicated the localization of *ppc* transcripts between the upper epidermis and the top end of the long palisade layer (CIAT 1993) and this may support the hypothesis that the palisade cells are capable of re-fixation/recycling of all respiratory CO_2 in light and in CO_2-free air that was observed over a range of PPFD and temperatures (El-Sharkawy and Cock 1987a, 1990, and Fig. 11). Complete or partial apparent re-fixation/recycling in light of respiratory CO_2 (both photorespiration and mitochondrial dark respiration) was recognized earlier in different C_3 and C_4 species (see Meidner 1962, Moss 1962, Tregunna *et al.* 1964, El-Sharkawy and Hesketh 1965, 1986, Forrester *et al.* 1966, El-Sharkawy *et al.* 1967, 1968, Volk and Jackson 1972) and in C_3–C_4 intermediates (see Devi and Raghavendra 1993). Nevertheless, more studies are warranted using *in situ* hybridization and immuno-fluorescence techniques to elucidate the spatial distribution of the photosynthetic key enzymes within the cassava mesophyll (CIAT 1993, López *et al.* 1993, Tenjo *et al.* 1993). Cassava and its wild relatives showed low photorespiration, relative to C_3 species (Fig. 12, El-Sharkawy and Cock 1987a, CIAT 1992, 1995, El-Sharkawy *et al.* 1992a, El-Sharkawy 2004), high percentage (40–60 %) of leaf-fed ^{14}C incorporated in C_4 acids after 5–10 s in the light, and elevated activities of PEPC (Cock *et al.* 1987, El-Sharkawy and Cock 1987a, 1990), but lack the typical C_4 leaf Kranz anatomy that is required for the compartmentalization of the key C_3 ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and C_4 PEPC (El-Sharkawy and Hesketh 1965, 1986, Laetsch 1974, Hatch 1977, 1987). We suggested that cassava and *Manihot* species are probably evolving biochemically towards the C_4 photosynthetic pathway with C_3–C_4 intermediate photosynthetic behaviour (Cock *et al.* 1987, El-Sharkawy and Cock 1987a, 1990, El-Sharkawy 2004, 2005).

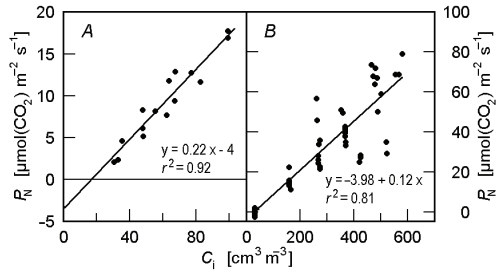

Fig. 12. (*A*) Relationship between leaf net photosynthetic rate (P_N) and intercellular CO_2 concentration (C_i) in cv. M Col 1684 (*A*) and in the wild *Manihot rubricaulis* (*B*)**.** Plants were grown in 40 000 cm^3 pots outdoors at CIAT HQ. Measurements were made under laboratory conditions (*A*) or in field during the dry period (*B*) using infrared gas analyzer. Data from 5 leaves at leaf temperature of 28–30 °C (*A*) or 30−32 °C (*B*) and PPFD of 1 500 µmol m^{-2} s^{-1}. Note the linear response and the low photorespiration (CO_2 compensation concentration was 20–22 or 30–33 µmol mol^{-1}, respectively; CIAT 1992, 1995, El-Sharkawy *et al.* 1992a).

Effects of water stress

Photosynthetic enzymes: In the above lysimeter studies, the activities of PEPC, RuBPCO, and the C_4 decarboxylase NAD-ME were measured at three weeks after initiation of water stress. Wide range of variations in activities of the three enzymes were observed among cultivars in both water treatments, with the largest variation in RuBPCO. Activities were reduced by water stress, with the highest reduction in RuBPCO, in extracts of one-month-old leaves that had developed before stress started (Table 4; CIAT 1993). The PEPC/RuBPCO ratio, which may indicate the relative importance of these two enzymes, was also reduced by stress. However, when leaves that developed under stress (8 weeks) were assayed, PEPC activity across all clones was 13 % higher than in unstressed crops, with differences among accessions (CIAT 1993, El-Sharkawy 2004). On the other hand, activity of RuBPCO was 42 % lower in the stressed crops. This differential effect of stress on the activities of these two key photosynthetic enzymes resulted in a much higher PEPC/RuBPCO ratio in the stressed crops as compared to the unstressed ones. These data indicate that under prolonged water deficit the relative importance of the C_4 PEPC *versus* the C_3 RuBPCO becomes more pronounced and support the hypothesis that the C_4 PEPC

Table 4. Activities of phospho*enol*pyruvate carboxylase (PEPC), ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), and NAD-malic enzyme (NAD-ME) in leaf extracts of field-grown cassava as affected by 3 weeks of water stress commencing at 92 d at Santander de Quilichao. Means ± SD [mmol(NADH) kg^{-1}(Chl) s^{-1}].

Clone	Unstressed				Stressed			
	PEPC	RuBPCO	NAD-ME	PEPC/RuBPCO	PEPC	RuBPCO	NAD-ME	PEPC/RuBPCO
CM 4013-1	6.17±10.00	5.17±0.83	6.67±1.00	1.19	5.17±0.50	6.83±1.50	3.17±0.83	0.76
CM 4063-6	9.50±1.00	62.00±1.83	6.50±1.00	0.15	6.83±0.67	28.17±1.67	2.83±1.50	0.24
SG 536-1	11.17±0.83	6.50±3.33	9.17±3.00	1.72	9.50±2.00	13.50±3.33	6.50±8.33	0.70
M Col 1505	7.50±0.17	19.67±1.33	2.67±0.33	0.36	8.17±1.67	8.17±2.00	4.83±1.00	1.00
Mean	8.50	23.33	6.33	0.86	7.50	14.17	4.33	0.68
% by stress					–12	–39	–32	–21

may play a significant role in photosynthetic activity under drought coupled with high air temperatures (CIAT 1993, El-Sharkawy 2004). This is of paramount importance in reducing both photorespiratory and mitochondrial dark CO_2 losses and in increasing net carbon uptake, and hence productivity (see Brown *et al.* 1975). Moreover, the recent evidence about the possible localization of PEPC in the upper end of the long palisade parenchyma further supports the role of PEPC involvement in re-fixation/recycling respiratory CO_2 when the highly dense abaxial stomata (particularly in hypostomatous leaves that normally possess >400 stomata per mm^2, El-Sharkawy *et al.* 1984a) are closing under drought, high solar irradiances, and high temperatures coupled with dry air.

Recent findings showed that cassava leaves which had developed under water deficits (in growth chambers) had a higher mitochondrial immuno-gold P-protein labelling density of the photorespiratory enzyme, glycine decarboxylase (GDC), in the palisade cells than in spongy mesophyll cells (Ueno and Agarie 1997). This may add another dimension to the C_3–C_4 intermediate hypothesis in cassava and the essential role of PEPC in recycling respiratory CO_2 within the palisade cells (see El-Sharkawy and Cock 1987a, 1990, and Fig. 11*A*–*E*). Nevertheless, Ueno and Agarie (1997) concluded that the tested 'cabinet-grown' cassava cultivars are C_3 and not C_3–C_4 intermediates. This conclusion is refutable on the basis of two important aspects. First, the patterns of distribution and confinement of GDC observed in some C_3–C_4 intermediate species with Kranz-like leaf anatomy are not necessarily applicable to other C_3–C_4 intermediates lacking such anatomy. Secondly, the observed GDC labelling patterns in ´cabinet-grown` plants are not, however, incompatible with the role of PEPC in the re-fixation/recycling of respiratory CO_2 in cassava leaves. Moreover, the expression and patterns of distribution of GDC within different mesophyll cells are influenced by the leaf developmental stage and the environments under which plants are grown (see Rylott *et al.* 1998). Our hypothesis of C_3–C_4 intermediate cassava does not exclude the presence of RuBPCO and the associated enzymes of the photorespiratory cycle in the palisade cells, nor does it restrict them to the spongy/bundle sheath cells in the absence of perfect C_4 leaf Kranz anatomy and a lack of complete separation and compartmentation of the key C_4 and C_3 enzymes in palisade and spongy/bundle sheath chlorenchyma cells (El-Sharkawy and Cock 1987a, El-Sharkawy 2004). Possibly, a limited CO_2-concentrating mechanism (*via* cytosolic PEPC) in palisade cells may operate as indicated by the disappearance at high temperatures of the post-illumination CO_2 burst (PIB) in CO_2-free air and the pronounced CO_2 surge in short period in darkness *via* the upper leaf surface (see Fig. 11*E*, El-Sharkawy and Cock 1987a). Under these conditions the oxygenase reaction by RuBPCO (in palisade cells chloroplasts) might have been suppressed. When the adaxial stomata were blocked, CO_2 releases *via* the lower surface in light and CO_2-free air were substantial at a wide range of PPFD (Fig. 11*A*,*B*, El-Sharkawy and Cock 1987a). In these cultivars with amphistomatous leaves, gas exchanges (both CO_2 and H_2O) *via* either leaf surface, measured at saturating PPFD and normal air, were substantial and in proportion to stomatal densities on both sides (El-Sharkawy *et al.* 1984a).

The cassava photosynthetic system with its intermediate behaviour is perhaps one of those interesting discoveries, and the only one in cultivated plants so far, that points to the need for a more comprehensive classification system in relation to photosynthetic pathways. In cassava and its wild relatives, there are wide genetic variations in the activities of the C_4 PEPC that correlate with leaf photosynthesis under extended water stress in the field (Calatayud *et al.* 2002, El-Sharkawy 2004). These attributes should be exploited in selection and breeding for more enhanced photosynthetic capacity, at least for identification of parental materials (see Tables 3 and 4, CIAT 1990–1994, El-Sharkawy and Cock 1990, López *et al.* 1993, El-Sharkawy 2004). The C_4 decarboxylation enzymes NAD-ME and NADP-ME also show activities in cassava, with differences among cultivars, comparable to those observed in C_4 and C_3–C_4 species. Wild species such as *Manihot rubricaulis* and *Manihot grahami* represent good genetic sources for elevated PEPC activities with a developed second palisade layer at the lower side of their amphistomatous leaves

(see Table 3, Calatayud *et al.* 2002, El-Sharkawy 2004, C. Castillo, J. Mayer, M.A. El-Sharkawy, unpublished). The existence of two palisade layers and the distribution of stomata on both leaf sides may add an adaptive advantage in terms of carbon uptake (see Parkhurst 1978, Solárová and Pospíšilová 1979, Pospíšilová and Solárová 1980, Mott *et al.* 1982, Tichá 1982, El-Sharkawy *et al.* 1984a, Gutschick 1984, Mott and O'Leary 1984). Similarly, the known genetic diversity in RuBPCO characteristics should be taped as well (Paul and Yeoh 1987, 1988). As biochemical assays are often expensive and difficult to use in screening large breeding populations, molecular biology techniques and genetic markers/mapping tools could be of use in identifying desirable genetic traits (see Beeching *et al.* 1993).

Leaf water status, canopy photon interception, and leaf photosynthesis: Predawn Ψ_L (Fig. 13 and CIAT 1992) remained around –0.5 MPa for all cultivars throughout most of the stress period of three months, with virtually no differences between the stressed and unstressed crops. Midday leaf water potential (Fig. 13) in all cultivars in both stressed and unstressed crops oscillated between –0.6 MPa (when presumably lower leaf-to-air VPD during wet period coincided with time of measurements) and –1.6 MPa (at much higher VPD during dry/sunny periods), with slight reductions often observed in the stressed crops. These values are within the ranges previously reported for cassava under extended periods of soil water shortages in the field (Connor and Palta 1981, Porto 1983, Cock *et al.* 1985, El-Sharkawy *et al.* 1992b, Cayón *et al.* 1997, De Tafur *et al.* 1997a) and are higher than those normally observed in other field crops under stress, indicating that cassava conserves water and prevents extreme leaf dehydration as a result of its stomatal sensitivity to stress (*i.e.* stress avoidance mechanisms). The phenomenon of osmotic adjustment (OA) in mature leaves which probably developed under water stress and other edaphic-stresses (which has been observed in several other field crops, see Hsiao 1973, Hsiao *et al.* 1976, Jones and Turner 1978, Turner *et al.* 1978, Ackerson and Hebert 1981, Morgan 1984) is not operating in field-grown cassava as predawn and midday bulk Ψ_L always remained above –0.8 and –2.0 MPa, respectively, during prolonged water deficits; hence OA is of little importance as a possible mechanism underlying cassava tolerance to drought. In recent studies with 'potted greenhouse-grown cassava', Alves (1998, 2002) found that the largest increases in solutes after a few days of water deficit occurred in the youngest and folded leaves (not fully expanded) with the smallest increases in the mature leaves, pointing to the small importance of OA in mature leaves. Nevertheless, such studies need to be done on field-grown plants if results are to be extrapolated to field conditions (El-Sharkawy 2005).

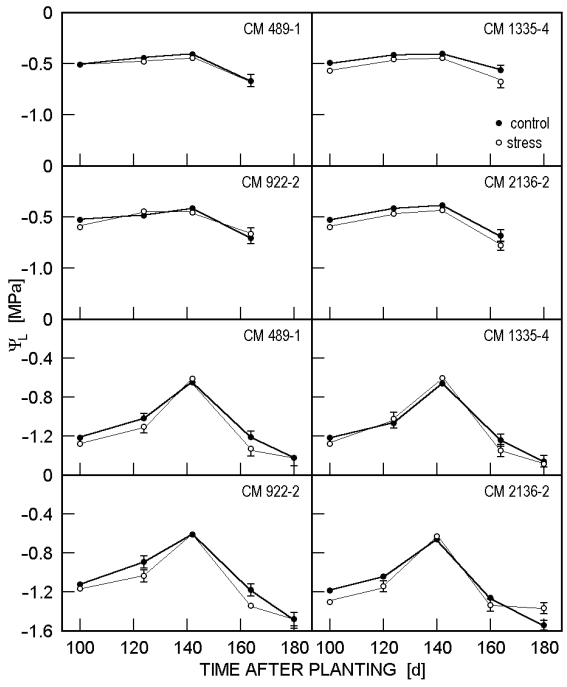

Fig. 13. Leaf water potential (Ψ_L) in water-stressed and well-watered cassava crops during the stress period initiated at 90 d after planting. Measurements were made with a pressure chamber at a field laboratory. Means of 5–10 leaves from upper canopy. *Upper four panels*: predawn; *lower four panels*: midday. Note the small differences between the two crops in predawn and midday Ψ_L and the increases in Ψ_L in high ambient humidity period. The predawn and midday values were above –0.8 and –2.0 MPa, respectively, indicating the striking stomatal control in cassava regardless of soil water status (CIAT 1991).

Because osmoregulation under stresses requires investment in the accumulation of solutes and assimilates for its development, McCree (1986) discussed the relative carbon costs involved in the process of OA in sorghum grown under both water deficit and salinity and concluded that the metabolic costs of storing photosynthates and using them for OA was less than the cost of converting them to new biomass, although the cost increased slightly under salinity. Under drought, changes in biosynthesis of plant growth regulators such as abscisic acid (ABA) contents and distribution within plant organs and tissues (particularly in roots, leaves, and buds) may play an important role in sensing changes in both soil water and atmospheric humidity and thus in controlling stomatal movements, leaf formation and extension, root growth, bud dormancy, besides other biological functions such as involvement in PEPC activation and expression, and in possible switching/induction from C_3 to CAM or C_4 photosynthesis in some species (see Jones and Mansfield 1972, Huber and Sankhla 1976, Ackerson 1980, Walton 1980, Radin *et al.* 1982, Zeiger 1983, Henson 1984a,b, Radin 1984, Davies *et al.* 1986, Schulze 1986, Turner 1986, Jones *et al.* 1987, Zeevaart and Creelman 1988, Zhang and Davies 1989, Chu *et al.* 1990, Chapin 1991, Taybi and Cushman 1999, Alves and Setter 2000, Ueno 2001).

The adaptive 'stress avoidance mechanism in cassava', operating *via* stomatal sensitivity to both edaphic and atmospheric water deficits, is of paramount importance for the crop's tolerance to prolonged drought (>3 months) enhanced by hot dry air as occurs in seasonally dry and semiarid zones (see El-Sharkawy 1993, De Tafur 1997a,b). Coupled with this mechanism is the deep rooting system (below 2 m soil depth) that allows the crop to extract storage water when available (see Connor *et al.* 1981, Porto 1983, El-Sharkawy and Cock 1987b, CIAT 1983–1994, El-Sharkawy *et al.* 1992b, De Tafur *et al.* 1997a, Cadavid *et al.* 1998). Another important characteristic in conserving water under extended stress is the large reduction in photon interception (Fig. 14, CIAT 1991–1995) through reduction in leaf canopy mainly resulting from restricted new leaf formation, smaller leaf size, and leaf fall (Connor and Cock 1981, Porto 1983, Palta 1984, El-Sharkawy and Cock 1987b, El-Sharkawy *et al.* 1992b). Although reduction in leaf area leads to water conservation, it also leads to reduction in total biomass and yield (see Fig. 8, Table 2; Connor and Cock 1981, Connor *et al.* 1981, Porto 1983, El-Sharkawy and Cock 1987b, CIAT 1991–1995, El-Sharkawy *et al.* 1992b, 1998b, El-Sharkawy and Cadavid 2002). Nevertheless, cassava can recover rapidly, once released from stress, by forming new leaves, which increase photon interception and canopy photosynthesis, thus compensating for previous losses in biomass, particularly root yield (Fig. 14; El-Sharkawy and Cock 1987b, CIAT 1991–1995, El-Sharkawy *et al.* 1992b, 1998b, El-Sharkawy 1993, El-Sharkawy and Cadavid 2002).

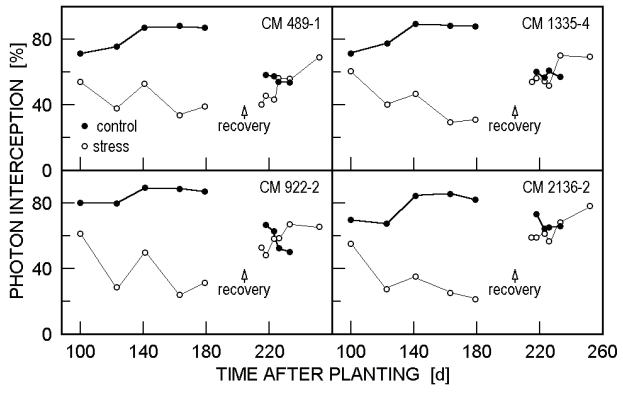

Fig. 14. Photon interception in water-stressed and well-watered cassava crops. Measurements were made with light sensors placed on top of canopy and at soil level in the middle of plots. Note the large reductions in photon interception over time in the stressed crops because of lower rate of leaf formation, small size of new leaves and fall of old leaves, and the increases after recovery from stress because of formation of new leaves (CIAT 1991).

Cassava leaves also remain reasonably active during water shortages in the field (Fig. 15). Stressed leaves were capable of maintaining P_N around 40–60 % of that of the non-stressed leaves during the entire stress period, with cultivar differences (the hybrid CM 489-1 showed the least reductions). Upon recovery from stress, however, P_N in previously stressed leaves can approach the rates of the unstressed ones. Furthermore, the newly formed leaves of the previously stressed crop showed even higher P_N than those of the unstressed crops (Fig. 15). This higher P_N in new leaves coincided with higher g_s to water vapour, higher mesophyll conductance to CO_2 diffusion, and higher N, P, Ca, and Mg contents in leaves, compared to leaves in unstressed crops (see CIAT 1990, El-Sharkawy 1993, Cayón *et al.* 1997). Moreover, Cayón *et al.* (1997) reported greater mobilization of K out of newly developed leaves (on average, new leaves in previously stressed crops consistently had 0.79 % K as compared to 0.96 % in unstressed ones), suggesting larger demands for assimilates in storage roots as K is normally translocated along with sugars to sinks (see Giaquinta 1983). Thus, leaf P_N is controlled also in this case by sink demand and strength (see Burt 1964, Thorne and Evans 1964, Nösberger and Humphries 1965, Humphries 1967, Herold 1980, Ho 1988, Wardlaw 1990,

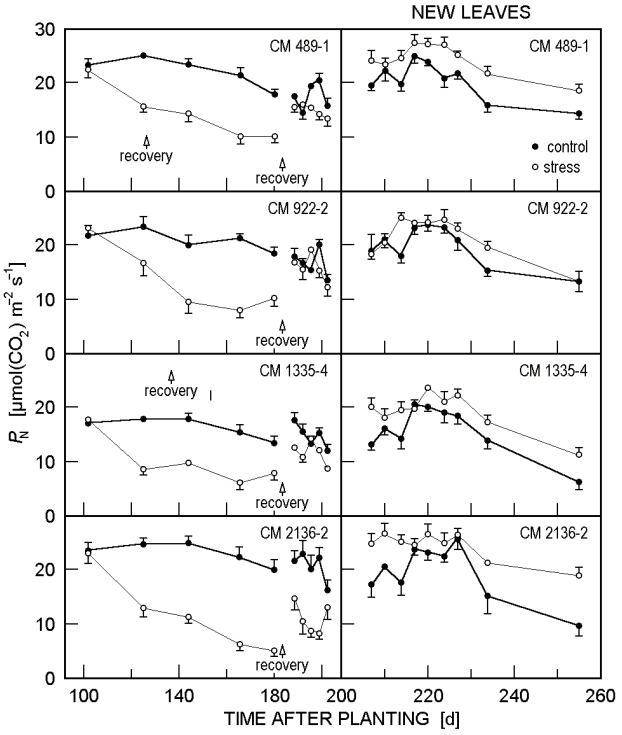

Fig. 15. Leaf net photosynthetic rate (P_N) in upper canopy as affected by midseason water stress. Measurements were made during the dry period with portable infrared gas analyzers. Means of 5–8 leaves from different plants. Note the progressive decreases in P_N over time due to water stress and the recovery after termination of stress in old leaves, and the consistently higher P_N in newly developed leaves in previously stressed crops as compared to the controls. There were apparent cultivar differences (CIAT 1991).

Pellet and El-Sharkawy 1994, El-Sharkawy 2004). Thus, the dynamics of K in leaves of field-grown cassava might be used as an indicator of root sink strength and source-sink relations.

These remarkable physiological responses to mid-season water stress point to the potential of cassava to tolerate prolonged drought and to its resilience and ability to recover from stress when water becomes available, such as in sub-humid zones with intermittent dry spells or in seasonally dry zones with well-marked bimodal rainfall distribution. Under these conditions, longer leaf life coupled with resistance to pests and diseases (Byrne *et al.* 1982, El-Sharkawy 1993), *i.e.* better leaf retention, is advantageous in saving dry matter invested in leaves and in allowing partitioning of excess photosynthates toward storage roots. In semiarid areas, the crop can survive but with larger losses in leaf canopy and smaller dry matter in the storage roots. In these ecosystems, a second wet cycle is needed to allow recovery of growth and the complete filling of the roots.

Evaluation of germplasm for tolerance to prolonged early water stress (2–6 months after planting), mid-season stress (4–8 months after planting), and terminal stress (6–12 months after planting) on undisturbed soils

Productivity, nutrient use efficiency, photosynthesis, and water uptake: Three-year field trials were conducted at CIAT-Quilichao experimental station, on large land areas next to the field lysimeters, to study the effects of prolonged water stress imposed at different growth stages on cassava productivity, nutrient uptake and use efficiency, leaf P_N, and patterns of water uptake (CIAT 1992, 1993, Caicedo 1993, El-Sharkawy *et al.* 1998b, El-Sharkawy and Cadavid 2002). Fig. 16 illustrates the dynamics of dry matter accumulation in storage roots over the growth cycle of four contrasting clones. Under early stress (initiated at 2 months after planting and terminated at 6 months) root yields at 6 months were significantly smaller than those of the control for all clones in both growth cycles. The same trends were observed in shoot biomass but with larger reductions than observed in roots (see CIAT 1992–1993, Caicedo 1993, El-Sharkawy *et al.* 1998b, El-Sharkawy and Cadavid 2002). Under mid-season stress (initiated 4 months after planting and terminated at 8 months) yields at 8 months were also significantly smaller compared to those of the well-watered controls in both cycles, except for CMC 40 (or MCol 1468, the name in Colombia). Reductions in shoot biomass were less pronounced compared to those in the early stress treatments (Caicedo 1993, El-Sharkawy *et al.* 1998b, El-Sharkawy and Cadavid 2002). Leaf area, as determined from periodic harvests, was also significantly smaller in both early and mid-season stress treatments, compared to the controls, resulting in much reduced canopy photon interception values (Fig. 17*A*,*B*; CIAT 1992, El-Sharkawy and Cadavid 2002). After cassava crops were allowed to recover, new leaf area formed rapidly in the previously stressed crops, with values in the early to mid-season treatments similar to or greater than those of the controls, thus resulting in greater photon interception values (Fig. 17*A*,*B*). In the early stressed crops increases in shoot biomass were less and remained lower than at other water regimes, indicating adverse effects of the early stress treatment on shoot biomass recovery (Caicedo 1993, El-Sharkawy *et al.* 1998b, El-Sharkawy and Cadavid 2002). Under the terminal stress treatment (from 6 months after planting until final harvest at 12 months), with the exception of CMC 40 whose yield was larger under stress, final root yield at 12 months was smaller compared to the controls, with the largest reduction in CM 523-37 (or ICA Catumare, the name in Colombia). Genotype by water regime interaction was

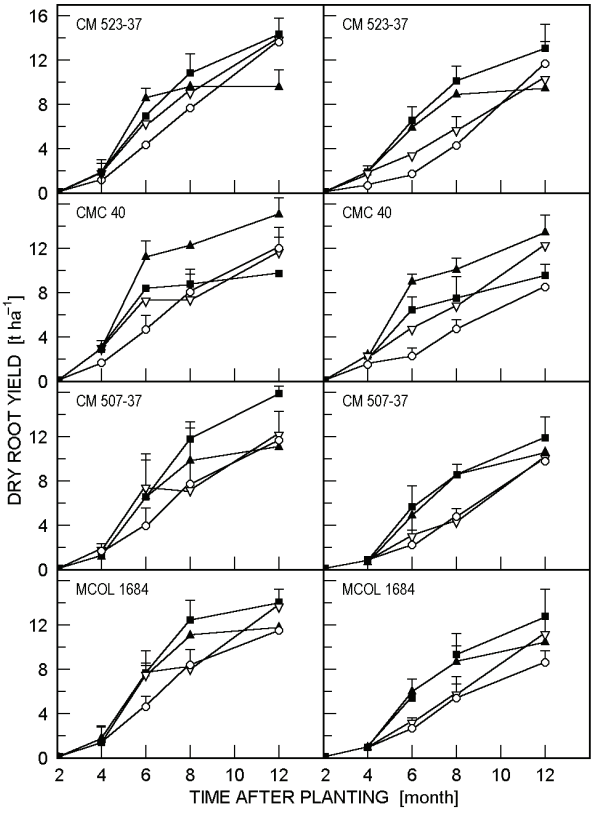

Fig. 16. Storage root dry matter yield in response to prolonged water stress imposed at different stages of growth: 2–6 early, 4–8 mid-season, 6–12 terminal, months after planting in four cassava cultivars. Crop cycles: first (*left panels*), second (*right panels*). ■ control, ○ early stress, ∇ midseason stress, ▲ terminal stress. Note the reduction in yields during stress and the recovery at final harvest in early and midseason stress. There were cultivar differences with cv. CM 40 (M Col 1468) having higher yield under stress (El-Sharkawy and Cadavid 2002).

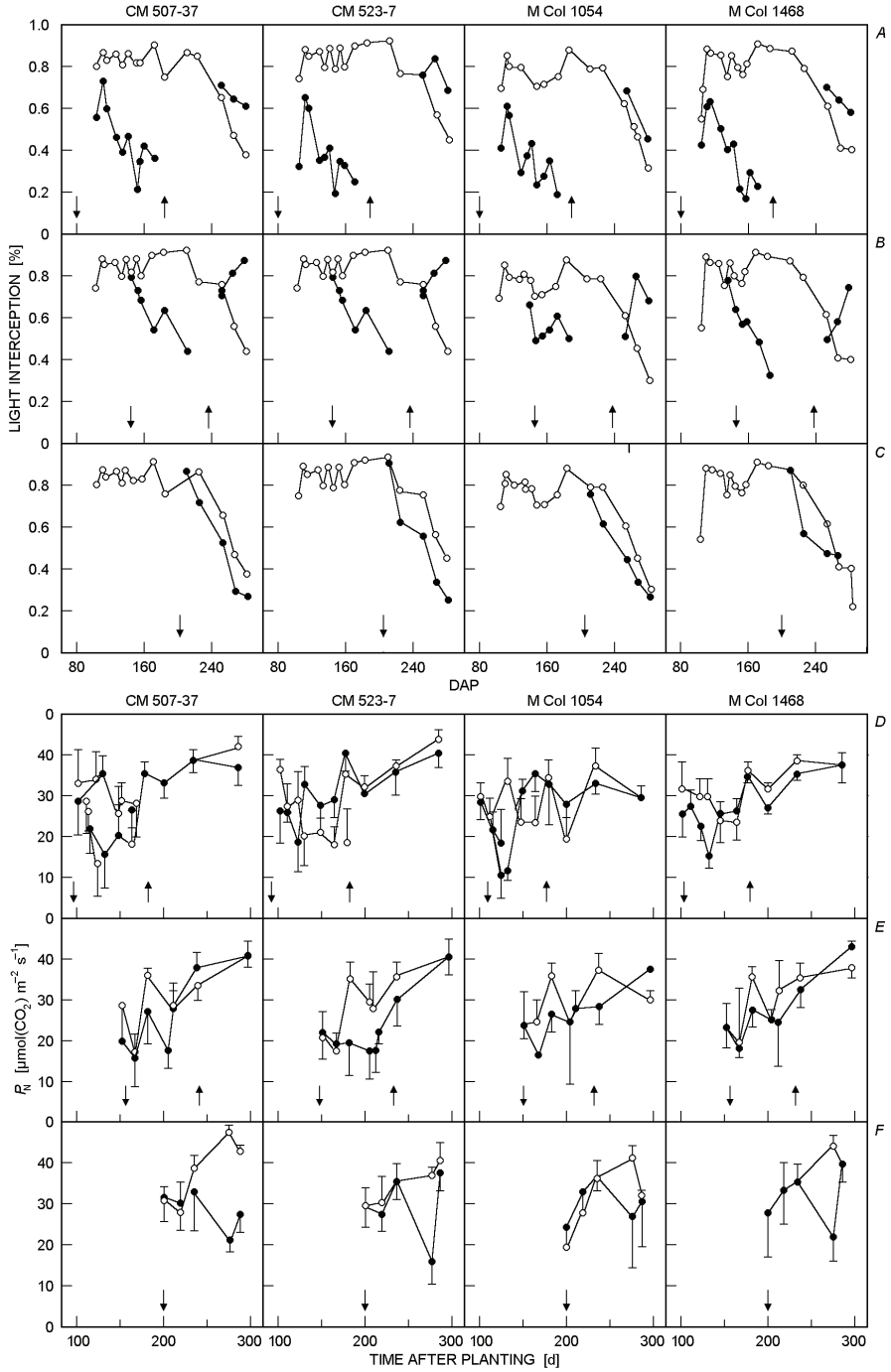

Fig. 17. Photon interception (*A–C*) and net photosynthetic rate, P_N (*D–F*) of cassava crops (four cultivars) as affected by early (*A*), midseason (*B*), and terminal (*C*) water stress. ● stress, ○ control, ↓ stress start, ↑ recovery, DAP = days after planting. Note in *A* the large reduction in photon interception in early stress and the recovery in both early and midseason stresses that exceeded the control. Greater reduction was found in P_N during terminal stress as compared to early and midseason stress treatments (CIAT 1992).

significant (p<0.01), indicating the sound breeding strategy for specific edapho-climatic zones. Similar findings were recently reported in the Sudan Savanna zone of Nigeria (Okogbenin *et al.* 2003).

Across clones in both years final yields were not significantly different among water regimes, indicating the capacity of cassava to tolerate extended periods of water deficit in sub-humid and seasonally dry warm-climates with bimodal precipitation patterns. The clone CMC 40 had the largest yield and shoot biomass under terminal stress with the smallest leaf area, compared to CM 523-37. The large yield and biomass in CMC 40, with a smaller mean leaf area, could be attributed mainly to its high leaf P_N observed in the field under diverse environments (El-Sharkawy *et al.* 1990, 1992a, Pellet and El-Sharkawy 1993a). Moreover, upper canopy leaves of CMC 40 showed higher activities of both C_3 and C_4 main enzymes compared to those in CM 523-37. They were 137 for RuBPCO and 52 for PEPC in CMC 40 *versus* 60 and 27 in CM 523-37 [mmol kg^{-1}(Chl) s^{-1}] (CIAT 1992, López *et al.* 1993). These findings point to the importance of selecting and breeding for high P_N, particularly

under stress. Variations among clones in leaf P_N, as measured in the field, were observed (Fig. 17*D*–*F*).

Nutrient uptake and nutrient use efficiency (NUE) as affected by extended water shortages and genotype architecture: Plant nutrient contents at final harvest were much smaller in stressed crops, particularly at the early stages (El-Sharkawy *et al.* 1998b, El-Sharkawy and Cadavid 2002). The resulting higher NUE for both root and total biomass in all elements were mainly the result of larger reduction in shoot biomass and stable root yields with larger HI. Across clones, increases in NUE, the result of early stress, were over 30 and 10 % in roots and total biomass, respectively (CIAT 1993). The same trends were observed with mid-season stressed crops, but with smaller values than in early stress. In terminal stress, which started after peak crop growth at 6 months, and after the bulk of nutrient uptake took place between 2–5 months (Howeler and Cadavid 1983, Howeler 2002), there were minimal increases in nutrient use efficiency in terms of root production, with the exception of magnesium which showed 25 % increases. These findings clearly illustrate the beneficial effect of water stress on conserving soil fertility as well as on nutrient use efficiency. Cassava is known for its high tolerance to both water stress and to poor soils (CIAT 1990–1995, Howeler and Cadavid 1990, El-Sharkawy 1993, Pellet and El-Sharkawy 1993a,b, 1997, Cadavid *et al.* 1998, Howeler 2002, El-Sharkawy 2004). Its capacity to accumulate more dry matter per unit of water and nutrient absorbed than most other food crops, points to its inherent advantage in marginal edapho-climatic conditions. Furthermore, these data have important implications for a breeding strategy for low-input agriculture production systems (Hershey and Jennings 1992). Selection and breeding for plant types with less demand for water and nutrients (*i.e.* medium to short-stemmed cultivars) may contribute to stabilizing and sustaining reasonable productivity in resource-limited environments.

Table 5. Dry root yield and dry top biomass [t ha^{-1}] for 15 cassava clones (high, intermediate, and low top biomass clones; means of five clones) for 126–303 d after planting (DAP) at Santander de Quilichao. First cycle, 1994–1995. NS – not significant.

Clones	Root yield for DAP			Top biomass
	126	182	303	303 DAP
High top	1.63	2.64	11.32	6.60
Intermediate top	2.32	2.80	10.90	3.70
Low top	2.21	3.02	10.39	2.60
LSD 5 %	0.55	NS	NS	0.95

In a two-year field trial at the CIAT-Quilichao station, a group of clones with tall stems (large top biomass), medium stems (medium top biomass), and short stems (small top biomass) were evaluated for productivity and nutrient use efficiency (CIAT 1996–1997, El-Sharkawy *et al.* 1998a). Differences in root yields among this group of clones (planted at 10 000 plants per ha) were small because of larger HI in the medium and short-stemmed cassava, compared to the tall ones, with tendency for early root filling in both the medium and short clones (Table 5). The larger shoot biomass in tall cultivars led to

Table 6. Nutrient use efficiency for root production at 10 months after planting [kg(dry root) kg^{-1}(total nutrient uptake)] for groups of tall, medium, and short cassava cultivars. Five cultivars in each group. Means of two years (1994–1996) (El-Sharkawy *et al*. 1998a).

Group	N	P	K	Ca	Mg
Tall	110	715	132	347	589
Medium	133	837	149	439	686
Short	131	885	161	430	669
LSD 5 %	17	85	22	77	91

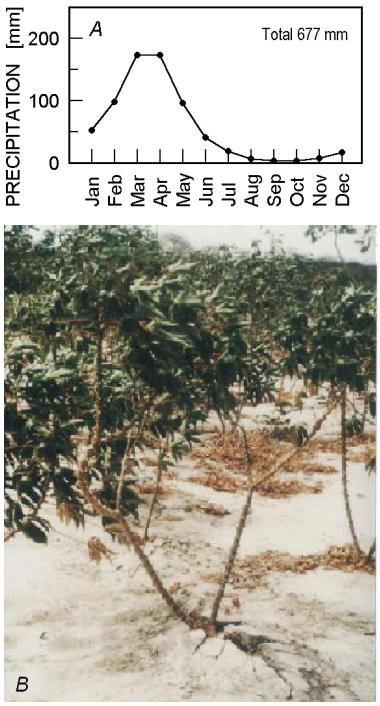

Fig. 18. Screening for drought tolerance for the semiarid ecosystems at Quixadá, Ceara, north-eastern Brazil. (*A*) Long-term (30 years) annual precipitation. 80 % occurs in 4 months and the rest of the growing season is dry coupled with high air temperatures, high evapo-transpiration potential, and high solar radiation. The soils are sandy with low water holding capacity and very low in nutrients. (*B*) Cassava germplasm at 8 months showed good leaf retention and sustainable canopy. Several clones were selected with high yield potential, tolerance to drought, low HCN content, and tolerance to major pests and diseases. Yield was >12 t ha^{-1} fresh root at 12 months with dry matter content >25 % that could be greatly enhanced with a second wet cycle (El-Sharkawy 1993, De Tafur *et al.* 1997b, CIAT cassava breeding database).

more nutrient uptake and lower nutrient use efficiency in terms of root production compared to medium and short-stemmed cultivars (Table 6, total plant nutrient uptakes were 15–30 % smaller in the medium and short cultivars, see El-Sharkawy *et al.* 1998a). These data support breeding and selection for medium to short plant architecture that would be advantageous for higher use efficiency of both native soil nutrients and applied fertilizers, particularly when crop residues are not recycled to soils. Moreover, short-stemmed cassava could be beneficial for both increasing productivity and reducing soil erosion when planted at densities higher than those normally used in pure stands and intercropped crops (current farming practices use about 5 000 and 10 000 plants per ha, in intercropped and pure-stand cassava, respectively) *via* rapid canopy closure at early growth stage when soils are prone to erosion. This breeding objective should be combined with higher leaf P_N, longer leaf life coupled with host plant resistance/tolerance to pests and diseases (*i.e.* better leaf retention, see Lenis *et al.* 2005 and Fig. 18), particularly for developing improved germplasm targeted to seasonally dry and semiarid zones (Byrne *et al.* 1982, Cock and El-Sharkawy 1988a,b, El-Sharkawy *et al.* 1990, 1992b, Hershey and Jennings 1992, El-Sharkawy 1993, 2004).

Water uptake and WUE: Patterns of water uptake across clones during extended water stress treatments imposed at different stages of growth are shown in Fig. 19. In all stress treatments, cassava withdrew more water from the deep soil layer (2-m depth), after the upper layers were almost depleted. Moreover, the water uptake from this deep layer increased as stress progressed, particularly in the terminal stress treatment, indicating the deep rooting behaviour as previously reported (Connor *et al.* 1981, El-Sharkawy and Cock 1987b, CIAT 1991–1994, El-Sharkawy *et al.* 1992b, De Tafur *et al.* 1997a, Cadavid *et al.* 1998). The available soil water in this soil ranges from 8 to 12 % by volume (see Connor *et al.* 1981, El-Sharkawy *et al.* 1992b), and hence the water uptake under any of these prolonged stress treatments would probably not exceed about 200 mm (the total available water in 2 m of soil), indicating the capacity of cassava to conserve and deplete deep soil water slowly over an extended period of stress. In two cultivars (MCol 1684 and its hybrid CM 507-37) subjected to 3-months of mid-season water shortage, El-Sharkawy *et al.* (1992b) reported that total water uptake, as estimated from periodic sampling of soil cores taken down to a 2 m depth, was around 100 and 160 mm during 35 and 96 d of stress, respectively. The latter value (160 mm) is equivalent to 70–75 % of the plant-available water to a depth of 2 m of this soil, and much less than the rate of pan evaporation of about 4.4 mm d^{-1} at the site of the trials. The estimated crop WUE, during the 43 d of peak canopy growth between 117 and 160 d after planting, were 4.4 and 4.8 g(total oven-dried biomass) kg^{-1}(water) in the stressed crops as compared to 3.7 and 4.9 g kg^{-1} in unstressed crops (for MCol 1684 and its hybrid CM 507-37, respectively). Because cassava has a long growth cycle and a small LAI during a significant portion of its growing season, seasonal crop WUE is low. Nevertheless, estimated cassava seasonal crop WUE [at about 2.9 g(total dry biomass) kg^{-1}(water), in field-lysimeter-grown crops] compares favourably with values found with the C_4 grain sorghum of about 3.1 g kg^{-1} and much higher than those in the C_3 field beans with about 1.7 g kg^{-1} (see El-Sharkawy and Cock 1986, El-Sharkawy 2004). Because of higher HI in cassava (from 0.6 to 0.7), WUE in terms of economic yield would be even higher than in both sorghum and field beans with lower HI values.

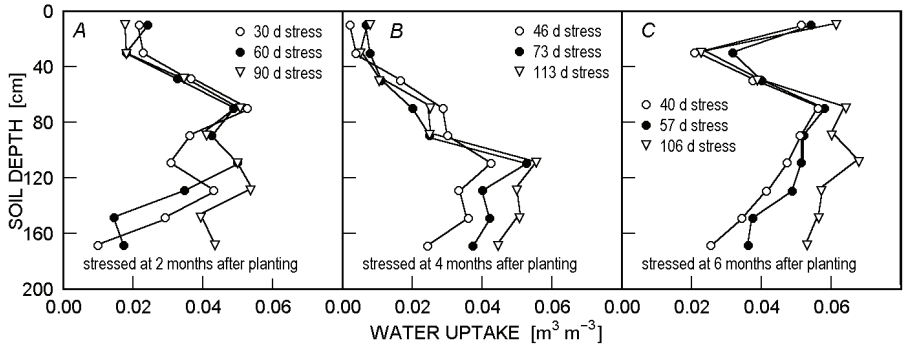

Fig. 19. Patterns of water uptake by cassava during extended water deficit at Santander de Quilichao. Note the greater water extraction from deeper soil layers that increased as water stress progressed over time, particularly in terminally stressed crops (El-Sharkawy *et al.* 1992b, CIAT 1993, De Tafur *et al.* 1997a).

These findings in field-lysimeter-grown crops were further substantiated by data in large field trials with 16 improved cultivars grown under rainfall of smaller than 1 000 mm in 10 months (60 % of which occurred in the 6th and 7th month of growth that might imply some water runoff and deep percolation into soil) in the seasonally dry zone, Patia Valley, Cauca Dept., Colombia (El-Sharkawy *et al.* 1990). Average total standing dry biomass (excluding fallen leaves) of over 30 t ha^{-1} and mean dry root yield of 20 t ha^{-1} were obtained. Assuming that all rainfall was used by the crops, the estimated evapotranspiration ratio (water loss per unit dry matter) of 270–300 for total biomass and about 500 for root yield were obtained. Such estimates for total biomass are com-

parable to values for C_4 and much smaller than values of C_3 crops grown in large containers at Akron, Colorado, USA, almost a century ago (see Shantz and Piemeisel 1927, Stanhill 1986). Moreover, estimated transpiration ratio for economic yield was much smaller in cassava than values in the C_4 cereals such as millet, sorghum, and maize.

The deep rooting characteristics of cassava, combined with partial stomatal closure in response to both edaphic and atmospheric water deficits, as well as reduced leaf canopy and photon interception but with reasonable leaf P_N, is of paramount importance where the crop has to endure several months' prolonged drought in the seasonally dry tropical ecosystems where excess rains often recharge deeper soil layers. This conservative pattern of water use results in optimal seasonal crop WUE (El-Sharkawy and Cock 1986, El-Sharkawy 2004). Boyer (1996) reviewed and discussed advances in drought tolerance in field crops and the possible mechanisms underlying enhanced crop WUE. Deep rooting behaviour accounted for a major part of differences in drought tolerance among species. These inherent mechanisms may partially explain why stressed cassava is still capable of producing reasonably well, as compared to other cereals and grain legume food crops, and further strengthens the strategy of expanding cultivation of cassava in drought-prone areas where severe food shortages might occur (Hershey and Jennings 1992, El-Sharkawy 1993).

Selection for tolerance to low-fertility soils

To alleviate pressures on natural resources, particularly in marginal soils where most cassava is produced with little or no external inputs, selection for tolerance to low fertility is warranted (Hershey and Jennings 1992, El-Sharkawy 1993, 2004). Potassium (K) and phosphorus (P) are the two most limiting nutrients mainly because of the large removal of K in harvested roots (>60 %) and the low P concentrations in most acidic soils in the tropics (Howeler 1985, CIAT 1988–1997, Howeler and Cadavid 1990, Pellet and El-Sharkawy 1993a,b, 1997, El-Sharkawy and Cadavid 2000, Howeler 2002). Large screening trials of cassava germplasm were conducted in the low-fertility soils at CIAT-Quilichao station over 10 years to evaluate tolerance to P (CIAT 1986−1996, Hershey and Jennings 1992, Pellet and El-Sharkawy 1993a,b, El-Sharkawy 2004), and later for K (CIAT 1992–1996, El-Sharkawy and Cadavid 2000).

Evaluation of core germplasm for tolerance to soils low in K and responses to K fertilizer

There were large responses to K application in all clones tested in yield and dry matter (across accessions average dry root yield with K application was 10.3 *vs*. 5.9 t per ha without K fertilizer and 38.1 and 36.2 % dry matter, respectively) indicating the low K in these soils (CIAT 1995). However, there were wide genetic differences in yield and in tolerance levels as estimated by the calculated low-K adaptation index (*i.e*. yield with zero K×yield with 100 kg K/mean yield with zero K×mean yield with 100 kg K). Two accessions (CM 2777-2 and CM 3372-4) had high tolerance with adaptation indices 50 % higher than the overall mean index (1.0) and they represent a good genetic source for improving germplasm. Pellet and El-Sharkawy (1997) identified clones having large yields with and without fertilizer, indicating that selection for large yield is not detrimental to soil fertility compared to that in land races/cultivars.

Furthermore, El-Sharkawy and Cadavid (2000) reported the existence of genetic variation in response to K in a 5-year trial with a few clones showing high yield potentials with and without K application as well as high K use efficiency in terms of root production. One clone (CM 507-37) had good leaf retention and fine rooting systems (El-Sharkawy and Cock 1987b, El-Sharkawy *et al.* 1992b), indicating the importance of these traits. Moreover, clone CMC 40 (or MCol 1468) showed the highest NUE under extended water stress at different growth stages resulting from larger biomass and yield (El-Sharkawy *et al.* 1998b, El-Sharkawy and Cadavid 2002), but low efficiency in wet conditions (Pellet and El-Sharkawy 1997, El-Sharkawy and Cadavid 2000). Thus, genotypic by environment interaction is important in this case.

Evaluation of core germplasm for tolerance to soils low in phosphorus and responses to phosphorus fertilizer

Fig. 20 presents data on yield with and without P application in a group of 33 accessions. Few clones had large yield with and without P fertilizer indicating high tolerance to low-P, as shown by their enhanced low-P adaptation indices. Cassava relies on vesicular arbuscular mycorrhiza (VAM) for P uptake (see Howeler *et al.* 1982, Howeler and Sieverding 1983). However, Pellet and El-Sharkawy (1993b) reported that cultivar differences in P uptake were related to differences in fine root length density rather than to infection rates with VAM; and uptake efficiency (estimated as uptake per unit root length) did not differ among cultivars. This finding

indicates again the importance of the fine rooting system in cassava plant-soil relation. Furthermore, these authors concluded that yield response to P was regulated by the balance between potentials of shoot growth and storage roots; and adaptation to low-P could be improved by selection for high fine root length density, moderate shoot growth, and stable high HI values. This conclusion was further substantiated by the enhancement in NUE under

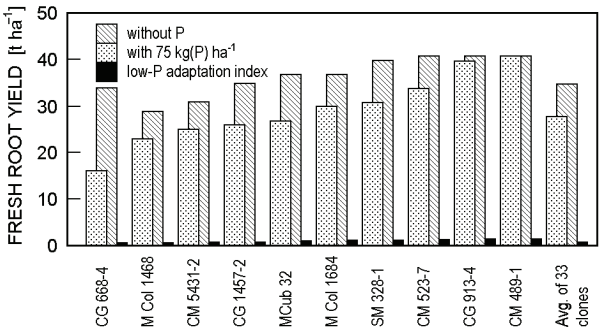

Fig. 20. Screening cassava germplasm for tolerance to low-P soils. Note the several clones with good yield potential at low and high P concentrations. More than 1 600 accessions were screened and the selected clones were used in breeding program. Low-P adaptation index was calculated as: (yield with zero P × yield with 75 kg P ha^{-1})/(mean yield with zero P × mean yield with 75 kg P ha^{-1}) (CIAT 1992).

Highlights and conclusions

The research reviewed here on cassava photosynthesis, physiology/eco-physiology, and responses to environmental stresses was conducted in collaboration with a multidisciplinary team at CIAT and with national programs where a diverse germplasm bank of the crop has been assembled and conserved since 1970. The research objectives revolved around the strategy adopted for developing new technologies for enhancing productivity under diverse environmental conditions, representing most edapho-climatic zones under which cassava cultivation is occurring, particularly stressful environments.

Under favourable environments in the lowland and mid-altitude tropical zones when climatic and edaphic conditions are near optimum for the crop to realise its inherent potential, cassava is highly productive in terms of root yield and total biomass (yields >15 t ha^{-1} oven-dried roots with 85 % starch in 10–12 months are attainable in experimental trials with improved germplasm). Physiological mechanisms underlying such high potential productivity are: (*1*) high leaf photosynthetic potential, comparable with efficient C_4 crops [P_N under high humidity, wet soil, high leaf temperature, and high solar radiation exceeds 40 µmol(CO_2) m^{-2} s^{-1}]; (*2*) long life (>60 d) of leaves that remain active during most of their life span; (*3*) sustainable leaf canopy that optimizes photon interception during a significant portion of the growth cycle; (*4*) high harvest index (>0.5) coupled with a strong root stress resulting from larger reductions in shoot biomass than in roots, *i.e*. higher HI, as well as in medium and short-stemmed cultivars (see Table 6; El-Sharkawy *et al.* 1998,a,b, El-Sharkawy and Cadavid 2002). Moreover, in another group of accessions it was found that low-P adaptation indices were correlated with standing shoot biomass at harvest, excluding fallen leaves, with number of harvested roots/plant, and with seasonal average upper canopy leaf photosynthesis (CIAT 1990, El-Sharkawy 2004), indicating the importance of both carbon assimilation source and capacity as well as root sink capacity in selection and breeding for tolerance to infertile soils.

Clone CM 489-1 with high adaptation index to low-P (Fig. 20) had high P_N at different amounts of P, large yield, and high nutrient and solar radiation use efficiencies (Pellet and El-Sharkawy 1993a,b, 1997). Moreover, this clone had the largest yields with and without mid-season extended water deficits (Fig. 8, Table 2, El-Sharkawy 1993) and less reduction in leaf P_N during water stress (Fig. 15). Among several clones tested in the field under extended water deficits, the C_4 PEPC activity was the highest in leaves of CM 489-1 that correlated with the P_N of the same leaves (El-Sharkawy 2004). These findings indicate that it is possible to select for and assemble in one genotype several desirable plant traits. Complementary multidisciplinary/multi-institutional research is crucial in this case and could enhance research efficiency and benefit/cost ratios (El-Sharkawy 2005).

sink (*i.e*. larger number of storage roots/plant).

Under stressful environments in seasonally dry and semiarid tropics, productivity is reduced with more reduction in above ground parts (shoots) than in storage roots (*i.e*. higher HI). Under these conditions the crop possesses some inherent adaptive mechanisms for tolerance. Most important is the remarkable stomatal sensitivity to changes in atmospheric humidity as well as in soil water. By closing stomata under water stress, the leaf remains hydrated and photosynthetically active, although at reduced rates, over most of its life span. Coupled with this 'stress avoidance mechanism' is the deeper rooting capacity where stored water at the 2-m depth can be extracted at a slow rate leading to not only the survival of the crop during long dry periods (>3 months) but also to reasonable yield with an efficient WUE and NUE. Moreover, leaf canopy is much reduced under prolonged stress, contributing to lower crop water consumption. When recovered from stress, cassava rapidly forms new leaves with higher P_N that leads to compensation for yield reductions resulting from previous prolonged stresses. Productivity over a wide range of germplasm and in different environments correlates with upper canopy leaf P_N, as measured in the field, and the relation is mainly the result of non-stomatal factors, *i.e*. biochemical/anatomical leaf traits. Among these leaf traits are elevated activities of the C_4 enzyme PEPC. Genetic

variations within cassava germplasm and wild *Manihot* exist in leaf photosynthesis as well as in enzyme activities, and this could be exploited in breeding programs. LAD under stress, coupled with host plant resistances/tolerances to pests and diseases, is a critical trait as yield correlates with plant leaf retention capacity (Lenis *et al.* 2006). Deeper rooting capacity is another important trait for selection and breeding improved germplasm targeted to drier zones.

In cooler zones as at high altitudes in the tropics and in the lowland subtropics, cassava growth is slower and the crop takes longer to achieve reasonable yields. Under these conditions, leaf formation is slower coupled with much reduced leaf P_N but with longer leaf life (Irikura *et al.* 1979, El-Sharkawy *et al.* 1992a, 1993). There are wide genetic variations in photosynthesis that could be of value in selecting and breeding more cool-climate tolerant genotypes. Combining enhanced leaf P_N with the normally longer leaf life in cool climates may improve productivity.

Compared to tall cassava, selection for medium and short-stemmed cassava is advantageous for saving on nutrient uptake, leading to higher NUE for root production without sacrificing yield potential. Core germplasm was screened for tolerance to soils low in P and K, with the identification of several accessions with good level of tolerance and in the same time are responsive to fertilizer application.

Results also point to the importance of field research *versus* studies carried out on plants grown indoors (greenhouses and growth cabinets), without calibration under representative environments to avoid acclimation problems (see El-Sharkawy 2005). This becomes even more critical when data from indoor-grown plants are used for extrapolation to predict responses in the field and when used in developing crop models.

Yet, because a lot remains to be done in order to further improve productivity while conserving dwindling natural resources such as water and land, maintenance research (*i.e.* research needed to upgrade previous findings and technologies, to serve sustainable agricultural, economic, and social developments, as well as to enhance food supply to meet increasing human demands) must continue with more support, particularly in developing countries. Basic research, especially when conducted in collaboration with multidisciplinary/multi-institution teams, within well-planned strategies and focused toward the fulfilment of a set of high priority goals/objectives, can be a cost-effective and a successful endeavour with high returns, although at a slower pace. International research and development organisations, as well as donor agencies and the private sector, should take a leading role in financing and supporting basic research particularly oriented toward serving the technological needs in resource-limited developing countries.

Scientists, especially those who are productive and innovative, should remain loyal to their research benches and fields as long as they can without being tempted by time-wasting non-scientific affairs. Nevertheless, scientists should also be aware and participate in formulating science-related policies, research strategies, and managerial decisions in order to avoid counterproductive policies and poor management often impacting negatively on science output. A case in point is that all of the above huge volume of scientific research on this vital world crop was done over only 15 years within a closely organised, reliably and adequately funded multidisciplinary program team of active and full time scientists from a wide range of disciplines working together towards a common aim, "that is the advancement of scientific frontiers in the interests of some of the world poorest farmers". The present situation in the CGIAR research centres where the eternal search for research funds has become now the responsibility of individual scientists who spend a great portion of their time to compete on the world stage for ever more limited funds for strictly time defined non-core research projects (*i.e.* rather than core funded integrated research programs) has been one of the main factors in the dispersal of the former excellent crop research teams in cassava and beans at CIAT (and perhaps at another centres too). The aim of researchers now is to get financial support for projects that mostly serve the interests of the donors (and perhaps also fit the narrow interests of individual scientists) rather than focusing on and investigating the more important problems *via* integrated productive research based on multidisciplinary approach. It is sad to say, but it is likely that the great effort outlined above in the advancement of the science of cassava is now mostly unsustainable. The CGIAR system is better advised to reconsider its current counterproductive research policy based on a short-sighted strategy *via* advocating short-term projectized, non-core funded, research. Without the steadfast funding from a few donor agencies in late 1940s through 1950s and the commitments of many national and international scientists who have recognized, assembled, conserved, and improved the genetic base underlying productivity of some important food crops, the 'Green Revolution' of the 1960s that saved most of the populations in many developing countries from famine, probably would have never happened. Moreover, the relatively small sum of money that had been invested in this revolution by a few developed countries had indirectly resulted in substantial socio-economic gains within their own societies (Wortman 1981). In discussing the opportunity to build corps of U.S. citizens trained and experienced in development of developing countries, Wortman (1981) concluded "for the American people, the advantage will be to improve American participation in world trade and investment, thus strengthening the U.S. home economy, as well as U.S. home employment, through production of U.S. export products".

Some concluding remarks

Finally I conclude with some pertinent quotes that have important implications for research efficiency, scientist productivity, research management style, and donor policy in light of the declining financial resources in support of agricultural research: (*A*) The best person to decide what research should be done is the person doing the research; the next best is the research director, who is probably wrong more than half the time. After that you leave the field of qualified people, with the level of scientific credibility decreasing rapidly. At the top is the committee of managers and associates who are wrong most of the time (C.E. Mees). (*B*) Another wasteful use of scientific manpower is that productive scientists are overloaded with far too many committees, study panels, and advisory duties, as well as fund raising and formal administrative activities (Warren Weaver). (*C*) The investigator may be made to dwell in a garret, he may be forced to live on crusts and wear dilapidated clothes, he may be deprived of social recognition, but if he is given time, he can steadfastly devote himself to research. Taking away his free time utterly destroys him as a contributor to knowledge (W.B. Cannon). (*D*) A scientist looking at non-scientific problems is just as dumb as the next guy (Lee A. Dubridge). (*E*) Science can neither survive with intermittent charity from donors nor with arrogant political managers (M.A. El-Sharkawy).

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