CHAPTER 3

Cassava Productivity, Photosynthesis, Ecophysiology, and Response to Environmental Stresses in the Tropics:

A Multidisciplinary Approach to Crop Improvement and Sustainable Production

Mabrouk A. El-Sharkawy[1], *Sara M. de Tafur*[2], *and Yamel López*[3]

Introduction

Cassava (Euphorbiaceae: *Manihot esculenta* Crantz) is also called manioc, yuca, or mandioca. It is widely grown for its starchy roots, which are used as a staple food and animal feed. Crops are cultivated throughout the tropics and subtropics of Africa, Asia, and Latin America, between latitudes 30° N and 30° S, and from sea level to more than 2000 m above sea level (masl)[4]. Growers are mostly farmers who live in areas of marginal environments that characteristically possess highly eroded, low-fertility, acidic soils. Farmers are usually too resource-poor to afford applications of agrochemicals (El-Sharkawy 1993, 2004; Ruppenthal et al. 1997; Fermont 2009).

Because cassava has an inherent tolerance of various edaphoclimatic stresses, the crop is expanding into more marginal lands, particularly in sub-Saharan Africa (Romanoff and Lynam 1992), where other staple food crops yield poorly (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 carbohydrates (protein is less than 3% in dried roots), mainly for human consumption. It is prepared fresh, as in the case of sweet cultivars, which have low contents of cyanogenic glucosides. It is also processed, particularly in the case of bitter cultivars, which are high in cyanogenic glucosides into dried products such as flour, starch, or animal feed (Dufour 1988; Essers 1995; Balagopalan 2002; Westby 2002). Cassava roots are highly perishable once harvested (van Oirschot et al. 2000), and must be used immediately or processed into dried products. Sometimes, however, pruning the crop 3 weeks before harvest can reduce deterioration.

Regardless of its attributes and potential productivity, the cassava crop has received little attention from policymakers or researchers in the developing countries where it is widely grown. Even so, limited work was carried out in parts of Africa, Asia, and Latin America until late 20th century (Verteuil 1917, 1918; Nijholt 1935; Cours 1951; James 1959; Hunt et al. 1977; Cock 1985), when cassava research increased exponentially.

Cassava is the most important source of dietary carbohydrates after rice, sugarcane, and maize for over 500 million people in the developing countries of the tropics and subtropics. Yet, the crop was overlooked by the so-called "Green Revolution" created through the efforts of international agricultural research centers in the 1960s. These centers aimed to improve major cereal crops such as wheat, rice, and maize with the help of and funding by a few international agricultural development and research agencies. In 1971, the Consultative Group on International Agricultural Research (CGIAR) was established under the sponsorship of the World Bank, the United Nations Development Programme (UNDP), and the Food and Agriculture Organization of the United Nations (FAO) (Wortman 1981). This Group gave high priority to research on other crops, including cassava, and on production ecosystems in the humid tropics of Africa (through the International Institute of Tropical Agriculture [IITA], based in Nigeria) and South America (through the Centro Internacional de Agricultura Tropical [CIAT], based in Colombia).

Given the necessary financial support, international multidisciplinary teams of scientists were able, for the first time, to conduct extensive research on cassava. They

1. Plant Physiologist, formerly of CIAT, Cali, Colombia. Present address: A.A. 26360, Cali, Colombia. E-mail: mabrouk99@hotmail.com
2. Plant Physiologist, Associate Professor of UN–Palmira, Colombia. E-mail: msmejiat@unal.edu.co
3. Plant Physiologist, Emeritus Professor of UN–Palmira. E-mail: lopezyamel@yahoo.com
4. For an explanation of this and other abbreviations and acronyms, see *Appendix 1: Acronyms, Abbreviations, and Technical Terminology*, this volume.

collaborated with the few, already existing, national research programs to improve germplasm collection and characterization, breeding, agronomy, cropping systems management, pest-and-disease control, and crop use. These activities were based on increased understanding of the physiological processes involved. Various researchers reviewed results on many aspects of cassava research in Africa, Asia, and Latin America over the last 3 decades. These authors include Kawano (2003) and others, working on different continents, who contributed chapters to the book entitled *Cassava*: *biology, production and utilization,* edited by Hillocks et al. (2002).

In this chapter, we review research, both published and unpublished, conducted at CIAT during more than 15 years on cassava productivity, physiology, and ecophysiology in response to environmental stresses normally encountered in the tropics. The review addresses a need to assemble and integrate this dispersed information for scientists in general and for those concerned with cassava in particular. Focus is on the strategy adopted to improve the crop, taking into account the conditions faced by cassava growers who lack the resources to use high-production inputs. This approach contrasts with the capital-intensive practices used in the Green Revolution crops.

Original results were regularly documented and reported in progress annual reports that were exchanged across countries (CIAT Reports 1983 to 1998), and published in peer-reviewed technical journals, reviews, students' theses, proceedings, and books (Porto 1983; El-Sharkawy and Cock 1984, 1986, 1987a, 1987b, 1990; El-Sharkawy et al. 1984a, 1984b, 1984c, 1984d, 1985, 1990, 1992a, 1992b, 1993, 1998a, 1998b, 2008; Cock et al. 1985, 1987; Veltkamp 1985; Cock and El-Sharkawy 1988a, 1988b; Guzman 1989; El-Sharkawy 1990, 1993, 2004, 2005, 2006, 2010; Bernal 1991; Hershey and Jennings 1992; Caicedo 1993; López et al. 1993; Pellet and El-Sharkawy 1993a, 1993b, 1994, 1997; Tenjo et al. 1993; Tscherning et al. 1995; Cayón et al. 1997; de Tafur et al. 1997a, 1997b; Cadavid et al. 1998; El-Sharkawy and Cadavid 2000, 2002; Flörchinger et al. 2000; de Tafur 2002; El-Sharkawy and de Tafur 2007, 2010).

Cassava Research Strategy at CIAT

The multidisciplinary cassava program at CIAT was established in the early 1970s. Having a global mandate for cassava, the Center focused its research strategy on collecting, conserving, and characterizing worldwide available germplasm (most of it coming from Latin America). The program also selected and bred germplasm that was more broadly adapted to the various environments prevailing in the tropics and subtropics of both Latin America and Asia.

At first, breeding objectives were directed towards developing high-yielding cultivars for favorable conditions where biotic and abiotic stresses were absent (Kawano et al. 1978; Cock et al. 1979). This strategy focused on selecting for high yield per unit land area and comparing with traditional vigorous cultivars and/or landraces suitable for intercropping. Another trait selected for was high dry matter content (i.e., high starch content) in storage roots. Harvest indexes (HI, where HI = root yield/total plant biomass) were selected to be higher than those (<0.5) usually found in low-yielding traditional varieties and landraces (Kawano 1990, 2003). This early work showed that cassava germplasm is genetically diverse, with potential for high productivity in near-optimal environments and as having sufficient genetic resources for tolerating a range of pests and diseases. Thus, the need to transfer traits from wild relatives (advocated even as recently as 2010 by Nassar and Ortiz) is largely obviated.

However, most cassava production occurs in environments with varying degrees of stresses and with little, or no, production inputs from resource-poor farmers. Hence, later breeding strategy goals centered on selecting and developing cultivars with adequate and stable yields, and able to adapt to a wide range of biotic and abiotic stresses (Hershey 1984; Hershey et al. 1988; Hershey and Jennings 1992; Kawano et al. 1998; Jennings and Iglesias 2002; Kawano 2003). This strategy was stimulated by cassava's inherent capacity to tolerate adverse environments, particularly those where other major staple food crops such as cereals and grain legumes would fail to produce. The strategy also aimed to avoid and/or reduce the negative consequences on the environment caused when high-input (agrochemicals) production systems are adopted (El-Sharkawy 1993).

The strategy took advantage of the wide genetic diversity found within more than 5000 accessions that were conserved at the time at CIAT headquarters (CIAT HQ). These accessions were mostly of Latin American origin, or originated in the 7 or 8 edaphoclimatic ecozones in Colombia, each of which was also characterized by high pest and disease pressures. These ecozones represent most cassava production ecosystems in the tropics and subtropics (Hershey and Jennings 1992; El-Sharkawy 1993).

In light of this environmentally sound breeding strategy, research on cassava physiology has focused on

both basic and applied aspects of the crop under prevailing environments. The goal was to better understand and elucidate the characteristics and mechanisms underlying productivity and tolerance of stresses (Cock and El-Sharkawy 1988a, 1988b; El-Sharkawy 1993, 2004). Pingali (2010) suggested that molecular biology tools would certainly help in achieving this goal, as would a deeper understanding of the agricultural systems and biology of tropical crops (including cassava plant physiology). He pointed out that temperate-zone research laboratories in countries belonging to the Organisation for Economic Co-opreation and Development (OECD) are currently not investing in such knowledge.

Objectives included (1) characterizing materials from a core collection of cassava germplasm held at CIAT for tolerance of extended water shortages, either natural or imposed, and of low-fertility soils; (2) studying leaf photosynthetic potential in relation to productivity under various edaphoclimatic conditions; and (3) identifying plant traits that may be useful in breeding programs. The multidisciplinary and interinstitutional research approach adopted was pivotal in achieving these objectives.

Exchange of Gas between the Cassava Leaf and the Environment

Responding to air humidity and water stress

Under controlled laboratory conditions, leaves, still attached to their plants, were sampled from both well-watered and water-stressed cassava grown in large pots left outdoors. The leaves' central lobes were inserted into clip-on chambers connected to an infrared gas exchange system and then exposed to high air humidity. This was followed by a short period of low humidity. Rates of CO_2 uptake at saturating photons and normal air declined sharply. The response was more pronounced in stressed plants (Figure 3-1; El-Sharkawy and Cock 1984). This effect of short exposure to dry air was totally reversible. This reaction was also observed in several woody species (Davies and Kozlowski 1974), but was only partially reversible after a much longer exposure to dry air. It resulted in about an 80% reduction in leaf photosynthesis.

Terminal leaf water potential was measured with a pressure chamber and compared with that of the free lobes of the same leaves. The lobes tested in both

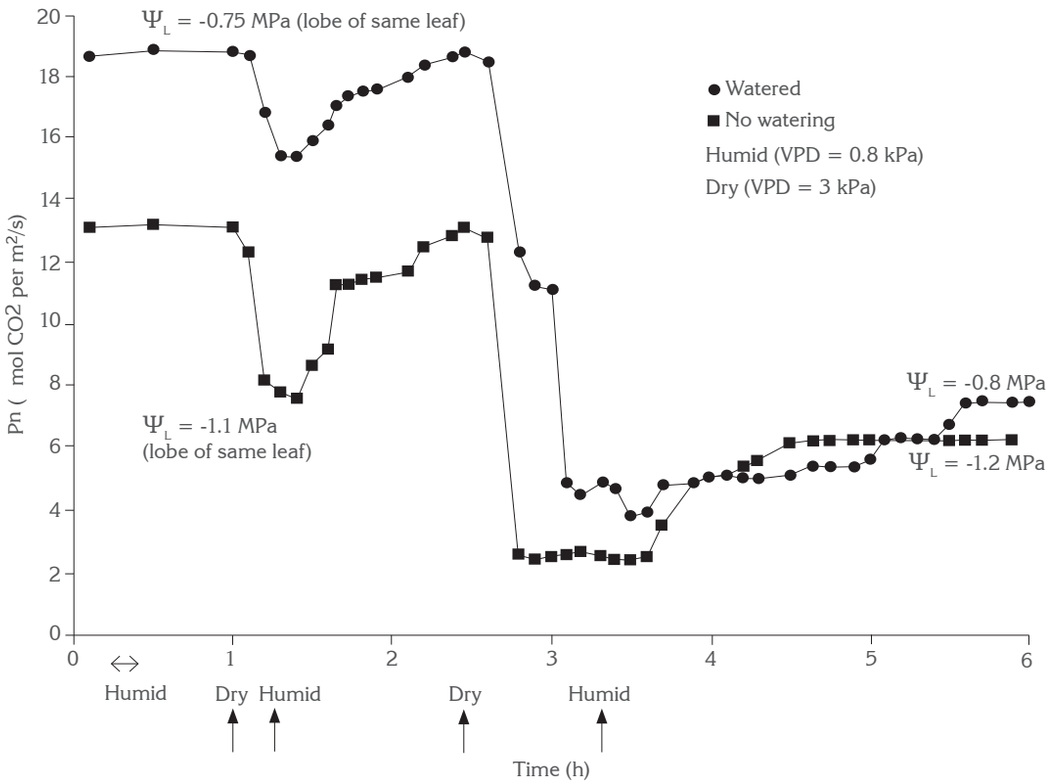

Figure 3-1. Response of leaf photosynthesis (Pn) to changes in air humidity, using plants of cassava cultivar M Col 88 grown in 40-liter pots (El-Sharkawy and Cock 1984).

well-watered and water-stressed plants remained unchanged during several hours of gas exchange monitoring. Results indicated that cassava stomata directly responded to changes in air humidity. This response was previously termed a feed-forward reaction (Cowan 1977; Farquhar 1978). It differs from the feed-backward response to changes in bulk leaf water potential. However, we did not determine abscisic acid (ABA) levels in leaves in this case. It was therefore not clear if ABA played a role in stomatal closure during this short exposure to dry air without changes in leaf water potential (Henson 1984).

When leaves were exposed to stepwise rises in leaf-to-air vapor pressure deficits (VPD), CO_2 uptake rates remained stable in the range of 1 to 1.5 kPa, and then rapidly declined above that range (Figure 3-2A). Transpiration initially increased with rising VPD up to 2 kPa, and then leveled off or declined with further increases in VPD (Figure 3-2B; Berg et al.1986; El-Sharkawy 1990). Leaf conductance also declined sharply at VPD greater than 2 kPa (Figure 3-2C).

These observations clearly showed that cassava is sensitive to changes in atmospheric humidity, irrespective of plant- or soil-water status. Furthermore, compared with 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). The response was related to stomatal density and maximum leaf conductance (El-Sharkawy et al. 1984d, 1985; El-Sharkawy and Cock 1986). Cassava leaves possess large numbers of stomata on the abaxial epidermis (>400 stomata/mm^2; Pereira 1977; Connor and Palta 1981; El-Sharkawy et al. 1984b; Guzmán 1989), which may underlie its strong response to humidity (El-Sharkawy et al. 1985; El-Sharkawy and Cock 1986).

The phenomenon of direct stomatal response to air humidity was observed since the last century (Haberlandt 1914; Thoday 1938; El-Sharkawy and Cock 1986). Numerous reports showed that several herbaceous and woody plant species tended to close their stomata in response to dry air, whether within plant communities, attached leaves, or isolated epidermal strips (Hoffman and Rawlins 1971; Hoffman et al. 1971; Lange et al. 1971; Schulze et al. 1972; Aston 1976; Hall and Hoffman 1976; Lösch 1977, 1979; Rawson et al. 1977; Sheriff and Kaye 1977; Lösch and Schenk 1978; Ludlow and Ibaraki 1979; Tibbitts 1979; Farquhar et al. 1980; Hall and Schulze 1980; Jarvis 1980; Tazaki et al. 1980; Bunce 1981, 1982, 1984; Leverenz 1981; Lösch and Tenhunen 1981; Fanjul and

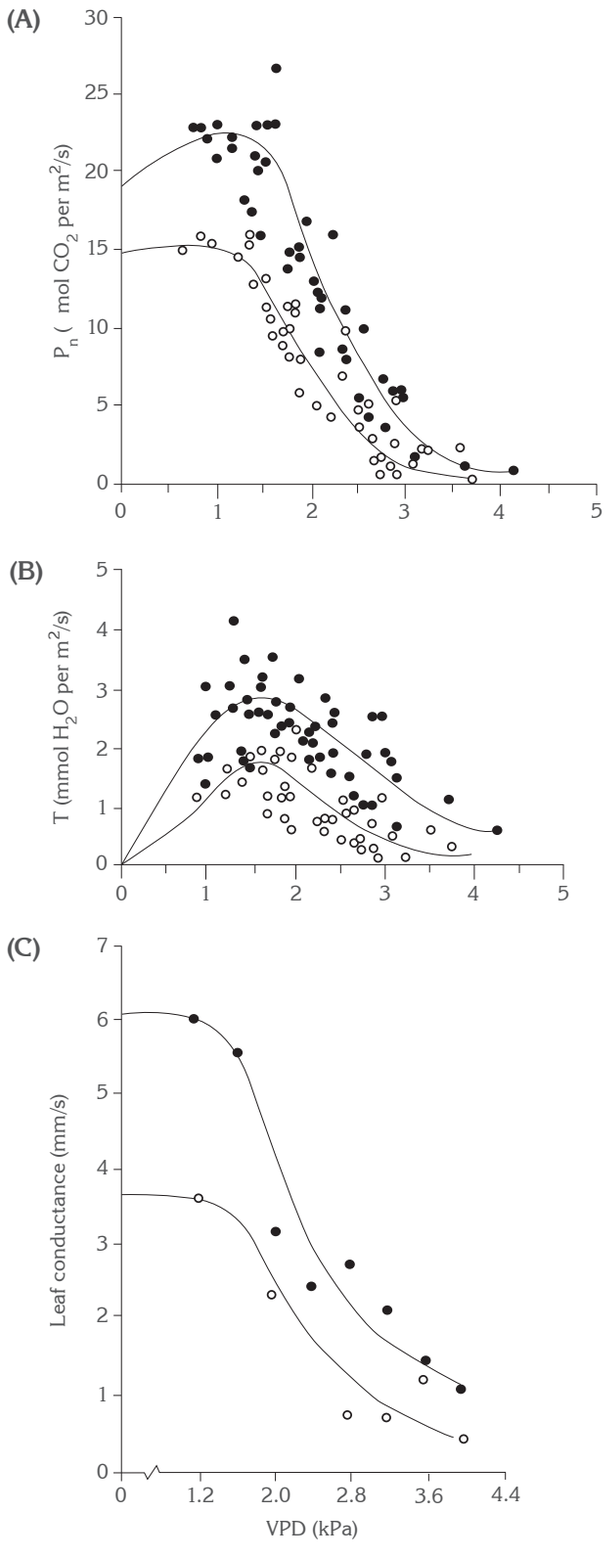

Figure 3-2. Responses of leaf photosynthesis (Pn) (**A**), transpiration (T) (**B**), and leaf conductance to water vapor (**C**) to stepwise increases in leaf-to-air vapor pressure deficit (VPD) in cassava cultivar M Col 90 (El-Sharkawy and Cock 1984).
○ refers to no watering; ● to well watered.

Jones 1982; Kaufmann 1982; Meinzer 1982; Schulze and Hall 1982; Gollan et al. 1985; Körner 1985; Körner and Bannister 1985; Jarvis and McNaughton 1986; Schulze 1986; Ward and Bunce 1986; Bongi et al. 1987; Hirasawa et al. 1988; Pettigrew et al. 1990; Held K 1991; Kappen and Haeger 1991; Tinoco-Ojanguren and Pearcy 1993).

This apparently widespread phenomenon indicated the need for detailed studies and for its consideration when modeling plant community/environment ecosystems (Jarvis and McNaughton 1986).

Mechanisms underlying stomatal response to air humidity

Stomatal movement is controlled by (1) stomata sensing changes in air humidity and (2) the so-called "peristomatal transpiration", first hypothesized by Seybold (1961/1962), where water is lost from the cuticle of the guard and subsidiary cells and their adjacent epidermal cells. The possible mechanisms underlying these activities were reviewed and discussed by many workers (Meidner and Mansfield 1968; Lange et al. 1971; Meidner 1976; Sheriff 1977, 1979, 1984; Lösch and Schenk 1978; Maier-Maercker 1979a, 1979b, 1983; Tyree and Yianoulis 1980; Lösch and Tenhunen 1981; Zeiger 1983).

Support for Seybold's hypothesis on the role of peristomatal transpiration was demonstrated through extensive research by German workers who used intact leaves and isolated epidermal strips systems without water stress (Lange et al. 1971; Maier-Maercker 1979a, 1979b, 1983; Lösch and Tenhunen 1981). Meidner and Mansfield (1968) argued that stomatal movements are unlikely to be affected by changes in atmospheric humidity, but instead by the water status of mesophyll tissue (feedback reaction).

Kramer (1983) cautioned against the hypothesized role of peristomatal transpiration until more information became available on the degree of cutinization of the mesophyll tissue (where most evaporation presumably takes place), and the inner and external walls of guard cells. Appleby and Davies (1983) demonstrated possible sites of evaporation from cuticle-free areas in the walls of guard cells of oak (*Quercus robur*), poplar (*Populus nigra*), and *Pinus sylvestris*, when these areas were exposed to the outside of the leaf during stomatal closure in dry air. Körner and Cochrane (1985) also reported relatively less cutinization of the external walls of guard cells in *Eucalyptus pauciflora*, which 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 a lower hydraulic conductivity within the leaf that probably accelerates water stress in the epidermis, regardless of leaf water content. Recently, Pieruschka et al. (2010) reported that the water balance in the epidermis is very sensitive to differences between the transpiration rate and the rate at which absorbed radiation produces water vapor inside the leaf. These authors suggested that leaf heat load is tightly linked to water transport from mesophyll cells, through the epidermis, to the leaf's environs.

This important finding further explains why cassava leaves orient themselves towards the sun in early morning and late afternoon (also called heliotropism or sun tracking) when VPD is lowest, and droop at mid-day (sun avoidance) when VPD is highest (El-Sharkawy and Cock 1984; Berg et al. 1986), thus optimizing water-use efficiency.

Tyree and Yanoulis (1980) used physical models of substomatal cavity to calculate water vapor diffusion patterns. They concluded that, because of high evaporation from guard cells, stomata could close in direct response to low humidity. They suggested that localized water stress or dehydration in guard cells may take place because of high leaf resistance to flow of water from minor leaf veins to guard cells.

A strong association between stomatal density (i.e., exposed epidermal areas) and the degree of sensitivity to changes in air humidity was observed in well-watered plants across many herbaceous and woody species (El-Sharkawy et al. 1985). Such an association may indicate the occurrence of localized dehydration in the stomatal apparatus and adjacent exposed epidermal cells. Hence, it supports a role for 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 (El-Sharkawy and Cock 1986) may accelerate water stress in the epidermis and stomatal apparatus. Hence, the striking sensitivity to changes in atmospheric humidity without any noticeable decrease in bulk leaf water potential (Figures 3-1 to 3-3; 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 conditions of high soil moisture and high bulk leaf water potential (El-Sharkawy 1990). Bunce (1985) also reported greater water loss from the outer surface of epidermis of herbaceous species under high wind speed, thus providing further evidence to support peristomatal transpiration.

Responses of field-grown cassava to air humidity, and implications for breeding for different edaphoclimatic zones and ecosystems

Cassava stomatal sensitivity to atmospheric humidity was also observed in field-grown cassava in soils with high moisture content at two sites: the mid-altitude, Palmira experiment station at CIAT HQ, Department of Valle del Cauca; and the low-altitude Carimagua ICA–CIAT station, Department of Meta (Figure 3-3A; Cock et al. 1985; Berg et al. 1986; El-Sharkawy 1990).

An array of cultivars, representing the core collection of cassava germplasm from different habitats, was grown at a third site: CIAT's mid-altitude experiment station at Santander de Quilichao, Department of Cauca. The cultivars showed significant differences in stomatal sensitivity to humidity (Figure 3-3C; El-Sharkawy 2004). Furthermore, total biomass and storage root yield were greater in high

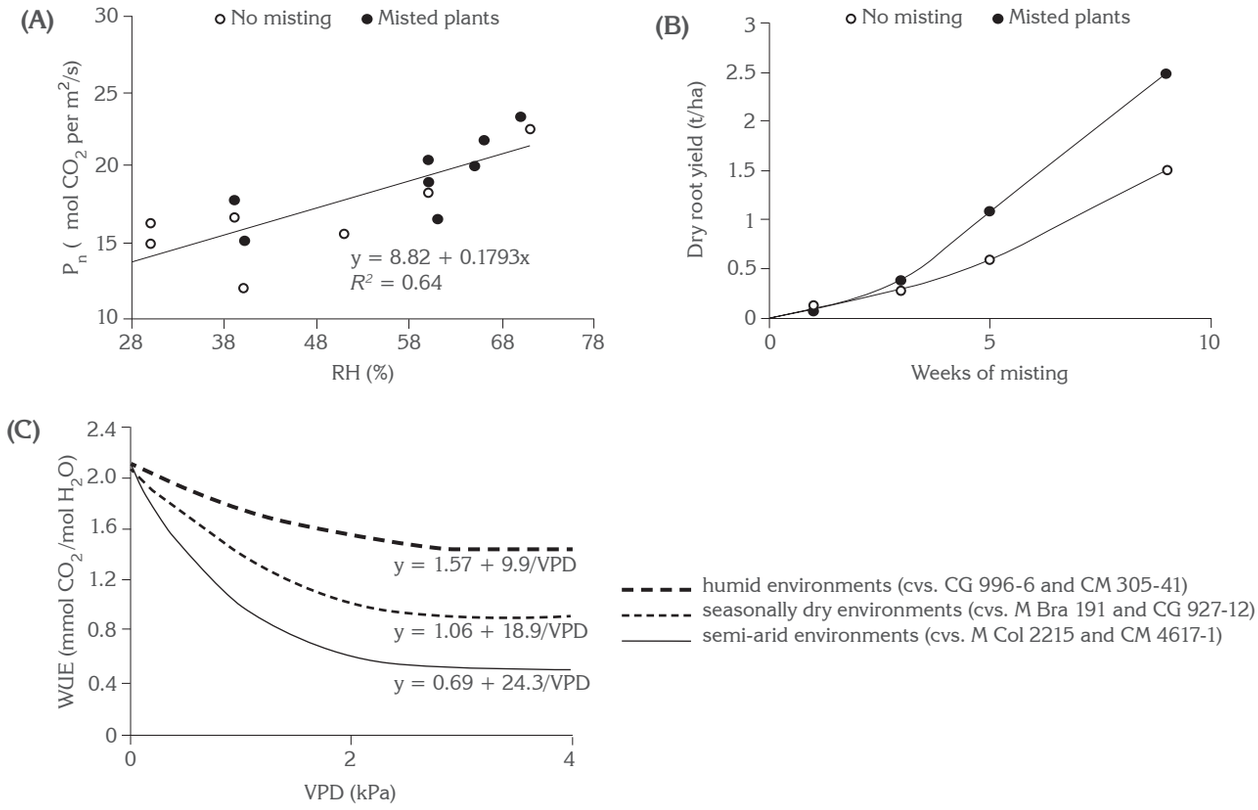

Figure 3-3. **(A)** Response of leaf photosynthesis (Pn) in cassava cultivar M Col 1684 to changes in air humidity in the field, with or without misting (Cock et al. 1985; El-Sharkawy and Cock 1986). (**B**) Oven-dried storage root yield of cv. M Col 1684 at periodic harvests after 3, 6, and 9 weeks of misting. Ages of plants at harvest were 65, 85, and 105 days, respectively. The differences in yield between the two crops were significant for all harvests ($P < 0.01$). Top biomass and leaf area index did not differ, whereas total biomass was significantly higher 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 vapor pressure deficit (VPD) in field-grown cassava in a mid-altitude, warm, subhumid climate. Levels of VPD progressively increased from morning to mid-day. Thirty-three clones were evaluated and were grouped according to humid, subhumid/seasonally dry, and semi-arid habitats. Sensitivity to VPD increased from humid to semi-arid habitats. Differences between plant groups illustrate the genetic diversity within cassava germplasm for response to changes in atmospheric humidity.

Adapted from El-Sharkawy 2004; MA El-Sharkawy, MC Amézquita, HF Ramírez, and G Lema 1991, unpublished.

humidity environments, particularly when enhanced by misting, leading to higher leaf photosynthesis. These findings indicated that stomatal sensitivity to changes in VPD was translated into growth at the canopy level (Figure 3-3B; Cock et al. 1985; El-Sharkawy and Cock 1986). Recent research on whole-plant-water relations of field-grown cassava under prolonged natural water deficit in Ghana (West Africa) showed that both canopy conductance and transpiration declined with increasing VPD (Oguntunde 2005; Oguntunde and Alatise 2007).

These findings have important practical implications for cassava breeding and improvement for different ecosystems and edaphoclimatic zones. For example, less sensitive cultivars should be selected and bred to maximize productivity in wet or humid zones such as the Amazon Basin, equatorial western Africa, and western Java in Indonesia; and in zones with short intermittent water deficits. For these cases, optimizing water-use efficiency is not of importance (El-Sharkawy and Cock 1986; El-Sharkawy 2004).

Less sensitive cultivars are those with hypostomatous leaves, that is, possessing lower stomatal density on leaf undersurfaces, and/or amphistomatous leaves, which possess equal conductance on both sides of the leaf blade. For more information on (1) leaf ontogenesis; (2) the impact on photosynthesis of stomatal density, size, and distribution patterns on both leaf sides; and (3) the comparative adaptive advantages of amphistomatic versus hypostomatic leaf characteristics, see Parkhurst 1978; Pospisilová and Solárová 1980; Mott et al. 1982; Tichá 1982; Gutschick 1984.

However, in subhumid or seasonally dry and semi-arid zones that characteristically have 3 months or more of water deficits, breeding and selecting for more sensitive cultivars is more advantageous. Such cultivars can conserve and deplete limited soil-water supplies more slowly. Thus, they optimize water-use efficiency, rather than maximize productivity, over a longer period during the growth cycle.

Because new leaf formation is highly 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 greater leaf retention. That is, leaves both live and last longer (El-Sharkawy 1993, 2004). Leaf retention was recently found, over a wide range of cultivars and breeding lines, to be positively correlated with productivity under naturally extended water deficits (Lenis et al. 2006). Moreover, leaves of plants subjected to imposed prolonged water stress (>2 months) in subhumid zones had 40% of the net photosynthesis found in well-watered plants. Yet, they were capable of completely recovering once the stress ended (CIAT Reports 1987 to 1994; El-Sharkawy 1993). Selecting for longer leaf life span helps save dry matter already invested in leaf canopy formation (Chabot and Hicks 1982). More assimilates would therefore be diverted towards storage roots, resulting in the crop having higher HI and harvestable yield (Cock and El-Sharkawy 1988a; El-Sharkawy 1993).

For seasonally dry zones, de Tafur et al. (1997b) reported a wide range of variation in net leaf photosynthesis among rainfed cassava, as measured in the field during the driest months. The photosynthetic rate (Pn) ranged from 27 to 31 μmol CO_2 per m^2/s, with significant differences among cultivars. In semi-arid zones, Pn ranged from 7 to 20 μmol CO_2 per m^2/s, with significant differences among cultivars. Such variation could be exploited to breed improved genotypes. Host-plant tolerance or resistance to pests and diseases must also be incorporated in cultivars targeted for seasonally dry and semi-arid zones to maintain, as much as possible, a functioning leaf canopy over an extended time (Byrne et al. 1982; Hershey and Jennings 1992; Bellotti 2002; Calvert and Thresh 2002; Hillocks and Wydra 2002).

Within cassava germplasm, a wide genetic diversity, useful for breeding programs, also exists for stomatal density, with a percentage of materials possessing amphistomatous leaves. Several accessions with a significant number of stomata on upper leaf surfaces have been identified. These, however, comprised less than 5% of more than 1500 landraces and cultivars that were screened in the field. The techniques used were the transient porometer and microscopic observations of leaf-surface replicas that were made by spraying leaves with collodion solution (El-Sharkawy et al. 1984b, 1985; Guzmán 1989).

Both porometry (Kirkham 2005) and leaf-surface replicas, combined with microscopic observation, are easy to handle in screening large numbers of breeding materials in the field for stomatal characterization. The leaf-replica method, however, has some limitations where leaves are hairy and stomata sunken (North 1956; Slávik 1971). The leaf-surface replica method was effective, using tissue-cultured young seedlings (El-Sharkawy et al. 1984b). It may facilitate early screening of larger populations. Zelitch (1962) described a similar technique for obtaining stomatal impressions, using silicon rubber, combined with cellulose acetate solution.

Response to Temperature

Responses of potted cassava grown outdoors in a high-altitude cool climate and in a mid-altitude warm climate

Cassava requires a warm climate for both optimal growth and productivity. However, it is also cultivated in cool climates at high altitudes in the tropics (>1700 m) and at low altitudes in the subtropics (Irikura et al. 1979). Growth and productivity depend largely on the leaf canopy's capacity to intercept solar radiation during most of the growth cycle. They also depend on leaf photosynthetic potential and performance under prevailing field conditions (Cock et al. 1979; El-Sharkawy et al. 1990; de Tafur et al. 1997a, 1997b; El-Sharkawy 2004). We therefore studied the effects of temperature on leaf photosynthesis during growth.

To obtain temperature differences under natural conditions, we took advantage of the nearness of a high-altitude site (2000 m; 17 °C mean annual temperature), located 18 km from CIAT HQ, itself located at about 965 masl, with a mean annual temperature at 24 °C. Several cultivars, representing several habitats, were grown in large pots (>40 L), which contained a mixture, by weight, of 40% top soil, 33% compost, 27% sand, and sufficient fertilizer. The potted plants were left outside and kept well-watered throughout their growth at the two sites. Solar radiation at the high-altitude location was similar to that of CIAT HQ in terms of duration and intensity (El-Sharkawy et al. 1992a, 1993).

Measurements of leaf gas exchange were conducted under controlled conditions in the physiology laboratory at CIAT. An open-ended, infrared, CO_2 analyzer was used to test responses to both leaf temperature at saturating photons levels (>1800 μmol per m^2/s) and to light intensity. Figures 3-4A to 3-4C (El-Sharkawy et al. 1984c, 1992a, 1993; El-Sharkawy and Cock 1990) illustrate responses of leaves grown in the cool climate and then acclimated for 7 days to the warm climate. They also show responses of leaves of the same plants that were later developed in the warm climate. The two cultivars were adapted to contrasting habitats: M Col 2059 from a cool, humid, high-altitude zone (Colombia) and M Bra 12 from a hot, humid, low-altitude zone (Brazil).

In both cultivars, net leaf photosynthetic rates were substantially lower in leaves that had developed in the cool high-altitude climate than for those that developed in the mid-altitude warm climate (Figures 3-4A and 3-4B). Leaves that had developed first in the cool climate and were then acclimated for 7 days in the warm climate partially recovered their photosynthetic capacities. Rates, however, remained much lower than those of the leaves developed in the warm climate.

In the hot-climate cultivar (M Bra 12), maximum rates in all sets of leaves were higher than those in the cool-climate cultivar (M Col 2059). This trend was also observed over the wide range of leaf temperatures tested. A wide temperature optimum of 25–40 °C and peaks at 30–35 °C were observed in the hot-climate cultivar for all sets of leaves. In the cool-climate cultivar, there was an apparent upward shift in optimal temperature in both the acclimated and warm-climate leaves. In contrast, a wide plateau occurred for the non-acclimated cool-climate leaves. In both cultivars and in all sets of leaves, rates declined rapidly at temperatures higher than 40 °C, reaching zero at 50 °C.

During the 7 days of acclimation in the warm climate, changes in non-stomatal components of photosynthesis (photosystems I and II, and CO_2 fixation reactions) were more likely than changes in physical stomatal characteristics (Berry and Björkman 1980). Moreover, the photosynthetic rates in cool-climate leaves were much lower at all photon levels and had less saturation irradiance than either the acclimated or warm-climate leaves (Figure 3-4C).

The differences in radiant energy saturated rates 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 at 1800 μmol per m^2/s. The same phenomenon was observed in several field-grown cassava cultivars in a warm climate when leaf photosynthesis was measured during the high rainfall period (Figure 3-5; El-Sharkawy and Cock 1990; El-Sharkawy et al. 1992a, 1993). Pereira (1977) also reported increases in cassava leaf net photosynthesis with rising photon flux density up to 2000 μmol per m^2/s. Maximum photosynthetic rates of several cultivars of field-grown cassava were more than 40 μmol CO_2 per m^2/s, with a mean C_i/C_a ratio of 0.42 (Table 3-1). These values are comparable with those observed in C_4 species and much less than those obtained in C_3 species.

These data indicate that cassava possesses high photosynthetic capacity, which is fully expressed only in hot humid climates with high solar radiation. Thus, when grown in environments, either natural or artificial, that deviate from these fundamental climatic

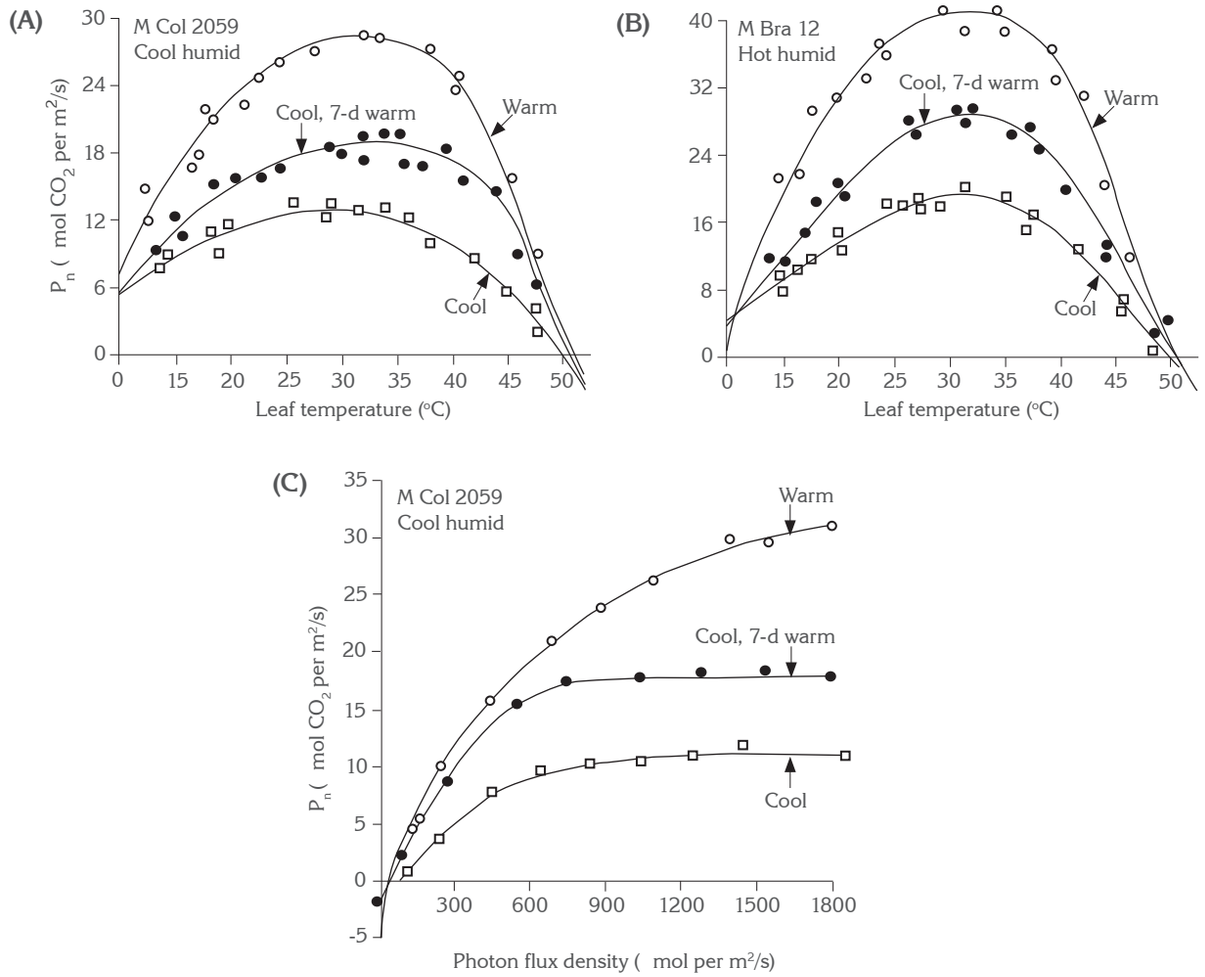

Figure 3-4. Response in terms of net photosynthetic rate (Pn) of cassava to leaf temperature. **(A)** Cultivar M Col 2059 in a cool habitat; **(B)** cv. M Bra 12 in a hot humid habitat; **(C)** response in terms of net photosynthetic rate (Pn) to PAR irradiance in cv. M Col 2059. □ refers to leaves developed in a cool climate; ● to leaves developed in a cool climate and then acclimated for 1 week in a warm climate; ○ to newly developed leaves in a warm climate.
Note that (1) an apparent upward shift in optimal temperature is observed from cool to warm-acclimated and warm-climate leaves; (2) the lack of light saturation in warm-climate leaves, compared with cool-and-warm-acclimated leaves; and (3) the higher maximum photosynthetic rates in all sets of leaves of cv. M Bra 12 from the hot-humid habitat, compared with the cool-climate cv. M Col 2059.
References: CIAT Report 1992; El-Sharkawy et al. 1992a, 1993.

requirements, its photosynthetic capacity is not fully expressed (Gleadow et al. 2009).

Studies of plants grown in the greenhouse or growth chamber showed much lower photosynthetic rates (from 15 to 20 μmol CO_2 per m^2/s), lower saturation radiation, lower optimal temperatures, and lower photosynthetic enzyme activity (Aslam et al. 1977; Mahon et al. 1977a, 1977b; Edwards et al. 1990; Angelov et al. 1993; Ueno and Agarie 1997; Gleadow et al. 2009). These studies are of limited value if their results are to be interpreted in relation to cassava's real potential 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 photosynthetic rates of potted cassava grown in growth chambers or greenhouses probably resulted from lower activities of photosynthetic enzymes. Such results have been long observed in other plant species. Other factors may have also played a part such as changes in leaf anatomy because of exposure to suboptimal irradiance and air temperatures during leaf development; imbalances in source-sink relations in the whole plant system; and pot size, which

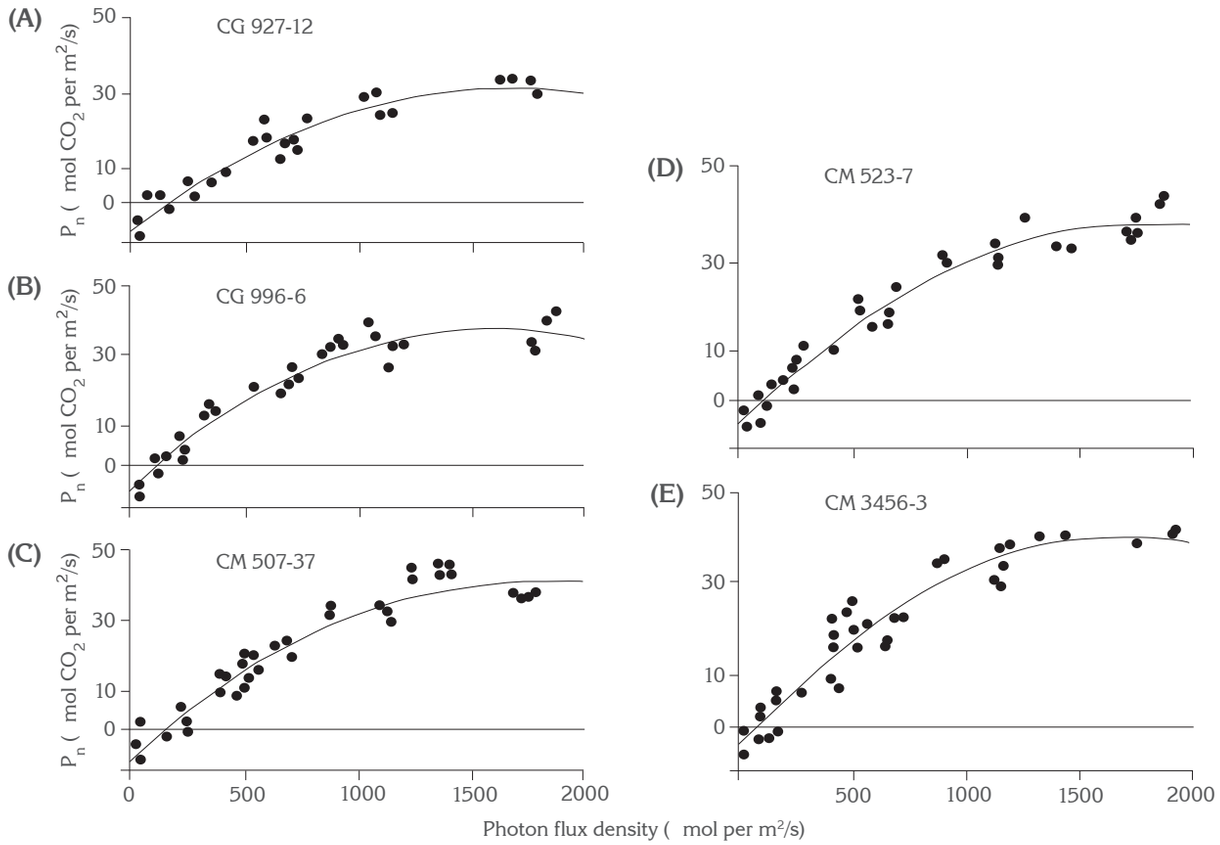

Figure 3-5. Responses in terms of leaf photosynthesis (Pn) to PAR irradiance in upper-canopy leaves of five cultivars of field-grown cassava during the rainy season (CIAT Report 1992; El-Sharkawy et al. 1992a, 1993).

may have encouraged feed-back inhibition of leaf photosynthesis because of restricted root sinks for assimilates (Nösberger and Humphries 1965; Humphries 1967; Neales and Incolls 1968; Moss and Musgrave 1971; Nobel 1976, 1980; Boardman 1977; Björkman et al. 1980; Herold 1980; Nobel and Hartsock 1981; Sesták 1985; Bunce 1986; Ho 1988; Wardlaw 1990; Evans 1993; Pellet and El-Sharkawy 1994; Gleadow et al. 2009).

El-Sharkawy (2005) recently reviewed and discussed the problems of plant acclimation/adaptation to environments that are normally encountered but bewilder scientists in general and plant photosynthesis researchers in particular. He pointed out the limited value of data collected on plants grown in environments that are inappropriate for optimal growth. He also emphasized their invalidity for use in crop modeling or extrapolating and predicting responses in natural environments if the necessary calibration is not carried out, that is, field data collected under the conditions in which plants or crops are normally grown must be taken into account for these purposes.

This conclusion on the invalidity of data obtained with plants inappropriately grown was further substantiated by recent findings in a wide range of long-term CO_2-enrichment field trials (Long et al. 2006). In these trials, the degree of enhancement in both leaf photosynthetic rate and yield of various crops by elevated CO_2 (as compared with crops grown at ambient CO_2) were much less than those previously observed with plants grown in greenhouses, growth chambers, or field enclosures, where air humidity and temperatures were probably also elevated. Such findings confirmed the limitations of using data from inappropriately grown plants for crop modeling or predicting anticipated effects of rising atmospheric CO_2 levels and air temperature on plant photosynthesis and productivity (i.e., the effects of global climate change) (Gleadow et al. 2009).

Table 3-1. Net leaf photosynthesis (P_n) of field-grown cassava at Santander de Quilichao, Department of Cauca, Colombia (warm subhumid), during the 1990/91 season. Maximum photosynthetic rates were obtained during wet periods and high air humidity. Note the C_i/C_a values, which are comparable with those of C_4 species and much lower than those of C_3 species, indicating cassava's high photosynthetic capacity, as expressed in near-optimal environments. In this group of cultivars, the average seasonal P_n was correlated with final root yield.

Cultivar	Maximum net photosynthesis (n = 6) μmol CO_2 per m^2/s	C_i/C_a (n = 6)	Seasonal average net photosynthesis (n = 30) μmol CO_2 per m^2/s
CG 996-6	49.7	0.37	33.8
M Bra 191	47.4	0.37	35.5
CM 4864-1	45.1	0.39	34.0
CM 4145-4	43.9	0.40	31.7
CM 3456-3	43.7	0.43	31.9
CM 507-37	43.7	0.38	28.7
CM 4716-1	43.6	0.42	31.8
M Col 1684	43.0	0.42	30.9
CM 4575-1	42.8	0.39	33.2
CM 4617-1	42.8	0.46	31.4
CM 523-7	42.3	0.45	30.1
CMC 40	42.3	0.44	30.3
CM 4701-1	42.2	0.45	30.9
CM 4711-2	41.3	0.45	30.9
CG 927-12	39.3	0.43	26.2
Mean of all cultivars	43.5	0.42	31.4
$LSD_{0.05}$	1.70	0.08	1.80

C_i/C_a = intercellular CO_2 divided by atmospheric CO_2. This ratio is commonly used to differentiate plant species according to their photosynthetic capacities, that is, the lower the ratio, the higher the capacity.

SOURCES: El-Sharkawy et al. (1992a, 1993).

Screening Cassava Germplasm for Leaf Photosynthesis

Field evaluation of cassava germplasm for leaf photosynthesis in subhumid, high-altitude, cool climates, and mid-altitude warm climates

Once we ascertained the importance of field research and the need to assess cassava's potential photosynthesis under representative environments, we studied photosynthesis in recently matured upper-canopy leaves. The germplasm used was selected from a core collection of the cassava genebank held at CIAT. Materials comprised groups of cultivars, landraces, and improved CIAT breeding materials grown at three sites used by CIAT's cassava breeding program to evaluate genetic performance. We wanted to identify, in the field, those cultivars and lines with high photosynthetic potential. They would then be used as parental materials in crosses and breeding procedures to improve productivity (El-Sharkawy 1993) in combination with other major breeding objectives such as yield stability; broad adaptation; and tolerance or resistance to edaphoclimatic stresses, pests, and diseases (Hershey and Jennings 1992; Jennings and Iglesias 2002).

This objective was justified by our previous research at different sites in subhumid, seasonally dry, and semi-arid environments. We measured photosynthesis in the field, using portable infrared gas analyzers across a wide range of germplasm and edaphoclimatic conditions.

Results showed significant correlations between upper-canopy leaf photosynthesis and total biomass and storage root yields (Figure 3-6; CIAT Reports 1987 to 1995; El-Sharkawy and Cock 1990; El-Sharkawy et al. 1990, 1993; Pellet and El-Sharkawy 1993a; de Tafur et al. 1997b; de Tafur 2002; El-Sharkawy 2004, 2006). Moreover, the findings at CIAT were corroborated by later research at IITA (Nigeria), where photosynthetic rates of upper-canopy leaves were correlated with storage root yields across diploid, triploid, and tetraploid cassava cultivars (Ekanayake et al. 2007).

Tables 3-2 to 3-4 present data on upper-canopy leaf photosynthesis measured in two climates: (1) the

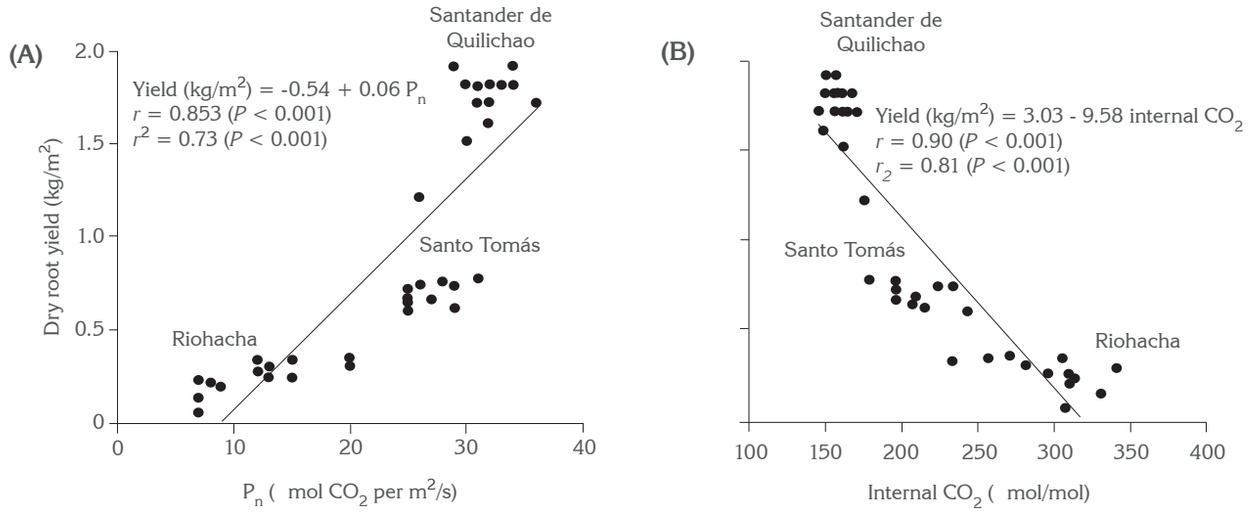

Figure 3-6. Relationships between dry root yield and upper-canopy leaf photosynthesis (P_n) **(A)** and intercellular CO_2 **(B)** for 38 cassava cultivars grown at three sites in Colombia: Santander de Quilichao (subhumid, 15 varieties), Santo Tomás (seasonal dry, 10 varieties), and Riohacha (semi-arid, 13 varieties) (El-Sharkawy et al. 1993; de Tafur et al. 1997b; de Tafur 2002; SM de Tafur and MA El-Sharkawy 1995, unpublished).

Table 3-2. Net leaf photosynthesis (P_n; μmol CO_2 per m^2/s), stomatal conductance (mmol per m^2/s), and internal CO_2 (μmol CO_2/mol) for some cassava clones with relatively high photosynthetic capacity. Note that, for this group of clones, the P_n rates are higher, and the stomatal conductance and internal CO_2 are lower than the trial means. The values indicate the importance of non-stomatal factors (i.e., biochemical and anatomical) in selecting for enhanced photosynthetic capacity. This group of clones will improve the genetic base of cassava for the cooler ecosystems of high-altitude tropics and subtropics.

Clone	P_n	Stomatal conductance	Internal CO_2
SM 1061-1	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 of all accessions (n = 107)	12.3	312	183
$LSD_{0.05}$	1.3	32	14

SOURCE: CIAT Report (1994).

high-altitude cool climate of Cajibío, Department of Cauca (about 1800 m; mean annual temperature at about 19 °C); and (2) the mid-altitude warm climate at two sites (CIAT Quilichao experiment station, Cauca, and CIAT Palmira station at HQ, Valle del Cauca), where altitudes range between 965 and 1000 m, and the mean annual temperature is about 24 °C. Crops were grown under rainfed conditions with minimal applications of fertilizer. Measurements were made on several occasions, mainly during dry periods, and averaged. Chambers enclosing central leaf lobes or part thereof (depending on the type of equipment and leaf chambers used) were always directed towards the sun between 09:00 and 12:00 local time when photon flux densities were greater than 1000 μmol per m^2/s. The plants used were 4 to 6-month-old plants, that is, of an age when leaf canopies nearly close (i.e., high leaf capacity source) and rates of storage root bulking are at their highest (high root sink demand).

At all sites, average leaf photosynthesis varied significantly among screened cultivars and landraces, but with rates greatly reduced in the high-altitude cool climate, thus confirming results and patterns observed with potted cassava grown in a high-altitude cool climate (Tables 3-2 to 3-4 and Figure 3-4). The accessions evaluated in the high-altitude cool climate were local traditional cultivars or landraces collected from cool-climate regions in several countries. They also included improved CIAT materials bred and selected for better adaptation to high-altitude cool climates.

The materials with rates (15.7 to 17.3 μmol CO_2 per m^2/s) that ranked higher than the overall mean photosynthetic rate (12.3) were four CIAT improved clones and a Peruvian cultivar (M Per 501) (Table 3-2). This finding indicates a narrow genetic base for this ecosystem. It also shows the relative effectiveness of the CIAT cassava program's strategy to breed for specific

Table 3-3. Net leaf photosynthesis (P_n; μmol CO_2 per m^2/s) in the upper-canopy leaves of cultivars from the core collection of cassava germplasm and grown at Santander de Quilichao in 1993/94. Measurements were carried out 5 to 6 months after planting, using portable infrared gas analyzers. Values are means of 7 to 11 measurements made during the dry period. Compare these higher P_n values, obtained within a warm subhumid habitat, with those obtained in the cool subhumid habitat shown in Table 3-2. Cultivar M Mal 48, from Malaysia, had the highest P_n rate and the highest dry root yield in this trial.

Clone	P_n	Clone	P_n
M Mal 48	27.6	M Tai 1	24.4
M Bra 900	27.6	M Pan 51	24.3
M Bra 12	26.8	M Bra 383	24.2
M Bra 191	26.7	M Ind 33	24.1
M Mal 2	26.4	CM 849-1	23.6
HMC-1	26.0	M Col 1684	23.4
CMC 40	25.8	M Mex 59	23.2
M Col 2061	25.4	M Ven 25	23.2
M Gua 44	25.4	M Bra 885	23.1
M Chn 1	25.3	M Cub 51	22.8
M Col 22	25.1	M Col 2215	22.3
M Arg 13	25.0	M Cub 74	22.3
M Ven 45A	24.8	M Per 205	22.0
M Col 1505	24.8	M Ptr 19	21.3
M Bra 110	24.6	M Ecu 82	21.0
$LSD_{0.05}$	4.8		

SOURCE: CIAT (1994).

edaphoclimatic zones and ecosystems. It also points out the importance of including leaf photosynthesis as a selection criterion for parental materials when enhancing productivity (El-Sharkawy and Cock 1990; El-Sharkawy et al. 1990; El-Sharkawy 2004).

The enhanced photosynthesis in these few clones could not be attributed to stomatal control because their average stomatal conductance (271 mmol per m^2/s) was significantly lower than the overall mean of accessions (312 mmol per m^2/s; Table 3-2). However, the intercellular CO_2 concentration was much reduced in these clones, thus indicating possible control by non-stomatal factors such as leaf anatomy and biochemistry (e.g., enzyme activity). As leaf formation is much slower but leaf life much longer in high-altitude cool climates than under warm-climate conditions (Irikura et al. 1979), selection for enhanced photosynthesis and tolerance of low temperature becomes even more important in this case. In the mid-altitude warm-climate sites, particularly at CIAT–HQ, average photosynthesis rates were much higher than in the cool-climate site (Tables 3-3 and 3-4). Measurements were all made during the dry period, when rates were lower than the maximum rates observed under wet conditions (Table 3-1).

Most of the materials evaluated at CIAT–HQ comprised cultivars and landraces from Brazil, with eight accessions from Argentina and one accession each from Colombia (HMC-1) and Bolivia (M Bol 1). The mean photosynthesis rate was significantly higher in the smaller group of germplasm materials from Argentina (26 μmol CO_2 per m^2/s) than in the germplasm from Brazil (Table 3-4), many accessions of which had lower rates than their original overall mean of 22 μmol CO_2 per m^2/s. Even so, several Brazilian accessions fell into the highest photosynthesis range, particularly M Bra 12 (Figure 3-4B) and M Bra 110.

These two materials could be used for crossing and breeding for warm-climate ecosystems. In contrast, the accessions from Argentina, presumably more adapted to subtropical ecosystems, better tolerated low winter temperatures than the warm-climate germplasm from tropical ecosystems. They could therefore be used in crossing and breeding for enhancing photosynthesis in germplasm for high-altitude cool-climates.

The P_n of this group of accessions was highly and negatively correlated with intercellular CO_2 (Figure 3-7). As P_n was measured in normal air, the calculated intercellular CO_2 concentration represents the balance

Table 3-4. Net leaf photosynthesis (P_n; μmol CO_2 per m^2/s) of upper-canopy leaves and intercellular CO_2 (μmol CO_2/mol) for 53 accessions from the core collection of cassava germplasm and grown at CIAT headquarters, Palmira, Colombia, in the 1991/92 season. Measurements of 4-month-old plants were made, using portable infrared CO_2 analyzers during dry periods. Compare these higher P_n rates in the warm subhumid habitat with those obtained in the cool subhumid habitat, as shown in Table 3-2. Note that most accessions from Argentina and Brazil had relatively high P_n rates, compared with trial means. Note that, in this group of clones, P_n was highly negatively correlated with intercellular CO_2 ($r^2 = 0.90$, $P < 0.0001$), indicating that differences in P_n were caused by non-stomatal factors, that is, anatomical and/or biochemical factors such as enzyme activity and leaf anatomy. Regression: intercellular $CO_2 = 315 - 7.83\ P_n$.

Accession	P_n	Intercellular CO_2	Accession	P_n	Intercellular CO_2
		Accessions with high P_n			
M Arg 11	32	73	M Bra 85	26	112
M Bra 12	30	82	M Arg 9	26	110
M Bra 110	30	74	M Bra 190	26	97
HMC-1	29	87	M Bra 124	26	100
M Arg 2	28	95	M Bra 403	25	131
M Bra 132	28	88	M Bra 162	25	108
M Bra 172	27	83	M Arg 5	25	115
M Arg 13	27	105	M Bra 242	24	133
M Bra 359	27	127	M Bra 299	24	133
M Bra 71	26	111	M Bra 165	24	114
M Arg 7	26	119			
		Accessions with intermediate to low P_n			
M Bra 243	23	132	M Bol 1	21	147
M Bra 405	23	143	M Bra 309	21	148
M Bra 73	23	134	M Bra 453	21	159
M Bra 217	23	140	M Bra 400	21	169
M Bra 404	23	151	M Bra 329	20	159
M Bra 125	23	129	M Bra 435	20	173
M Bra 258	22	143	M Bra 337	19	177
M Bra 273	22	128	M Bra 158	19	137
M Bra 77	22	145	M Bra 325	18	169
M Bra 233	22	144	M Bra 237	17	173
M Arg 12	22	146	M Bra 355	17	195
M Bra 416	22	151	M Bra 450	17	196
M Arg 15	22	139	M Bra 328	17	182
M Bra 356	22	156	M Bra 311	16	188
M Bra 191	21	145	M Bra 315	16	191
M Bra 383	21	162	M Bra 335	14	187
$LSD_{0.05}$	6.2	23			

SOURCE: CIAT Report (1992).

between the supply from outside air via stomata and the demand from carboxylation reactions within the mesophyll. The lower intercellular CO_2 in accessions with high Pn indicates a faster carboxylation rate, probably because of higher rubisco activity inside the chloroplasts and/or higher activity of phospho*enol*pyruvate carboxylase (PEPC) in the cytosol of mesophyll cells. This finding indicates the need to select and breed for higher activity of key photosynthetic enzymes.

The accessions screened at Quilichao were a mix of cultivars and landraces, mostly from Latin America, but also from Asia (Table 3-3). Again, average photosynthesis rates 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 versus the overall mean for the trial at 10.6 t/ha). This clone has already been used for crossing and breeding at CIAT.

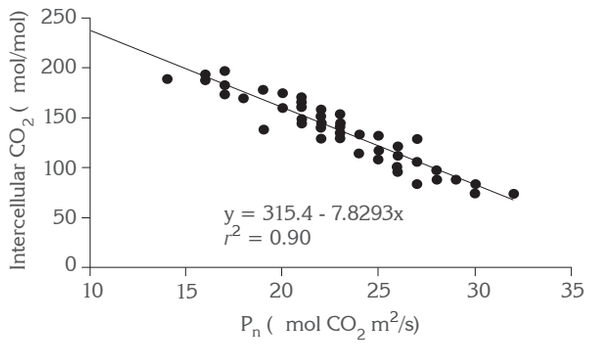

Figure 3-7. Relationships between P_n and intercellular CO_2 concentration (C_i) in 53 cassava accessions (Table 3-4). Note the negative correlation between P_n and C_i, which indicates that the association was caused by non-stomatal factors (i.e., biochemical and/or anatomical mesophyll traits) (SM de Tafur and MA El-Sharkawy 1995, unpublished).

Evaluating cassava germplasm for leaf area duration (seasonal average leaf area index) and productivity in a mid-altitude warm climate

To complement the joint physiology/breeding efforts to characterize cassava germplasm from the core collection and identify useful yield-determinant traits, a field trial was conducted at the CIAT–Quilichao experiment station (mid-altitude warm climate). Thirty clones were evaluated for leaf duration across the growth cycle (seasonal average leaf area index as measured with a leaf canopy analyzer). Table 3-5 (CIAT Report 1995) presents data on yield, shoot and total biomass, seasonal average leaf area index, and root dry matter content.

Wide variations among clones were found for standing shoot (i.e., top biomass, excluding dropped leaves) and total biomass, yield, dry matter content in roots, and seasonal leaf area index. Notably, several accessions from Brazil were among the highest ranked in terms of yield, total biomass, and dry matter contents in storage roots, thus highlighting the importance of Brazilian germplasm (El-Sharkawy and de Tafur 2010).

CIAT, to diversify the genetic base of the cassava genebank, has incorporated many of these accessions for their useful plant traits. Outstanding among these is clone M Bra 12, with its high leaf photosynthesis under both potted (grown outdoors in a mid-altitude warm climate) and field-grown conditions (Table 3-4; Figure 3-4B), high yield, and resistance to mites (Byrne et al. 1982). Two other accessions of Brazilian origin, M Bra 383 and M Bra 191, which ranked high in this group of clones, were also reported as being among the highest ranked clones (fourth and fifth, respectively, among 33 clones evaluated) for tolerance of soils low in phosphorus (CIAT Report 1990; El-Sharkawy 2004).

In this group of accessions, standing shoot biomass correlated with root yield ($r = 0.7$; $P < 0.001$). This finding confirms previous findings, and suggests the use of this trait as a proxy for leaf area formation and duration when evaluating large breeding populations (CIAT Report 1990; El-Sharkawy et al. 1990; El-Sharkawy 2004). In this group of clones, dry root yield also correlated with seasonal leaf area index (Figure 3-8; $r = 0.65$; $P < 0.001$), further corroborating earlier reports (Pellet and El-Sharkawy 1993a). It also supports the concept of breeding for longer leaf life and optimal leaf area duration to maximize productivity under favorable conditions and to ensure sustainable yields in stressful environments (El-Sharkawy and Cock 1987b; Cock and El-Sharkawy 1988a, 1988b; El-Sharkawy et al. 1992b; El-Sharkawy 1993, 2004; Lenis et al. 2006).

In conclusion, although leaf photosynthesis can be used as a selection criterion in cassava improvement programs, it may be difficult to handle when evaluating large breeding populations. It should be included at least in the evaluation and selection 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), and longer leaf life (greater leaf retention and duration over the growth cycle; El-Sharkawy and Cock 1987b; Cock and El-Sharkawy 1988a, 1988b; El-Sharkawy et al. 1992b; El-Sharkawy 1993, 2004; Lenis et al. 2006). Recent advances in molecular biology and the development and manufacture of more precise techniques, methods, and equipment can only enhance and speed up the elucidation of fundamental mechanisms underlying photosynthetic potential and associated beneficial traits, and their controlling genes.

Responses to Extended Water Shortages Imposed at Different Growth Stages in the Field

Unlike grain crops, cassava does not have specific water-stress sensitive growth stages beyond crop establishment. It is therefore highly tolerant of prolonged drought in areas that typically have low (<600 mm annually) and erratic precipitation, dry air and high temperatures (i.e., potential for high

Table 3-5. Seasonal average leaf area index (LAI), dry root yield, top and total biomass, and root dry matter content of 30 clones from the core collection of cassava germplasm. Plants were grown at Santander de Quilichao, Colombia, in the 1994/95 season. Leaf area duration, as estimated by seasonal average LAI, was significantly correlated with root yield (Figure 3-8), indicating the importance of this trait in selecting and breeding for improved cultivars (Lenis et al. 2006).

Clone	Seasonal average LAI (m^2/m^2)	Dry top biomass (t/ha)	Dry root (t/ha)	Total biomass (t/ha)	Root dry matter (%)
M Bra 383	1.3	6.0	15.5	21.5	41.7
M Bra 12	1.0	5.3	15.3	20.6	38.2
M Pan 51	1.8	10.2	14.7	23.9	40.8
CM 849-1	1.4	6.3	14.1	20.4	41.7
M Bra 191	1.5	5.7	14.0	19.7	41.3
M Mal 48	1.9	4.2	13.9	18.1	41.0
M Bra 885	1.6	5.4	13.8	19.2	41.6
M Ven 25	1.1	4.9	12.6	17.5	40.0
HMC-1	1.6	4.8	12.2	17.0	38.5
M Ind 33	1.6	5.9	11.6	17.5	36.6
M Bra 100	1.9	6.4	11.3	17.7	40.6
M Gua 44	1.2	4.6	11.2	15.8	38.9
M Cub 74	0.9	3.9	10.8	14.7	39.8
M Tai 1	1.2	5.6	10.6	16.2	37.5
M Mex 59	1.6	6.8	10.3	17.1	40.7
M Arg 13	0.9	2.4	9.3	11.7	39.1
M Mal 2	1.8	6.9	8.4	15.3	36.2
M Chn 1	1.0	1.9	8.0	9.9	36.2
M Col 22	1.4	1.7	7.8	9.5	37.8
M Ven 45A	1.0	5.1	7.7	12.8	37.4
M Col 1684	0.8	2.2	7.0	9.2	34.6
M Ecu 82	0.8	3.4	6.7	10.1	38.6
M Ptr 19	1.1	4.0	6.1	10.1	40.0
M Col 2215	0.7	2.4	5.9	8.3	40.3
CMC 40	0.9	1.9	5.6	7.5	36.7
M Col 2061	0.9	3.5	5.3	8.8	30.7
M Per 205	1.0	3.8	5.3	9.1	38.7
M Col 1505	0.7	2.1	5.1	7.2	40.6
M Cub 51	0.7	3.1	4.9	8.0	39.9
M Bra 900	0.9	1.3	4.7	6.0	31.4
Mean of all clones	1.2	4.4	9.7	14.0	39.6
$LSD_{0.05}$	0.5	2.0	3.5	4.6	3.6

SOURCE: CIAT Report (1996).

evapotranspiration), low-fertility soils, and high pest-and-disease pressure. Examples of areas with such conditions include Northeast Brazil, Colombian North Coast, the Peruvian coastal regions, some areas of sub-Saharan Africa, and parts of Thailand (El-Sharkawy 1993).

Under these conditions, other staple food crops such as grain cereals and legumes, will rarely survive and produce. That cassava can grow in such areas is contrary to the common assumption that it originated in the hot humid climates of the Amazon forests. Indeed, Allem (2002) suggests that cassava may, in fact, have originated in the open savanna forests of Brazil. This inherent capacity to withstand drought is also behind the crop's expansion into more marginal lands across many parts of Africa, Asia, and Latin America, where it is grown by resource-poor farmers.

We have already mentioned some inherent plant mechanisms that may underlie such tolerance. Most notable among them is the cassava plant's striking sensitivity to both changes in atmospheric humidity and soil-water deficits. It reacts by partly closing its

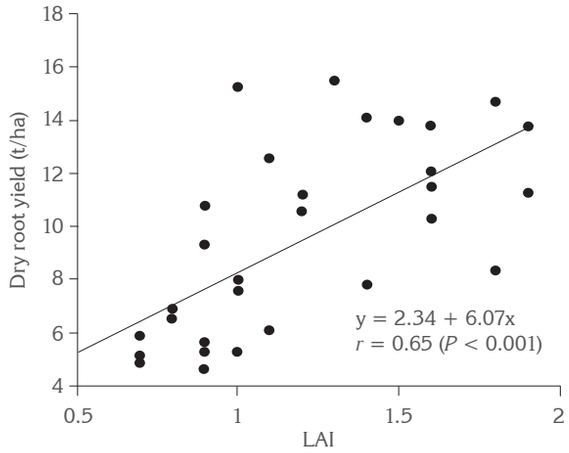

Figure 3-8. Relationships between final dry root yield of 30 clones from the core collection of cassava germplasm and seasonal average leaf area index (LAI). The significant correlation indicates the importance of leaf area duration for yield formation. Because of the small leaf area canopy in the first 3 months and in the final 8 to 12 months, the seasonal average canopy area still limits yield. It indicates the need to select and breed for a higher and more sustainable canopy that would last most of the growth cycle, combined with enhanced leaf photosynthesis, high harvest index, and strong root sink (larger storage root number/plant). Cultivar M Bra 12 had a notably higher yield with an LAI that was less than the overall average of the trial, indicating its high leaf photosynthetic potential (CIAT Report 1995; El-Sharkawy 2006).

stomata and restricting water losses, once it is exposed to dry air and/or dry soils. Thus, the leaf is protected from severe dehydration. Such sensitivity is also coupled with the leaf's ability to partly retain its photosynthetic capacities under prolonged water shortages (El-Sharkawy et al. 1992b; El-Sharkawy 1993; Caýon et al. 1997; de Tafur et al. 1997a, 1997b).

Moreover, the cassava plant, despite its sparse fine-root system, is able to penetrate soil layers at 2 m or deeper, unlike other crops such as cereals and tropical grasses (Tscherning et al. 1995). Thus, the plant can endure long periods of drought. Moreover, it is slow to deplete the deeper stored water, resulting in higher seasonal crop water-use efficiency, although at reduced productivity (Connor et al. 1981; El-Sharkawy and Cock 1986, 1987b; El-Sharkawy et al. 1992b; El-Sharkawy 1993, 2004).

We report here on further research with diverse germplasm that was exposed at various growth stages to long periods (3–6 months) of water shortages. We used a large field drainage lysimeter (30 × 15 × 2.3 m deep) at CIAT–Quilichao, which was excavated and refilled with the same soil layers (El-Sharkawy and Cock 1987b). We also used adjacent undisturbed larger areas.

Water stress was always initiated by covering soil with high moisture content with caliber-6 white plastics, which were manually kept free of rainwater and of ruptures or leaks during stress periods. Soil water was periodically monitored by sampling or by using a calibrated neutron meter at a 1.8–2 m depth. Leaf water potential was assessed with the standard pressure chamber technique (Kirkham 2005). Leaf gas exchange was measured with portable infrared gas analyzers, and leaf area coverage/index was measured with a solar-irradiance sensing analyzer (leaf canopy analyzer; LI-COR Biosciences, Inc., Lincoln, NE, USA). Periodic harvests were also conducted to determine yield and biomass (CIAT Reports 1987 to 1995; El-Sharkawy and Cock 1987b; El-Sharkawy et al. 1992b, 1998b; Cayón et al. 1997; de Tafur et al. 1997a, 1997b; El-Sharkawy and Cadavid 2002).

Evaluating Germplasm under Mid-Season Water Stress in a Field Drainage Lysimeter

Relationship between productivity and hydrocyanic acid (HCN) levels

Sixteen cultivars from a core collection of cassava germplasm were evaluated across two cropping cycles in a field drainage lysimeter. For each cycle, at 90 to 100 days after planting, 3 months of water stress was initiated (mid-season stress). Figure 3-9 illustrates dry root yield accumulation patterns for four representative accessions affected by stress during the growth cycle.

Water stress significantly reduced yield and shoot biomass in all accessions at the end of the stress (data not shown; CIAT Reports 1991, 1992; El-Sharkawy et al. 1992b). On recovery from stress, however, final yields of some accessions were equal to those of well-watered plants, while final yields in others were reduced (Table 3-6; El-Sharkawy 1993). Significant differences for final root yield were also found among cultivars, with the hybrid CM 489-1 having the highest yield under both stress (18 t/ha oven-dried roots) and non-stress (19 t/ha) conditions. Also noteworthy is CM 489-1, which showed high PEPC activity in leaf extracts under extended field water shortages.

In a group of cultivars, high PEPC activity correlated with leaf photosynthesis (P_N) (El-Sharkawy 2004). The parameter P_N also correlated with high

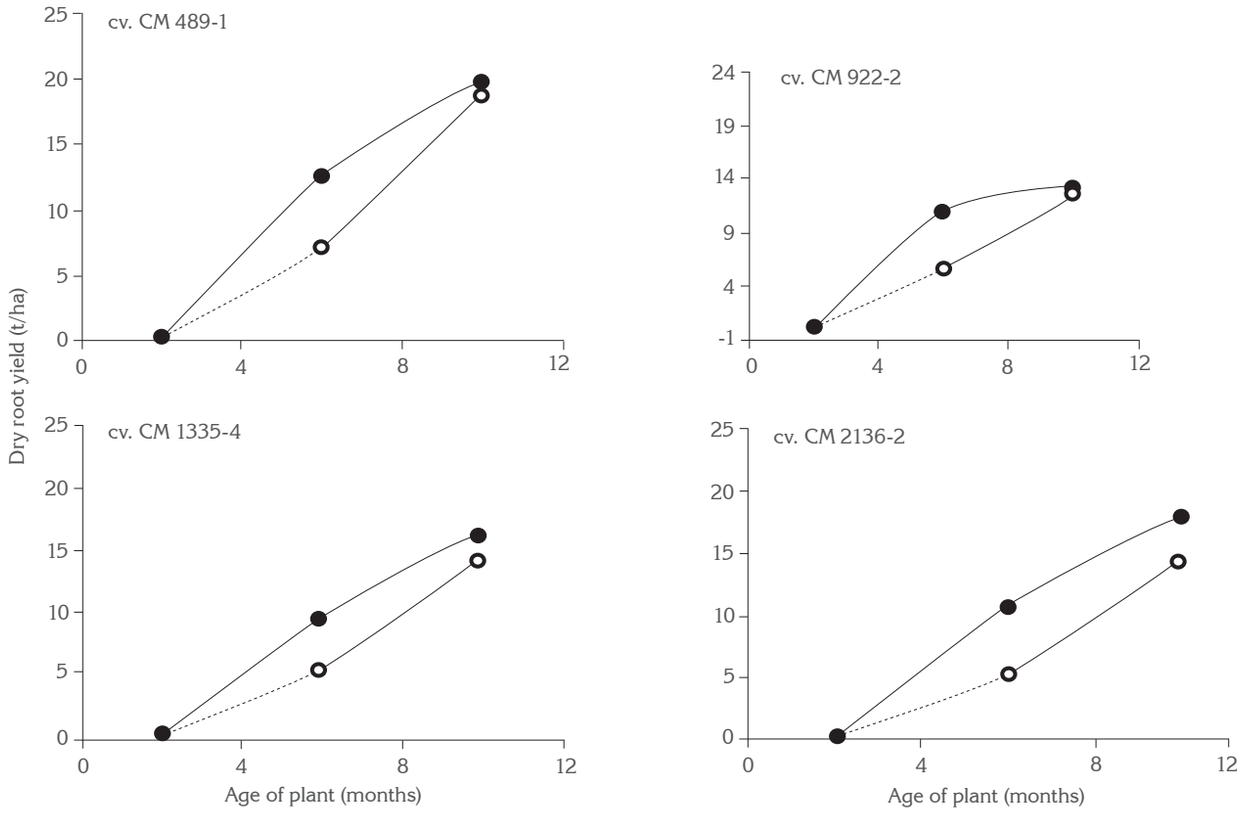

Figure 3-9. Dry root yield in a group of clones affected by 3 months of water stress, starting 90 days after planting (mid-season stress). Yield was significantly lower at the end of stress, but recovered rapidly with watering, so that final yields were approaching those of the controls. There were differences among cultivars, with cv. CM 489-1 having the highest yield in both water regimes (CIAT Report 1992; El-Sharkawy 2006). —●— refers to unstressed clones; —○— to stressed clones.

values for yield, nutrient-use efficiency in terms of root production, radiation-use efficiency in terms of total biomass production, and many harvestable storage roots per plant across a range of phosphorus fertilizer levels in acidic soils in subhumid warm climates (Pellet and El-Sharkawy 1993a, 1993b, 1994, 1997).

Across accessions, reductions as a result of water stress were much higher for shoot biomass (28%) than for roots (9%). However, HI increased about 6%, thus indicating cassava's potential to tolerate prolonged mid-season stress in subhumid zones and its ability to recover and compensate for possible losses in productivity. This is an advantage over other staple food crops (El-Sharkawy and Cock 1987b; CIAT Reports 1991, 1992; El-Sharkawy et al. 1992b). The genetic variability existing for stress tolerance should be exploited in breeding and improving cassava germplasm for dry environments (CIAT Reports 1991 to 1995; Hershey and Jennings 1992; El-Sharkawy 1993).

When exposed to extended water deficits, most cassava cultivars show increased HCN content (which indicates cyanogenic potential) in their storage roots. Thus, the roots become less suitable for human consumption if they not properly processed to eliminate most, if not all, HCN (Dufour 1988; Rosling 1994; Essers 1995). Some crop management practices can greatly reduce HCN in cassava roots, for example, where moderate amounts of N-P-K fertilizers and/or plant residues such as mulch are applied to low-fertility sandy soils in zones with long dry periods (Cadavid et al. 1998). Another practice is to apply K to clayey acidic soils low in K in subhumid zones (El-Sharkawy and Cadavid 2000). Selection for low HCN cultivars, however, remains a major objective of most breeding programs, particularly those targeting germplasm for stressful environments (El-Sharkawy 1993).

In our case, those less sensitive genotypes with low HCN that we identified (Table 3-6) offer adequate genetic sources for breeding sweet cultivars. Nassar (1986) also reported some wild species with low HCN

Table 3-6. Yield, top biomass (dry, t/ha), and hydrocyanic acid (HCN) content in roots at final harvest (11 months after planting) after 3 months of mid-season water stress, starting 90–100 days after planting. Averages are from the 1987/88 and 1988/89 seasons at Santander de Quilichao, Colombia. Note the increase in HCN contents due to stress and the differences among cultivars. Clones with lower HCN under stress are good genetic sources for selecting and breeding materials suitable for fresh consumption by humans, particularly in dry and semi-arid zones.

Clone	Unstressed			Stressed		
	Roots	Tops	Total HCN (mg/kg dry root)	Roots	Tops	Total HCN (mg/kg dry root)
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
Average	17.8	8.8	157	16.2	6.4	263
% change due to stress				-9	-28	+68

SOURCES: CIAT Report (1991); El-Sharkawy (1993).

and high protein in storage roots. In addition to traditional breeding, transgenic approaches have been used to produce cassava with reduced HCN levels (i.e., >90% reduction in cyanogenic contents in storage roots) in transformed cv. M Col 22 (Jørgensen et al. 2005). "Acyanogenic" (i.e., cyanogen-free) clones have also been generated (Siritunga and Sayre 2003). However, the role of cyanogenesis in cassava, as a potential deterrent to pests that feed on leaves and roots, needs to be assessed in the developed "acyanogenic" materials before they are released (Riis et al. 1995, 2003).

Pereira (1977) and Poulton (1990) have argued that high levels of HCN in plants may function as a defensive mechanism to protect crops against predators, herbivores, and rodents. HCN may also serve as a source of stored nitrogen, particularly for seeds. The presumably defensive role of HCN against pests and diseases has not been observed (Brekelbaum et al. 1978). Despite normally higher elevations of leaf HCN levels in most cultivars, water-stressed cassava crops in Northeast Brazil and North Colombia (which typically have several months of water shortages) showed higher rates of infestation by mites than did non-stressed crops (MA El-Sharkawy 1992, pers. comm.).

Thrips also fed on cassava, regardless of HCN levels in leaves (Schoonhoven 1978). Other pests with different feeding habits, whether on shoots or roots, may present different responses. Recent work at CIAT (Bellotti et al. 1988; Bellotti and Arias V 1993; Bellotti and Riis 1994; Bellotti 2002) showed that the subterranean burrower bug (*Cyrtomenus bergi*) preferred to feed on cassava roots low in HCN than on bitter cassava, particularly in soils with high moisture content, even though several sweet cultivars were determined as having potential resistance or tolerance of the bug (Riis 1997). One mechanism that may deter or prevent the bug from feeding on sweet cassava is high HCN content in the storage root peel instead of the parenchyma tissue (Riis 1997). The first two nymphal instars have short stylets, thus confining feeding mainly to the root peel (Riis 1990; Riis et al. 1995). Hence, selection for sweet cultivars having high HCN in the thicker root peel may be advantageous in this case.

Some cultural practices such as intercropping cassava with sunn hemp (*Crotalaria* sp.), which possesses natural insecticidal substances, was found to effectively reduce bug attack and damage to cassava roots. However, cassava yield was reduced because of competition and crowding by the *Crotalaria* (Bellotti et al. 1988; Bellotti 2002).

A social study was recently conducted on the Tukano Indians in northwestern Amazon Basin, Brazil. The Tukano cultivate more bitter cultivars, which are high in HCN, than they do sweet cultivars, perhaps because they are more prevalent rather than because of any inherent adaptive advantages. That is, no consistent relationships or patterns were demonstrated to exist for the Tukanos' preferring bitter cultivars over sweet ones, whether for resistance to predators, particularly pests and diseases, or other reasons (Wilson 2003). However, Wilson and Dufour (2002) suggest that higher yields, often observed in bitter cultivars grown in that region, form the Amazon Basin Indians' likely criterion for choosing high-HCN cassava.

However, to our knowledge, no conclusive evidence, based on sound research, exists as to whether bitter cultivars do, in fact, have an inherent and superior potential for productivity than sweet ones. That no consistent relationship exists between productivity and HCN contents in roots is furthermore supported by data in Table 3-6 and findings of trials with 14 other cultivars. These materials were tested across five consecutive growth cycles under different application rates of K fertilizer in acidic clayey soils in subhumid zones of Colombia (El-Sharkawy and Cadavid 2000).

Moreover, several tested clones, including HMC-1, HMC-2, M Cub 74, M Pan 70, M Col 1505, CM 91-3, CM 523-7, CMC 40, CM 1585-13, and those shown in Table 3-6, have high yields and moderate to low HCN levels in root parenchyma. Most of these clones are improved materials that indicate compatibility of selection and breeding for high yield with low HCN. Álvarez and Llano (2008) have suggested that the bread-making quality of bitter cultivars is different and possibly better than that of sweet cultivars. This might be a reason why the Indians grow bitter cultivars, that is, for culinary, not agronomic, purposes. More research is needed to uncover other possible reasons why bitter cassava is chosen in the Amazon Basin and elsewhere.

Photosynthesis and the C_3-C_4 Intermediate Characteristics of Cassava

Previous research on cassava photosynthesis shows that several cassava cultivars and wild species exhibit activity of the C_4 enzyme PEPC, ranging from 1.5 to >5 μmol per mg Chl/min. This is 15% to 25% of activities of C_4 species such as maize and sorghum. The research also demonstrated the importance of elevated PEPC activity, which may partly underlie cassava's high photosynthetic capacity and which correlates with productivity across environments and genotypes (Table 3-7; Cock et al. 1987; El-Sharkawy and Cock 1987a, 1990; CIAT Reports 1990 to 1994; El-Sharkawy et al. 1990, 1992a, 1993, 2008; Bernal 1991; López et al. 1993; Pellet and El-Sharkawy 1993a; de Tafur et al. 1997b; El-Sharkawy 2004).

The PEPC activity observed in cassava and its wild relatives are much higher than those observed in C_3 species such as field bean. Instead, they are comparable with activities found in several C_3-C_4 intermediate *Flaveria* species, which have a limited functional C_4 cycle. They are also two to three times higher than in C_3-C_4 plants with a C_4-like kranz anatomy, as found in *Panicum milioides* (Ku et al. 1983; Brown and Bouton 1993).

Table 3-7. Activity of C_4 phospho*enol*pyruvate carboxylase (PEPC) carboxylase in leaf extracts. Values are means of four leaves; ± are standard deviations. Note the much higher activity in cassava, compared with beans, a C_3 species, and the very high activity in wild *Manihot* (about 30% to 40% of activity in maize, a C_4 species). In a group of field-grown cultivars under prolonged water stress, P_n was significantly correlated with the PEPC activity measured in the same leaves (El-Sharkawy 2004), indicating the importance of selecting and breeding for elevated PEPC activity. Note that wild *Manihot* also possesses an additional short-palisade layer beneath the lower leaf surface and numerous stomata on both leaf surfaces—two traits advantageous for enhancing photosynthesis (El-Sharkawy 2004).

Species	PEPC activity (μmol NADH)	
	gfw/min	mg Chl/min
Maize (cv. CIMMYT 346)	15.0 ± 1.8	7.0 ± 3.6
Common beans (cv. Calima G 4494)	0.2 ± 0.07	0.3 ± 0.1
Cassava cultivars:		
M Mex 59	3.2 ± 0.6	2.2 ± 1.0
M Nga 2	1.3 ± 0.1	0.4 ± 1.0
Wild *Manihot* species:		
M. grahamii	4.0 ± 0.9	2.8 ± 1.2
M. rubricaulis	5.8 ± 0.6	3.4 ± 1.3

SOURCES: El-Sharkawy and Cock (1990); El-Sharkawy (2004); MA El-Sharkawy, L Bernal, and Y López (1988, unpublished).

The presence of a C_4 PEPC protein in cassava was further determined immunologically (Figure 3-10; CIAT Report 1991). In cassava, PEPC appears to be of at least two different forms (isoenzymes), compared with PEPC from maize. The presence of the enzyme, however, does not necessarily mean it is active. When we used the stain Fast Violet BB, which is relatively specific for oxaloacetate (i.e., the initial C_4 product), we could demonstrate that the PEPC is, indeed, active in cassava (Figure 3-10, at left, lanes 2 and 3 for cassava cultivar M Col 22, lane 1 for maize, and lane 4 for beans). Thus, we quantitatively confirmed activity in centrifuged leaf extracts (Table 3-7).

More recent research on the biochemical and molecular characteristics of cassava photosynthetic enzymes showed that a maize PEPC-specific antiserum (maize *ppc* probe, received from T. Nelson, Yale University, USA) cross-reacted with

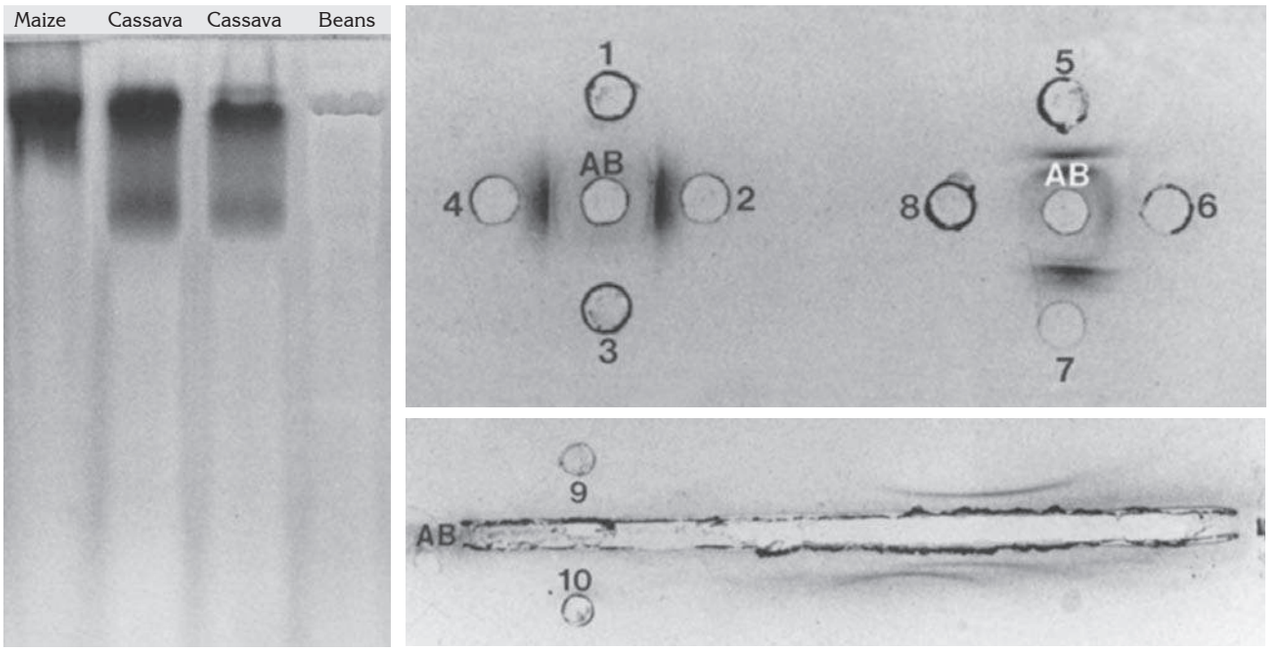

Figure 3-10. At right, immunological detection of phospho*enol*pyruvate carboxylase (PEPC) in leaf extracts: *upper right*, shows a double immunodiffusion, with wells 1, 3, and 8 for beans; wells 2, 4, and 5, for purified maize PEPC; well 6 for cassava cultivar M Col 22; well 7 for maize; AB = antiserum containing anti-PEPC. *Lower right*, shows immunoelectrophoresis in 1.2% agarose gel, where well 9 is for purified maize PEPC; well 10 is for cassava; AB = antiserum containing anti-PEPC. At left, simple PAGE patterns for PEPC in maize, cassava, and beans; the two forms of PEPC (isoenzymes) in cassava are apparent (Y López, MA El-Sharkawy, JH Cock, and H Ramírez 1987, unpublished; CIAT 1991; El-Sharkawy 2006).

cassava PEPC. The reaction indicated the presence of homologous antigenic determinants (CIAT Report 1993; López et al. 1993). This was also shown at the DNA level in Southern blot hybridization with a maize *ppc* probe and total, enzyme-digested, cassava-genomic DNA (Figure 3-11; CIAT Report 1993; López et al. 1993; Tenjo et al. 1993). These studies were repeated with about 60 more accessions, and included *me* (malic enzyme; Figure 3-12) and *mdh* (malate dehydrogenase) maize probes.

No polymorphisms were found that would have related the elevated activity of cassava PEPC to a higher copy number of the genes involved. Moreover, the corresponding gene sequences in cassava appeared similar to the maize probes used, as shown by good hybridization signals at high stringency (CIAT Report 1993).

So far, preliminary studies on the compartmentalization of PEPC in cassava have indicated the location of *ppc* transcripts between the upper epidermis and the top end of the long-palisade layer (CIAT Report 1993). The location tends to support the hypothesis that, over a range of photon flux densities and temperatures, the palisade cells are capable of refixing and/or recycling all respiratory CO_2 in light and in CO_2-free air (Figure 3-13; El-Sharkawy and Cock 1987a). Complete or partial apparent refixation and/or recycling in light of respiratory CO_2 (both photorespiration and mitochondrial dark respiration) was recognized earlier in different C_3 and C_4 species (Meidner 1962; Moss 1962; Tregunna et al. 1964; El-Sharkawy and Hesketh 1965, 1986; Forrester et al. 1966; El-Sharkawy et al. 1967, 1968; Jackson and Volk, 1969; Volk and Jackson 1972) and in C_3-C_4 intermediates (Devi and Raghavendra 1993).

However, more studies are needed, using *in situ* hybridization and immunofluorescent techniques, to elucidate the spatial distribution of the photosynthetic key enzymes within the cassava mesophyll (CIAT Report 1993; López et al. 1993; Tenjo et al. 1993). Cassava and wild relatives show low photorespiration (CO_2 compensation concentration was 20–30 ppm), relative to C_3 species (Figure 3-14; El-Sharkawy and Cock 1987a; CIAT Reports 1992, 1995; El-Sharkawy et al. 1992a; El-Sharkawy 2004); high percentage (40%–60%) of leaf-fed ^{14}C incorporated into C_4 acids after 5–10 s under light; and elevated PEPC activity (Cock et al. 1987; El-Sharkawy and Cock 1987a, 1990). However, they lack the typical C_4 kranz leaf anatomy that is

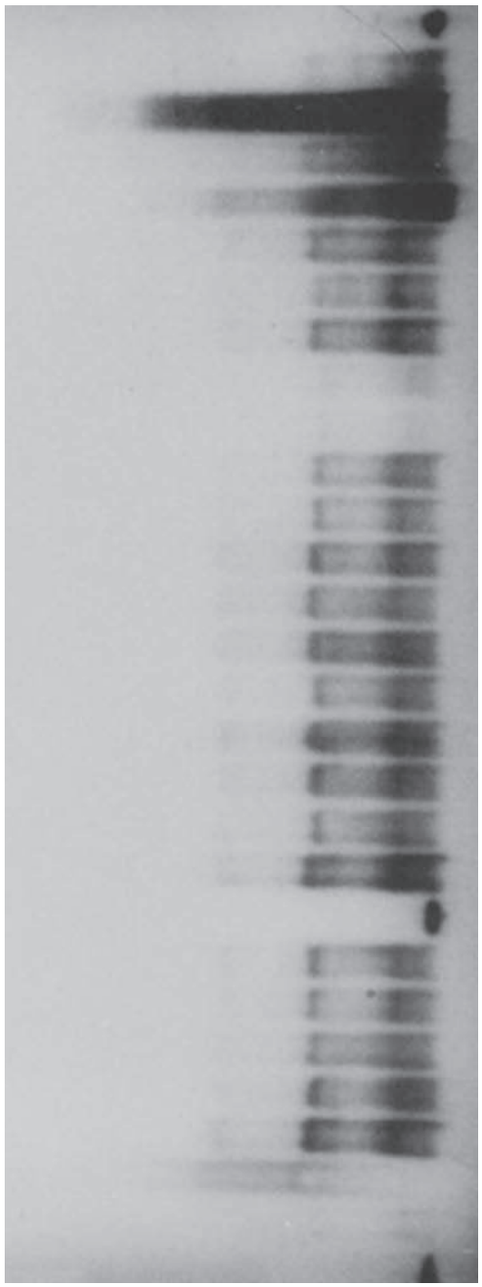

Figure 3-11. Southern hybridization of BamHI-digested cassava DNA, hybridized with a maize *ppc* probe (CIAT Reports 1993, 1994; JE Mayer, MA El-Sharkawy, RM de Estefano, and FA Tenjo 1993, unpublished). Note the variable degrees of hybridization with the maize *ppc* probe within cassava germplasm and wild *Manihot grahamii*.

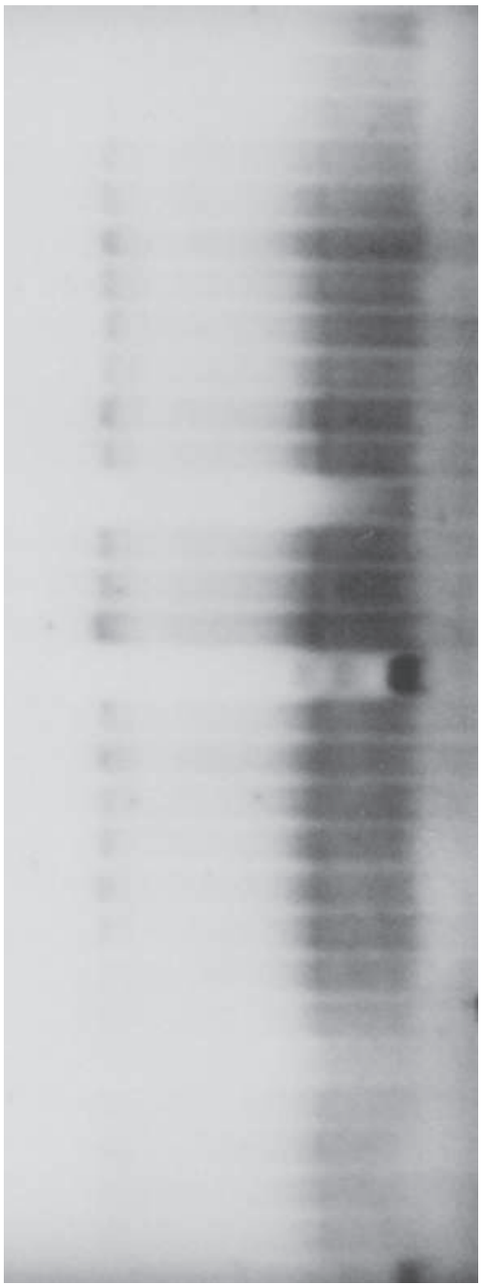

Figure 3-12. Southern hybridization of BamHI-digested cassava DNA, hybridized with a maize *me* probe (CIAT Reports 1993, 1994; JE Mayer, MA El-Sharkawy, RM de Estefano, and FA Tenjo 1993, unpublished). Note the variable degrees of hybridization with the maize *me* probe within cassava germplasm.

required to compartmentalize the key C_3 rubisco and C_4 PEPC enzymes (El-Sharkawy and Hesketh 1965, 1986; Laetsch 1974; Hatch 1977, 1987). We suggest that cassava and *Manihot* species are probably evolving biochemically towards a C_4 photosynthetic pathway and therefore demonstrate C_3-C_4 intermediate photosynthetic behavior (Cock et al. 1987; El-Sharkawy and Cock 1987a, 1990; El-Sharkawy 2004, 2005).

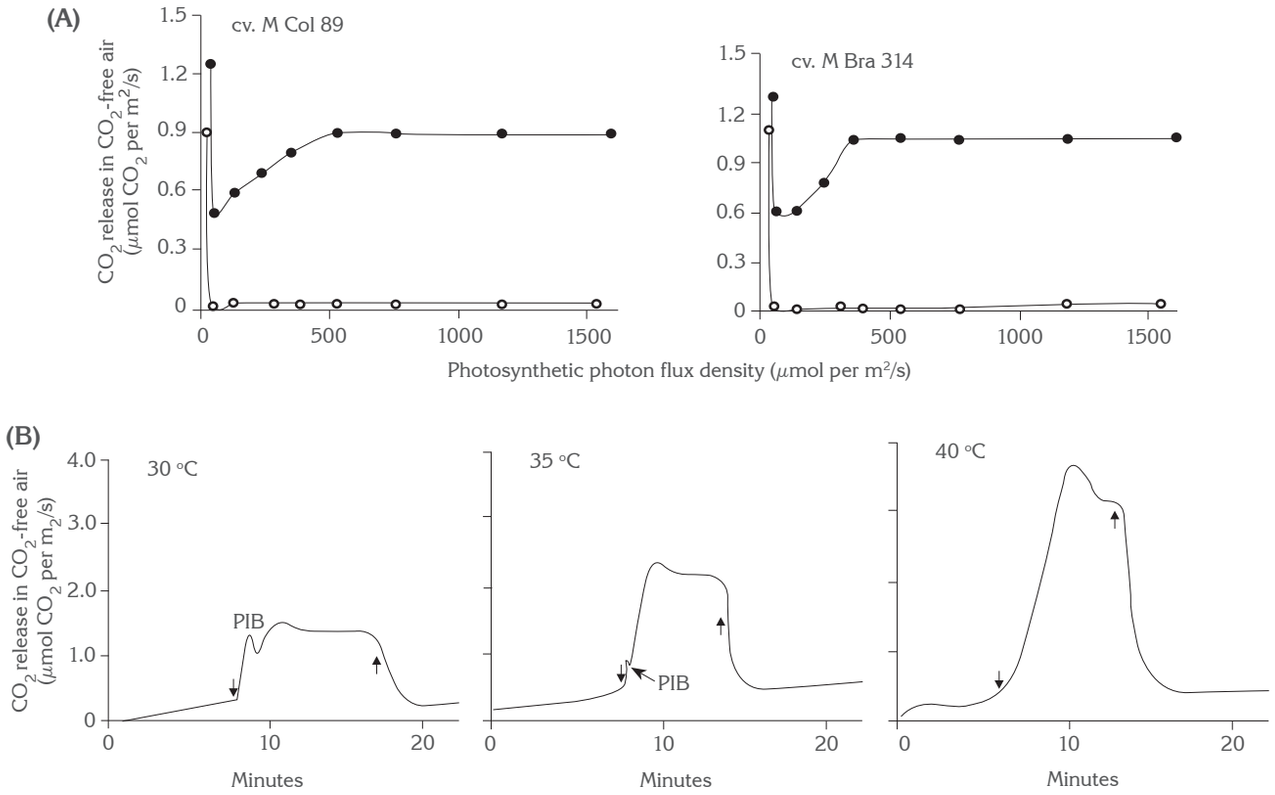

Figure 3-13. **(A)** Differential CO_2 releases in CO_2-free air from the upper (o) and lower (●) surfaces of amphistomatous cassava leaves (cvs. M Col 89 and M Bra 314) as a function of photon flux density at a constant leaf temperature of 27 °C. Note the consistent lack of CO_2 release from the upper surface of leaves of both cultivars when the abaxial stomata were blocked versus release from the lower surface. This indicates the complete refixation/recycling of respiratory carbon dioxide (both photorespiration and dark mitochondrial) within the long-palisade layer, which occupies more than 60% of leaf thickness. **(B)** Recorder's traces of CO_2 releases in CO_2-free air under light and dark from the upper surface of amphistomatous cassava leaves (cv. M Bra 314) at 30, 35, and 40 °C leaf temperatures. Photon flux density was 1200 μmol per m^2/s (↓ refers to light off; ↑ refers to light on; PIB to post-illumination burst of CO_2). Note the lack of carbon dioxide release under light, which was observed in several light–dark cycles over a longer period (>1 h); the decrease in PIB magnitude with rising leaf temperature and eventual disappearance at 40 °C; and the pronounced surge of carbon dioxide within 3 min of darkness. The lack of carbon dioxide release under light was attributed mostly to an efficient refixation/recycling system in the palisade cells (El-Sharkawy and Cock 1987a).

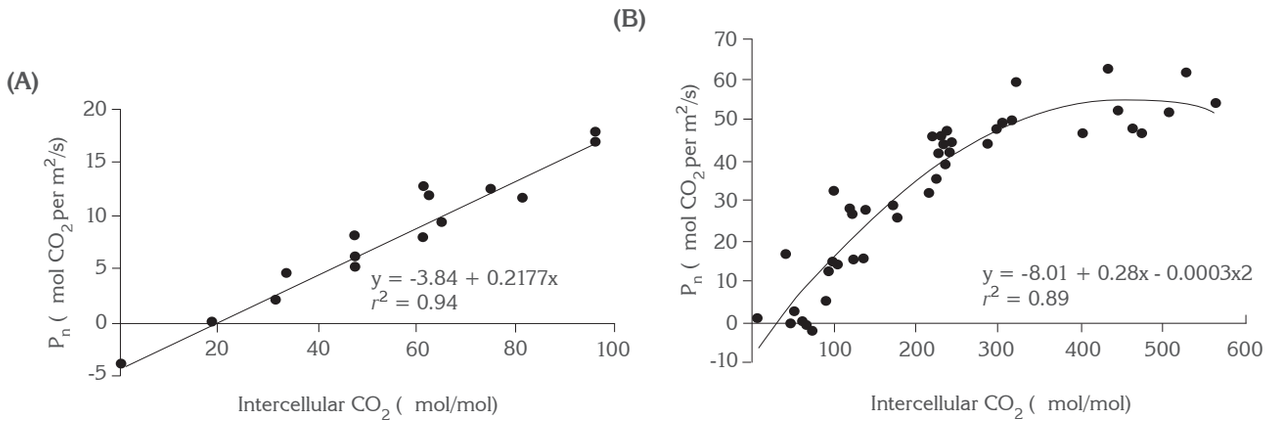

Figure 3-14. Relationship between leaf photosynthesis (P_n) and intercellular CO_2 concentration in cassava cultivar M Col 1684 **(A)** (CIAT Report 1992; El-Sharkawy et al. 1992a) and in wild *Manihot rubricaulis* **(B)** (CIAT Report 1995; SM de Tafur and MA El-Sharkawy 1995, unpublished). Note the linear response and the low photorespiration where the CO_2 compensation point was about 20 in **(A)** and 28–30 μmol/mol in **(B)**; a saturated P_n in *M. rubricaulis* at 400–500 μmol/mol intercellular CO_2 is apparent.

Effects of Water Stress

Photosynthetic enzymes

Three-month-old cassava plants were exposed to water stress for 3 and 8 weeks in the field. After 3 weeks of water stress, activities of PEPC, rubisco, and the C_4 decarboxylase NAD-ME were observed, overall, to have declined, particularly rubisco, in leaf extracts (Table 3-8; CIAT Report 1993). The average PEPC-to-rubisco ratio, indicating the relative importance of these two enzymes, was also reduced by stress.

However, after 8 weeks of stress, PEPC activity, averaged across all clones, was 13% higher than in unstressed crops, with differences among accessions (Table 3-9; CIAT Report 1993). However, rubisco activity was 42% less in the stressed crops. This differential effect of stress on the activities of these two key photosynthetic enzymes resulted in a much higher PEPC-to-rubisco ratio in the stressed crops than in the unstressed ones. These data indicate that, under prolonged water deficit, the relative importance of the C_4 PEPC versus the C_3 rubisco becomes more pronounced, lending support to the hypothesis that the C_4 PEPC enzyme may play a significant role in photosynthetic activity under drought with high air temperatures (CIAT Report 1993; El-Sharkawy 2004), probably by reducing both photorespiratory and mitochondrial dark CO_2 losses and by increasing net carbon uptake and hence productivity.

Moreover, recent evidence suggests that PEPC is possibly located in the upper end of the long-palisade parenchyma. This further supports the role of PEPC involvement in refixing or recycling respiratory CO_2 when highly dense abaxial stomata are partly closed under conditions of drought, high solar irradiance, and high temperatures with dry air, particularly in hypostomatous leaves, which normally possess >400 stomata/mm^2 (El-Sharkawy et al. 1984b). Besides increasing carbon uptake, such a mechanism for CO_2 recycling protects the leaves from photoinhibition by dissipating excess absorbed photons (Osmond et al. 1980; Osmond and Grace 1995). In the field, leaves of over 100 cassava cultivars remained photosynthetically active, although at much reduced rates, under prolonged drought with hot dry air and intense solar radiation (El-Sharkawy et al. 1990, 1992b; El-Sharkawy 1993; de Tafur et al. 1997a, 1997b).

Ueno and Agarie (1997) examined the mitochondria of palisade and spongy mesophyll cells in leaves from cassava plants cultivated in growth chambers and developed under brief water deficits. The palisade cells demonstrated a higher density of immunogold labeling of the P-protein subunit of the photorespiratory enzyme glycine decarboxylase (or GDC). These findings may add another dimension to the C_3-C_4 intermediate hypothesis in cassava and to the essential role of PEPC in recycling respiratory CO_2 within palisade cells (Figure 3-13; El-Sharkawy and Cock 1987a, 1990).

Even so, Ueno and Agarie (1997) concluded that the chamber-grown cassava cultivars were C_3 and not C_3-C_4 intermediates. This conclusion, however, is questionable 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. Second, the observed GDC labeling patterns in chamber-grown plants are not, however, incompatible with the role of PEPC in the refixing or recycling of respiratory CO_2 in cassava leaves. Moreover, the degree of expression and distribution of GDC within leaf tissues are probably affected by the developmental stages of leaves and by the environmental conditions during growth (Rylott et al. 1998).

Our hypothesis of C_3-C_4 intermediate cassava does not exclude the presence of rubisco and the enzymes associated with the photorespiratory cycle in the palisade cells. Nor does it restrict them to spongy or bundle-sheath cells in the absence of a perfect C_4 kranz leaf anatomy and lack of complete separation and compartmentalization of the key C_4 and C_3 enzymes in palisade, spongy, and 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 burst (PIB) of CO_2 in CO_2-free air and the pronounced CO_2 surge within a short period in darkness via the upper leaf surface (Figure 3-13B; El-Sharkawy and Cock 1987a). Under these conditions, the oxygenase reaction by rubisco (in palisade-cell chloroplasts) might be suppressed. When adaxial (upper leaf surface) stomata were blocked, CO_2 releases through lower leaf surfaces in light and CO_2-free air were substantial over a wide range of photon flux density (Figure 3-13A; El-Sharkawy and Cock 1987a).

Table 3-8. Activities of selected photosynthetic enzymes in leaf extracts from clones of field-grown cassava, comparing well-watered plants with those affected by 3 weeks of water stress, starting 92 days after planting at Santander de Quilichao, Colombia. Values are means ± SD; activities are expressed in μmol per mg Chl/min. Note the much higher reduction in C_3 rubisco activity, compared with C_4 PEPC activity in leaves that developed before stress was initiated.

Clone	Unstressed[a]				Stressed[a]			
	PEPC	Rubisco	NAD-ME	PEPC/ rubisco	PEPC	Rubisco	NAD-ME	PEPC/ rubisco
CM 4013-1	0.37 ± 0.60	0.31 ± 0.05	0.40 ± 0.06	1.19	0.31 ± 0.03	0.41 ± 0.09	0.19 ± 0.05	0.76
CM 4063-6	0.57 ± 0.06	3.72 ± 0.11	0.39 ± 0.06	0.15	0.41 ± 0.04	1.69 ± 0.10	0.17 ± 0.09	0.24
SG 536-1	0.67 ± 0.05	0.39 ± 0.20	0.55 ± 0.18	1.72	0.57 ± 0.12	0.81 ± 0.20	0.39 ± 0.50	0.70
M Col 1505	0.45 ± 0.01	1.18 ± 0.08	0.16 ± 0.02	0.38	0.49 ± 0.10	0.49 ± 0.12	0.29 ± 0.06	1.00
Average	0.51	1.4	0.38	0.86	0.45	0.85	0.26	0.68
% change due to stress					-12	-39	-32	-21

a. PEPC refers to phospho*enol*pyruvate carboxylase; rubisco to ribulose bisphosphate carboxylase; NAD-ME to the C_4 photosynthetic pathway, subtype nicotinamide adenine dinucleotide malic enzyme.

SOURCES: CIAT Report (1993); Y López and MA El-Sharkawy (1992, unpublished).

Table 3-9. Activities of selected photosynthetic enzymes in leaf extracts from clones of field-grown cassava, comparing well-watered plants with those affected by 8 weeks of water stress, starting at 92 days after planting at Santander de Quilichao, Colombia. Values are means ± SD; activities are expressed in μmol per mg Chl/min. Note the large reductions in C_3 rubisco activity, compared with the C_4 PEPC, in leaves that developed under stress, resulting in a higher PEPC/rubisco ratio. This photosynthesis-based biochemical assay is useful in selecting for tolerance of prolonged drought (Tables 3-10 and 3-11).

Clone	Unstressed[a]			Stressed[a]		
	PEPC	Rubisco	PEPC/ rubisco	PEPC	Rubisco	PEPC/ rubisco
CM 4013-1	0.86 ± 0.12	0.28 ± 0.10	3.10	1.18 ± 0.17	0.30 ± 0.01	3.9
CM 4063-6	0.89 ± 0.05	2.30 ± 0.03	0.39	1.42 ± 0.26	0.62 ± 0.02	2.3
SG 536-1	1.46 ± 0.42	0.44 ± 0.12	3.30	1.33 ± 0.22	0.25 ± 0.08	5.3
M Col 1505	1.09 ± 0.10	0.57 ± 0.13	1.90	0.96 ± 0.16	0.89 ± 0.14	1.1
Average	1.08	0.9	2.2	1.22	0.52	3.2
% change due to stress				+13	-42	+45

a. PEPC refers to phospho*enol*pyruvate carboxylase; rubisco to ribulose bisphosphate carboxylase.

SOURCES: CIAT Report (1993); Y López and MA El-Sharkawy (1992, unpublished).

In cultivars with amphistomatous leaves, exchange (measured at saturating photon flux density and normal air) of both CO_2 gas and H_2O vapor via either leaf surface was substantial. It was also proportionate to stomatal densities and stomatal conductance on both sides (El-Sharkawy et al. 1984b). Only in some uncultivated C_3-C_4 intermediate species such as those found in the genera *Flaveria*, *Panicum*, and *Diplotaxis,* did a kranz-like leaf anatomy with developed bundle sheaths appear (Ku et al. 1983; Brown et al. 1985; Brown and Hattersley 1989; Araus et al. 1990; Ueno et al. 2003). The GDC is probably confined to this anatomy (Hylton et al. 1988).

C4 photosynthesis in the absence of the typical kranz leaf anatomy in some uncultivated plants, and implications for understanding the origin of the C4 syndrome

Bienertia cycloptera, an uncultivated species of the Chenopodiaceae family, grows in salty depressions in Central Asia. Recent findings indicated that this plant has a functional C4 pathway but lacks the typical kranz anatomy where key C4 and C3 enzymes are presumably located and compartmentalized within the cytosol or chloroplasts of the same mesophyll cell (Voznesenskaya et al. 2001, 2002).

Likewise, the submersed monocot *Hydrilla verticillata,* which also lacks the kranz anatomy, was found to possess a functional C4 pathway with PEPC and rubisco being, respectively, present in the cytosol and chloroplasts of all cells. That is, the enzymes were not segregated into special and separate cell types, as is common in terrestrial C4 species (Salvucci and Bowes 1983; Magnin et al. 1997; Reiskind et al. 1997). Shifts from C3 to C4 photosynthesis can also occur under specific environmental conditions. In this submersed species, a CO_2-concentrating mechanism apparently operates in the chloroplasts where rubisco and decarboxylation enzymes are located. The mechanism may represent an ancient form of C_4 photosynthesis that evolved long before the kranz anatomy-dependent C_4 syndrome appeared in terrestrial species (Magnin et al. 1997; Reiskind et al. 1997).

If this is indeed an ancient mechanism, then an important question arises on the first induction step of C_4 photosynthesis on earth: was the CO_2-concentrating mechanism first induced and then did it evolve in unicellular aquatic organisms in response to limiting supplies of CO_2 in the water? Did this happen before C_4 plants evolved onto land in response to much reduced atmospheric CO_2, higher O_2, and stressful environments, as believed so far? Reinfelder et al. (2000, 2004) found functional C_4 photosynthesis in the unicellular marine diatom, thus possibly providing evidence to support the hypothesis.

Moreover, in some amphibious sedges such as *Eleocharis vivipara*, different culms on the same plant can have C_3, kranz-less, photosynthetic characteristics when they develop under water but C_4, kranz-type characteristics when formed in air (Ueno 2001). In this case, C_4 photosynthesis was apparently linked to kranz-type anatomy, but with the incomplete compartmentalization of rubisco, which was located in both mesophyll and bundle-sheath cells (Ueno 1996).

Hibberd and Quick (2002) also reported on the biochemical characteristics of C_4 photosynthesis, that is, high C_4 enzyme activity and the respective controlling genes. These were found to already exist and be expressed in the stem and petiole photosynthetic cells that surround vascular tissues of C_3 flowering plants. These features therefore indicate a possible first step in the induction and evolution of the C_4 syndrome.

The induction and evolution of the biochemical components of the C_4 syndrome in the plant kingdom perhaps took place long before the more complex structural and anatomical components had evolved in terrestrial plants. Extensive research has recently been conducted on the molecular mechanisms underlying the C_4 syndrome; its multiple families of genes and isogenes encoding different isoforms of PEPC in C_3, C_4, C_3-C_4 intermediate, and the crassulacean acid metabolism (CAM) systems; and the expression patterns of the respective controlling genes in different plant species, organs, tissues, and subcellular organelles (Hermans and Westhoff 1990, 1992; Rajagopalan et al. 1994; Westhoff et al. 1997; Sheen 1999; Westhoff and Gowik 2004). Such gene-level studies increase understanding of how C_4 photosynthesis evolved and is controlled. They may also pave the way for possible bioengineering of more efficient leaves in economically important crops.

In light of these advances and discoveries, the re-evaluation and revision of classification systems, previously used in the past to identify C_3, C_4, or C_3-C_4 intermediate plants, is therefore warranted. These classifications were based on only a few given criteria of leaf anatomy, subcellular structure and organization, physiology, and biochemistry. Cassava is a case in point, where its photosynthetic characteristics

comprise perhaps the only discovery so far of C_3-C_4 intermediate photosynthesis in cultivated plants. The case therefore points to the need for more comprehensive classification systems.

In cassava and its wild relatives, wide genetic variations exist for C_4 PEPC activity, correlating with leaf photosynthesis and yield under extended water stress in the field (Calatayud et al. 2002; El-Sharkawy 2004; El-Sharkawy et al. 2008). These attributes should be exploited when selecting and breeding for enhanced photosynthetic capacity, at least when identifying parental materials (Tables 3-7 to 3-10; CIAT Reports 1990 to 1994; El-Sharkawy and Cock 1990; López et al. 1993; El-Sharkawy 2004). Also notable are the C_4 decarboxylation enzymes NAD-ME and NADP-ME showing activity in cassava, with differences among cultivars (Table 3-10), and comparable with those observed in C_4 and C_3-C_4 species.

Wild species such as *Manihot rubricaulis* and *M. grahamii* represent good genetic sources for elevated PEPC activity. They also show more efficient leaf anatomy, having developed a second palisade layer, albeit short, on the lower side of their amphistomatous leaves (Table 3-7; Calatayud et al. 2002; El-Sharkawy 2004). The existence of two palisade layers and the distribution of stomata on both leaf sides may provide an adaptive advantage in terms of carbon uptake (Parkhurst 1978; Solárová and Pospisilová 1979; Pospisilová and Solárová 1980; Tichá 1982; El-Sharkawy et al. 1984b; Gutschick 1984; Mott and O'Leary 1984). For similar reasons, known genetic diversity in rubisco characteristics should also be recorded (Paul and Yeoh 1987, 1988).

Because biochemical assays are often expensive and difficult to use in screening large breeding populations, molecular biology techniques, genetic markers, and mapping tools would be useful for identifying desirable genetic traits (Beeching et al. 1993).

Leaf water status, canopy light interception, and leaf photosynthesis

Trials showed that pre-dawn leaf-water potential (Figure 3-15A; CIAT Report 1992) remain at about -0.5 MPa for all cultivars throughout most of a 3-month stress period, with virtually no differences between stressed and unstressed crops. Mid-day leaf water potential (Figure 3-15B) in all cultivars in both stressed and unstressed crops fluctuated between -0.6 MPa (during wet periods when, presumably, leaf-to-air VPD was lower) and -1.6 MPa (during dry or sunny periods when VPD was much higher), with stressed crops often showing slight reductions. These values fall in 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). They are higher than those normally observed in other field crops under stress. These findings indicate that cassava conserves water and prevents extreme leaf dehydration through stomatal sensitivity to stress, that is, the crop uses stress avoidance mechanisms.

The phenomenon of osmotic adjustment (OA) in mature leaves therefore appears to have developed under water and edaphic stresses, as has been observed in several other field crops (Hsiao 1973; Hsiao et al. 1976; Jones and Turner 1978; Turner et al. 1978; Ackerson and Hebert 1981; Morgan 1984). It does not operate in field-grown cassava, because pre-dawn and mid-day, bulk, leaf-water potential always remains above -0.8 MPa and -2.0 MPa, respectively, during prolonged water deficits. Hence, OA is of little importance as a possible mechanism underlying cassava's tolerance of drought. Recent studies with potted cassava grown in the greenhouse showed that, after few days of water deficit, the largest increases in solutes occurred in the youngest and not yet fully expanded leaves (Alves 1998, 2002). The smallest increases occurred in mature ones, confirming the lack of importance of OA to mature leaves. Even so, further study on field-grown plants is needed if results are to be extrapolated to field conditions and to obviate acclimation problems (El-Sharkawy 2005).

Osmoregulation requires the investment and accumulation of solutes and assimilates for its development under stress. McCree (1986) examined the relative carbon costs involved in OA in sorghum grown under either water deficit or salinity. He concluded that the metabolic costs of storing photosynthates and using them for OA were less than those of converting photosynthates to new biomass, although costs did increase slightly under salinity. Under drought, changes occur in the biosynthesis, contents, and distribution of plant growth regulators such as ABA within plant organs and tissues (particularly roots, leaves, and buds). Changes in regulators may be important for the plant's sensing changes in both soil water and atmospheric humidity. They may also enable the plant to control stomatal movements, leaf formation and extension, root growth, bud dormancy, and other biological functions such as

Table 3-10. Activities of selected photosynthetic enzymes in leaf extracts from cassava clones grown in the field at Santander de Quilichao, Colombia, 1992. Values are means ± SD. Note the wide range of genetic variation for enzyme activity that could be used to select and breed for enhanced photosynthesis and, hence, productivity. Also note that cultivar CMC 40 (also known as M Col 1468) had the highest rubisco and PEPC activities, and the highest root yield under prolonged terminal water stress (Table 3-11).

Clone	Activities (μmol per mg Chl/min)[a]				
	PEPC	Rubisco	NAD-ME	NADP-ME	PEPC/rubisco
CM 523-7	1.57 ± 0.10	3.62 ± 0.62	1.84 ± 0.1	0.41 ± 0.04	0.43
CM 507-37	1.91 ± 0.10	6.84 ± 0.66	1.37 ± 0.4	0.46 ± 0.18	0.28
M Col 1684	2.90 ± 0.19	6.96 ± 1.18	2.05 ± 0.6	0.36 ± 0.03	0.42
CMC 40	3.07 ± 0.27	8.16 ± 0.71	1.84 ± 0.6	0.24 ± 0.05	0.38

a. PEPC refers to phospho*enol*pyruvate carboxylase; rubisco to ribulose bisphosphate carboxylase; NAD-ME to the C_4 photosynthetic pathway, subtype nicotinamide adenine dinucleotide malic enzyme; NADP-ME to the C_4 photosynthetic pathway, subtype nicotinamide adenine dinucleotide phosphate malic enzyme.

SOURCES: CIAT Report (1992); López et al. (1993).

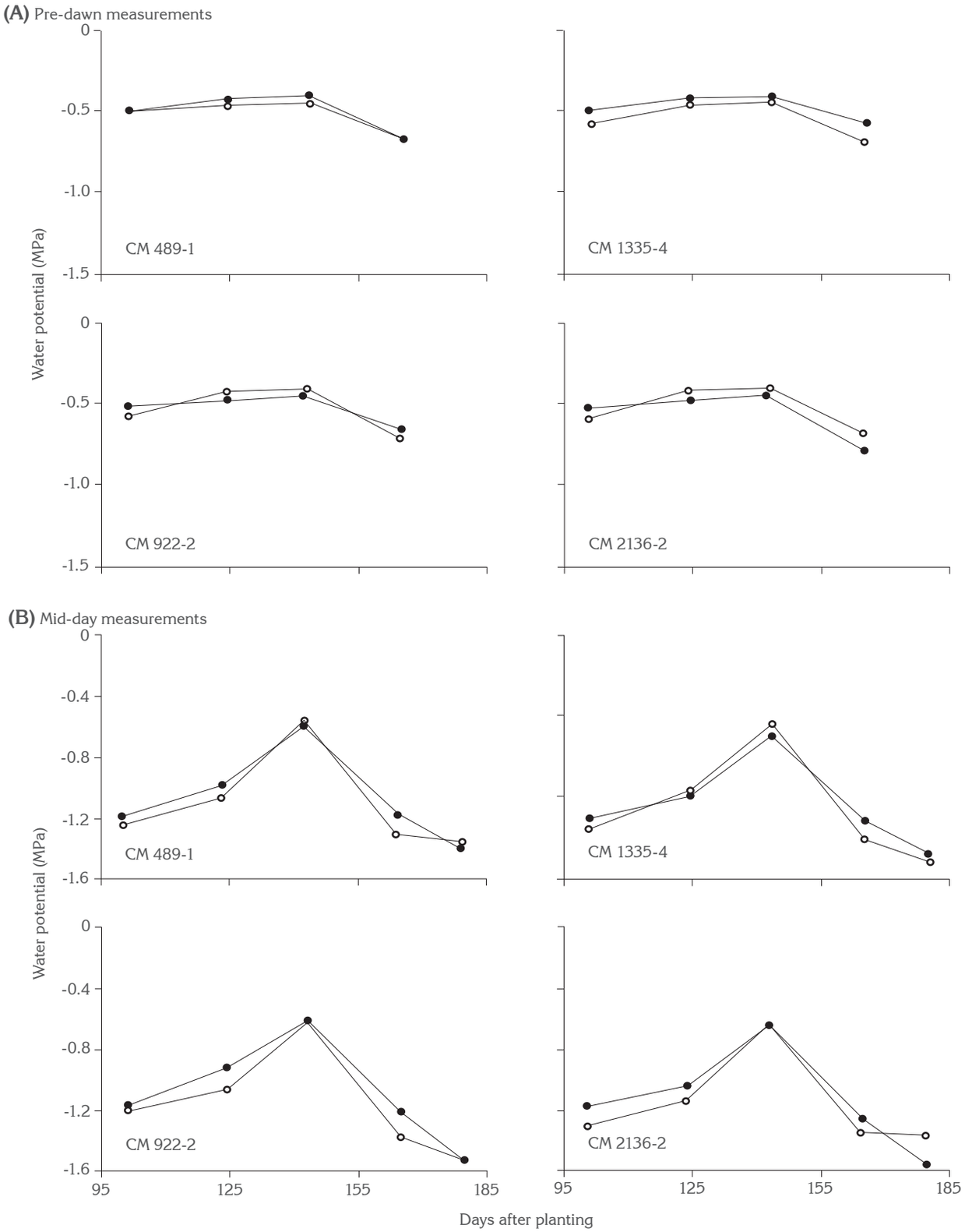

Figure 3-15. Leaf-water potential in water-stressed (○) and well-watered (●) cassava crops during stress, starting 90 days after planting. Values are means of 5 to 10 leaves from the upper canopy and taken at pre-dawn **(A)** and mid-day **(B)**. Note the small differences between the two water regimes and the four crops' pre-dawn and mid-day water potentials, and the increases in water potential during high ambient humidity. The pre-dawn and mid-day levels were more than -0.8 and -2.0 MPa, respectively, indicating the striking stomatal control in cassava, regardless of soil-water status (CIAT Report 1991; El-Sharkawy 1993).

PEPC activation and expression, and possibly the switching or induction from C_3 to CAM or C_4 photosynthesis in some species (Jones and Mansfield 1972; Huber and Sankhla 1976; Ackerson 1980; Walton 1980; Radin et al. 1982; Zeiger 1983; Henson 1984; 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, III, 1991; Taybi and Cushman 1999; Alves and Setter 2000; Ueno 2001).

The adaptive "stress avoidance mechanism in cassava" that operates via stomatal sensitivity to both soil and atmospheric water deficits is of paramount importance for the crop's tolerance of prolonged drought (>3 months) and hot dry air in seasonally dry and semi-arid zones (El-Sharkawy 1993; de Tafur 1997a, 1997b). Coupled with this mechanism is a deep rooting system (reaching soil layers more than 2 m deep) that allows the crop to extract available stored water (Connor et al. 1981; CIAT Reports 1983 to 1994; Porto 1983; El-Sharkawy and Cock 1987b; El-Sharkawy et al. 1992b; de Tafur et al. 1997a; Cadavid et al. 1998).

Another important mechanism for conserving water under extended stress is to significantly reduce light interception (Figure 3-16; CIAT Reports 1991 to 1995). The leaf canopy is reduced, mostly through restricted new leaf formation, smaller leaf size, and leaf drop (Connor and Cock 1981; Porto 1983; Palta 1984; El-Sharkawy and Cock 1987b; El-Sharkawy et al. 1992b)—this factor is also essential for reducing water consumption. Although reduced leaf area would conserve water, it would also reduce total biomass and yield (Figure 3-9; Table 3-6; Connor and Cock 1981; Connor et al. 1981; Porto 1983; El-Sharkawy and Cock 1987b; CIAT Reports 1991 to 1995; El-Sharkawy et al. 1992b, 1998b; El-Sharkawy and Cadavid 2002). Nevertheless, once released from stress, cassava recovers rapidly by forming new leaves, which increase light interception and canopy photosynthesis. Thus, previous losses in biomass, particularly root yield, are compensated (Figure 3-16; El-Sharkawy and Cock 1987b; CIAT Reports 1991 to 1995; El-Sharkawy et al. 1992b, 1998b; El-Sharkawy 1993; El-Sharkawy and Cadavid 2002).

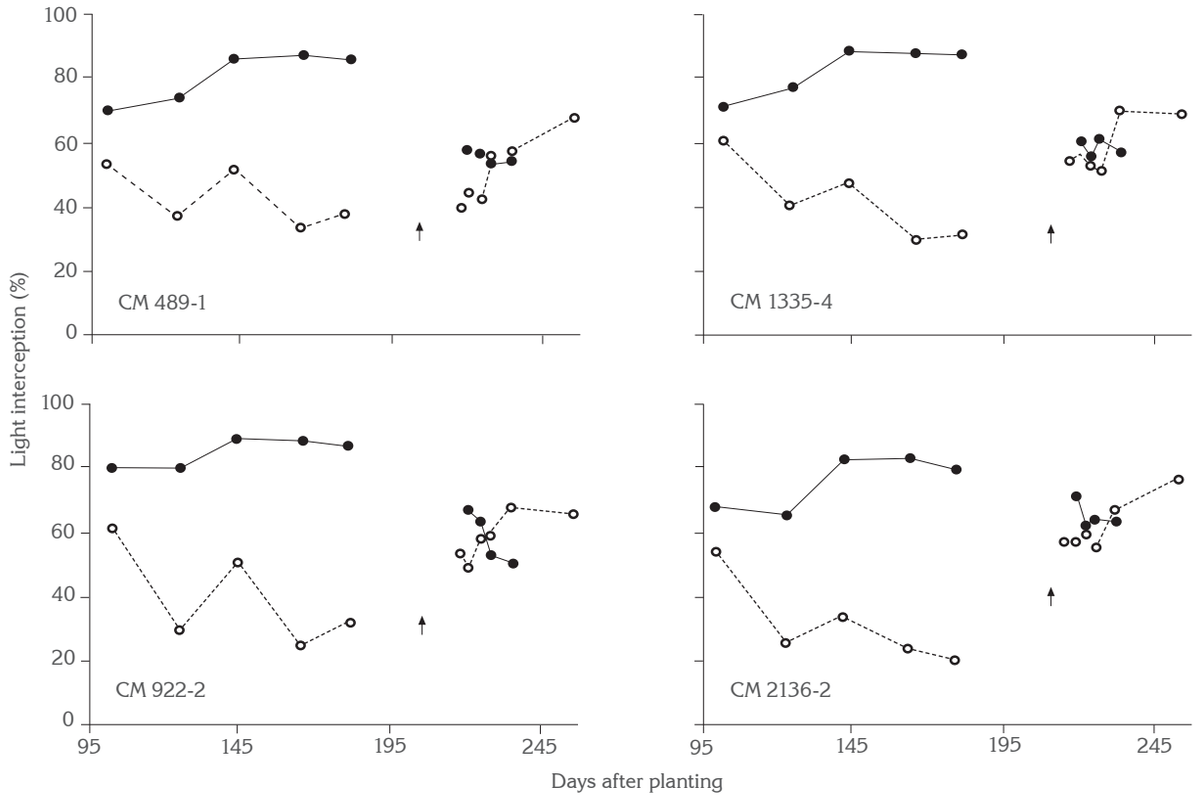

Figure 3-16. Light interception in water-stressed (o) and well-watered (●) cassava crops. Note the large reductions in light interception over time in the stressed crops because of a lack of leaf formation, the small size of new leaves, and the dropping of old leaves. Note also the increases after recovery (↑) from stress as new leaves formed (CIAT Report 1991; El-Sharkawy 1993).

Cassava leaves also remain adequately active during water shortages in the field (Figure 3-17). Stressed leaves can maintain a photosynthetic rate that is 40%–60% that of unstressed leaves over a 3-month stress period. Cultivars show differences, for example, hybrid CM 489-1 demonstrates smaller reductions than others. Once recovered from stress, previously stressed leaves can approach the rates of unstressed ones. Furthermore, newly formed leaves of previously stressed crops show even higher photosynthetic rates than those of the unstressed crops (Figure 3-17). These higher photosynthetic rates in new leaves coincide with higher stomatal conductance to water vapor; higher mesophyll conductance to CO_2 diffusion; and higher N, P, Ca, and Mg in leaves than unstressed crops (CIAT Report 1990; El-Sharkawy 1993; Cayón et al. 1997).

Moreover, Cayón et al. (1997) reported greater mobilization of potassium (an average of 0.79% K) in newly developed leaves of previously stressed crops, whereas new leaves from unstressed crops averaged 0.96% K. This finding suggests a higher demand for assimilates in storage roots, as K is normally translocated, together with sugars, to sinks (Giaquinta 1983). Thus, leaf photosynthesis is also controlled, in this case, by sink demand and strength (Burt 1964; Thorne and Evans 1964; Nösberger and Humphries 1965; Humphries 1967; Herold 1980; Ho 1988; Wardlaw 1990; Pellet and El-Sharkawy 1994; El-Sharkawy 2004). The dynamics of K in leaves of field-grown cassava may therefore be used as an indicator of root sink strength and source–sink relationships.

These remarkable physiological responses to mid-season water stress point to cassava's potential to tolerate prolonged drought, and its resilience and ability to recover from stress when water becomes available, as in subhumid zones with intermittent dry spells or in seasonally dry zones with well-marked bimodal rainfall distribution. Under these conditions, longer leaf life, that is, increased leaf retention, coupled with resistance to pests and diseases (Byrne et al. 1982; El-Sharkawy 1993), is advantageous in saving dry matter invested in leaves and in allowing partitioning of excess photosynthates towards storage roots.

The crop can also survive, but with higher losses of leaf canopy and less dry matter in storage roots, in semi-arid zones with an annual rainfall of less than 600 mm and with periods of water deficits of more than 4–5 months (e.g., Northeast Brazil, northeastern Colombia, and sub-Saharan Africa; El-Sharkawy 1993; de Tafur et al. 1997b; El-Sharkawy 2004). In this ecosystem, a second wet cycle is needed to allow recovery of growth and complete root bulking.

Evaluating Germplasm for Tolerance of Water Stress

Cassava germplasm can be evaluated for different traits while grown under different levels of water stress. The studies described in the next three sections were conducted under prolonged early water stress (occurring 2–6 months after planting); mid-season stress (4–8 months after planting), and terminal (i.e., end-of-season) stress (6–12 months after planting).

Productivity, nutrient-use efficiency, photosynthesis, and water uptake

Three-year field trials were conducted at CIAT–Quilichao experiment station to study the effects of prolonged water stress imposed at different growth stages on cassava productivity, nutrient uptake and use efficiency, leaf photosynthesis, and patterns of water uptake (CIAT Reports 1992, 1993; Caicedo 1993; El-Sharkawy et al. 1998b; El-Sharkawy and Cadavid 2002). Figure 3-18 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 yield at 6 months was significantly smaller than the control for all clones in both growth cycles.

The same trends were observed in shoot biomass but with greater reductions than those observed for roots (CIAT Reports 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), yield at 8 months was also significantly lower than for the well-watered control in both cycles, except for CMC 40 (also known as 'M Col 1468' in Colombia). Reductions in shoot biomass were less pronounced than those under early stress (Caicedo 1993; El-Sharkawy et al. 1998b; El-Sharkawy and Cadavid 2002).

Under both early and mid-season stress, leaf area, as determined from periodic harvests, was also significantly smaller than for controls, resulting in a much reduced canopy light interception (Figure 3-19; CIAT Report 1992; El-Sharkawy and Cadavid 2002). Once the cassava crops were allowed to recover, new

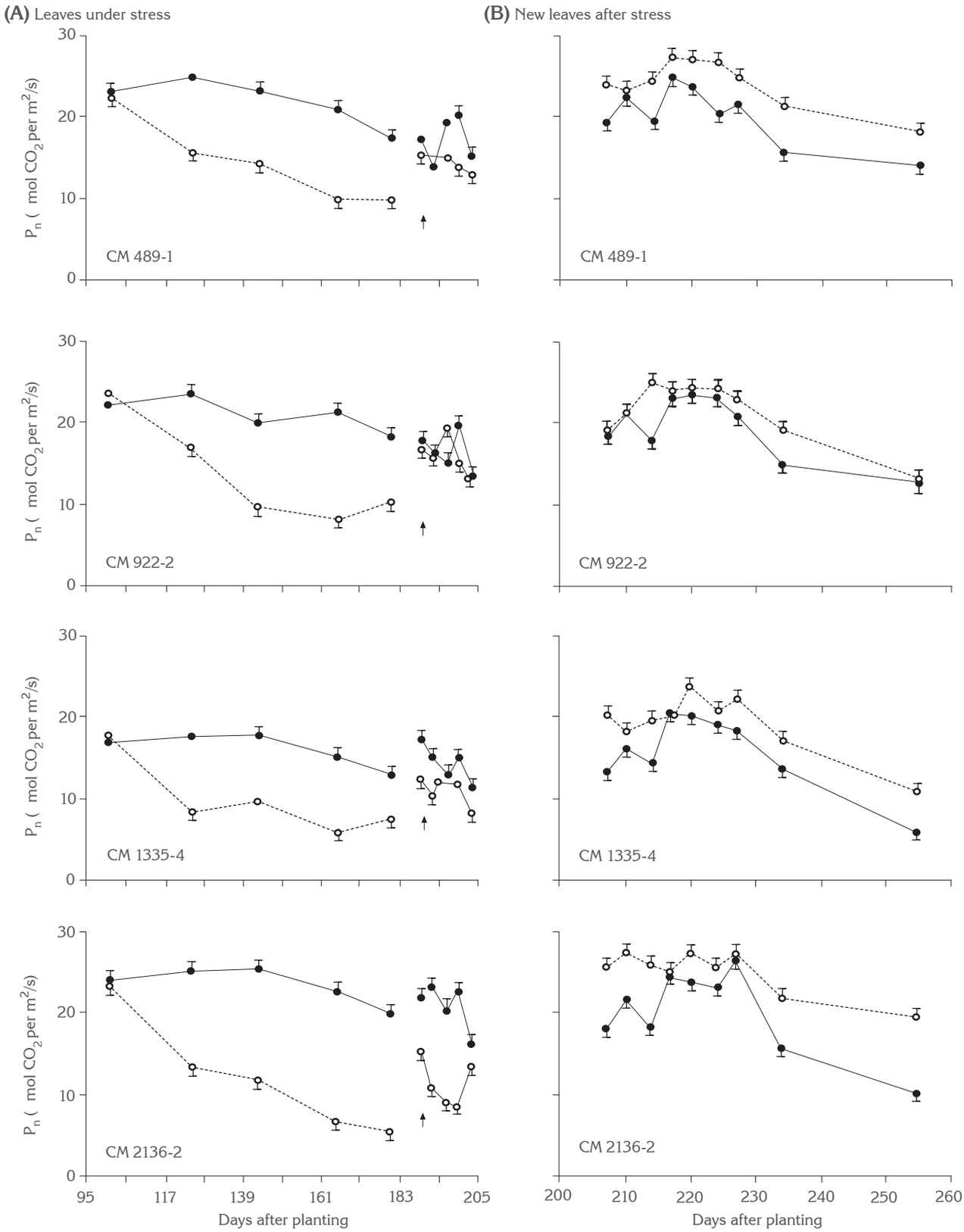

Figure 3-17. Leaf photosynthesis (P_n) in the upper canopy when affected by mid-season water stress (CIAT Report 1991; El-Sharkawy 2006). **(A)** Leaves under water stress; ↑ refers to recovery. **(B)** New leaves after stress period. ○ refers to leaves under stress; ● to control.

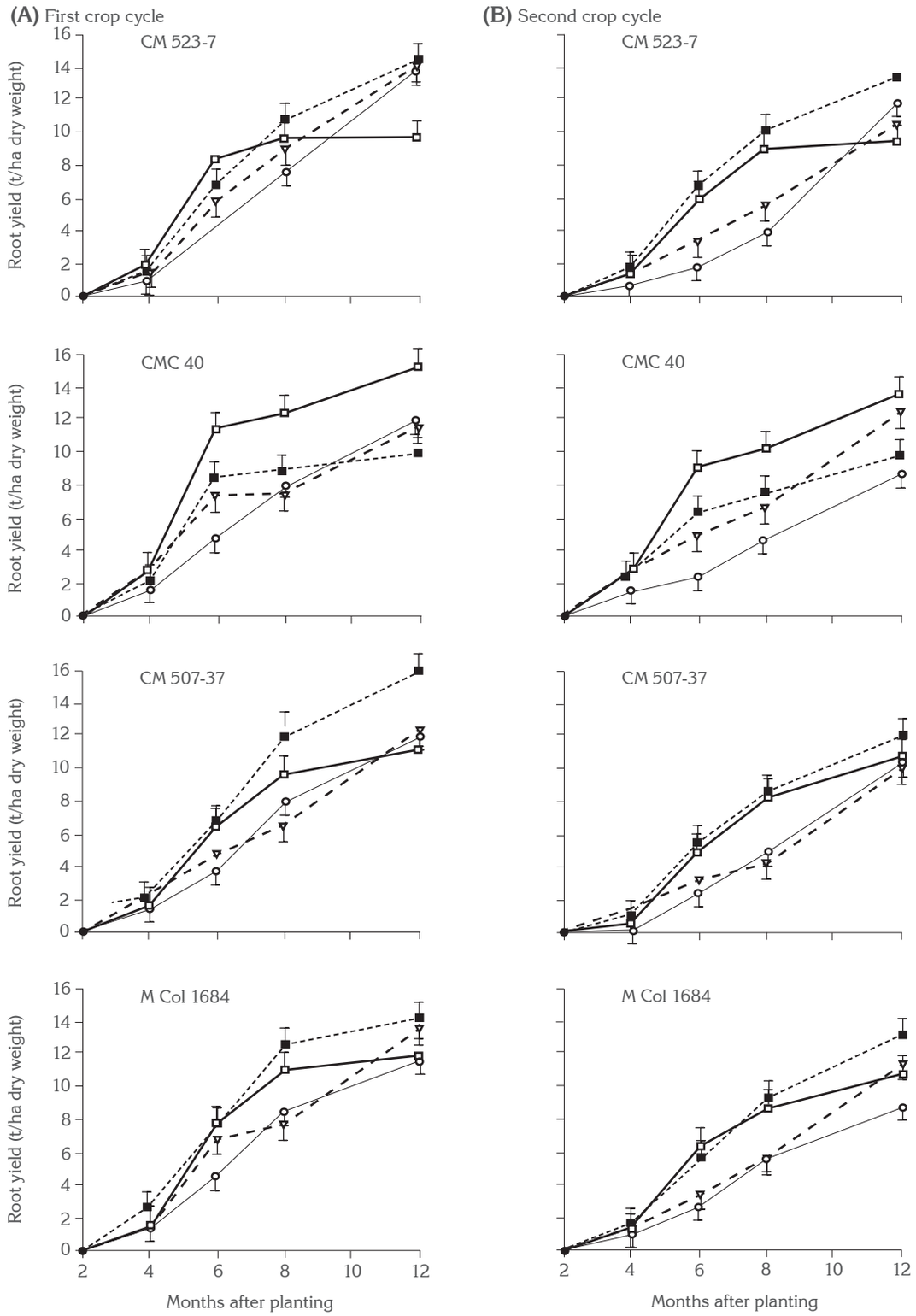

Figure 3-18. Storage root dry matter yield over time in response to prolonged water stress imposed at different growth stages (early, i.e., 2–6 months; mid-season, 4–8 months; terminal, 6–12 months after planting) and the control in four cassava cultivars. Vertical bars = ± SE (n = 4); **(A)** first crop cycle; **(B)** second crop cycle. ■ refers to control; ○ to early stress; ▽ to mid-season stress; □ to terminal stress. Note the reduction in yields during stress and recovery at final harvest from early and mid-season stress. Cultivars differed, with cv. CMC 40 (also known as M Col 1468) having the highest yield under stress. (El-Sharkawy and Cadavid 2002.)

leaf area formed rapidly, developing values similar to or higher than those of the controls, thus resulting in increased light interception (Figure 3-19). In the early stressed crops, increases in shoot biomass were lower, and remained lower, than under other water regimes. This indicated adverse effects of early stress on shoot biomass recovery (Caicedo 1993; El-Sharkawy et al. 1998b; El-Sharkawy and Cadavid 2002).

Under terminal stress (initiated 6 months after planting until final harvest at 12 months), final root yield at 12 months was less than the control. The largest reductions occurred in clone CM 523-7 (also known as 'ICA Catumare' in Colombia). An exception was CMC 40, whose yield was higher under stress. Genotype × water regime interaction was significant ($P < 0.01$), indicating the soundness of the strategy of breeding and selecting for specific edaphoclimatic zones. Similar findings were recently reported in the Sudanian savanna of Nigeria, using variations in the soil-water table as a variable for testing responses of cassava cultivars to water stress (Okogbenin et al. 2003).

Final yields of several clones across 2 years were not significantly different among water regimes (Table 3-11), indicating cassava's capacity to tolerate extended water deficit in subhumid and seasonally dry warm climates with bimodal precipitation patterns. Compared with clone CM 523-7, CMC 40 had the higher yield and shoot biomass under terminal stress, and the smaller leaf area, probably because of the high leaf photosynthesis observed in the field under diverse environments (El-Sharkawy et al. 1990; El-Sharkawy et al. 1992a; Pellet and El-Sharkawy 1993a).

Moreover, the upper-canopy leaves of CMC 40 showed greater activity for both C_3 and C_4 main enzymes than did the leaves of CM 523-7. In μmol per mg Chl/min, values were 8.2 for rubisco and 3.1 for PEPC in CMC 40 versus 3.6 and 1.6 in CM 523-7 (Table 3-10; CIAT Report 1992; López et al. 1993). These findings indicate the importance of selecting and breeding for high leaf photosynthesis, particularly under stress. Variations among clones for leaf photosynthesis, as measured in the field, were observed (Figure 3-20).

Nutrient uptake and use efficiency

Plant nutrient contents at final harvest were much less in stressed crops, particularly at early stages (El-Sharkawy et al. 1998b; El-Sharkawy and Cadavid 2002). The resulting higher nutrient-use efficiencies for all elements in both root and total biomass were caused mainly by increased reductions in shoot biomass and stable root yields, and higher HI (Table 3-12). Across clones, increases in nutrient-use efficiency due to early stress were >35% and >10% in roots and total biomass, respectively. However, among nutrient elements, the lowest percentage increases were for nitrogen-use efficiency and the highest for magnesium.

Similar trends were observed for mid-season stressed crops, but with lower values than for early stress. Under terminal stress, which started after peak crop growth at 6 months and after the bulk of nutrient uptake took place between 2 and 5 months (Howeler and Cadavid 1983; Howeler 2002), nutrient-use efficiency increased minimally in terms of root production, except for magnesium, which showed a 25% increase.

These findings clearly illustrate the beneficial effect of responses to water stress on conserving soil fertility, as well as on nutrient-use efficiency. Cassava is known for its high levels of tolerance of both water stress and poor soils (CIAT Reports 1990 to 1995; Howeler and Cadavid 1990; El-Sharkawy 1993; Pellet and El-Sharkawy 1993a, 1993b, 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 edaphoclimatic conditions.

Furthermore, these data have important implications for a breeding strategy for low-input, agricultural production systems (Hershey and Jennings 1992). Selection and breeding for plant types that demand less water and fewer nutrient resources (e.g., medium-statured to short cultivars) may help stabilize and sustain adequate productivity in resource-limited environments. In 2-year field trials held at CIAT–Quilichao, a group of clones that were tall (high top biomass), medium-statured (medium top biomass), and short (low top biomass) were evaluated for productivity and nutrient-use efficiency (CIAT Report 1996, 1997; El-Sharkawy et al. 1998a). Differences in root yields among this group of clones (planted at 10,000 plants/ha) were small because of higher HI in the medium-statured and short cassava than in tall cassava, with early root bulking tending to occur in both medium-statured and short clones (Table 3-13).

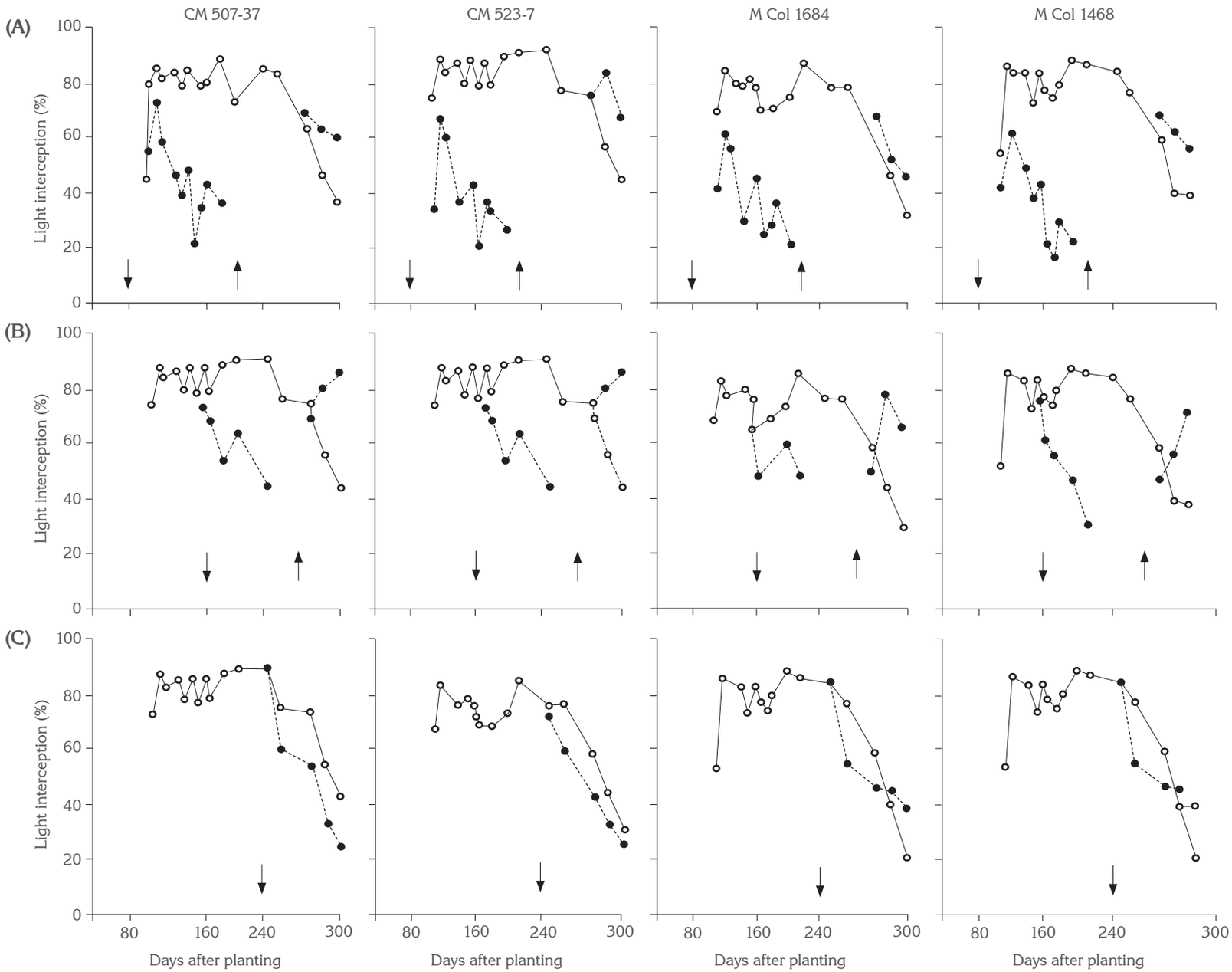

Figure 3-19. Interception of light (× 10^2 %) by four cassava cultivars affected by early water stress **(A)**; mid-season water stress **(B)**; and terminal water stress **(C)**. ● refers to stress; ○ to control; ▼ to stress starting; ▲ to recovery. (CIAT Report 1992; El-Sharkawy 2006.)

Table 3-11. Effect of prolonged water stress imposed on four cassava cultivars at different growth stages, Santander de Quilichao, Department of Cauca, Colombia, over the 1990/91, 1991/92, and 1992/93 seasons. Parameters evaluated were storage root yield and shoot biomass at 12 months after planting, and mean leaf area index (mean LAI) over the growth cycle. The data given below are from the 1991/92 and 1992/93 seasons. Note that cv. CMC 40 (also known as M Col 1468) had the highest root yield under prolonged terminal water stress and the highest activities for phospho*enol*pyruvate carboxylase (PEPC) and rubisco activities (Table 3-10).

Cultivar	Stress treatment (root yield at t/ha, dry weight)				Stress treatment (shoot biomass at t/ha, dry weight)				Stress treatment (mean LAI)			
	Control	Early	Mid-season	Terminal	Control	Early	Mid-season	Terminal	Control	Early	Mid-season	Terminal
CM 507-37	14.0	11.1	11.3	11.1	6.0	2.7	5.6	5.3	2.3	1.3	1.8	2.3
CM 523-7	13.8	12.8	12.1	9.7	5.3	4.2	5.2	4.5	2.3	1.4	1.3	2.0
CMC 40	10.0	10.4	12.1	14.6	7.8	3.9	5.7	6.7	1.7	1.1	1.1	1.7
M Col 1684	13.6	10.3	12.5	11.5	5.0	2.2	4.0	4.3	1.8	1.1	1.3	1.8
Average	12.9	11.2	12.9	11.7	6.0	3.3	5.1	5.2	2.0	1.2	1.4	2.0
$LSD_{0.05}$	NS*				0.8				0.3			
Treatment × cultivar	2.7				1.4				0.5			

* NS = not significant at 5%.

SOURCES: Caicedo (1993); El-Sharkawy et al. (1998b); El-Sharkawy and Cadavid (2002).

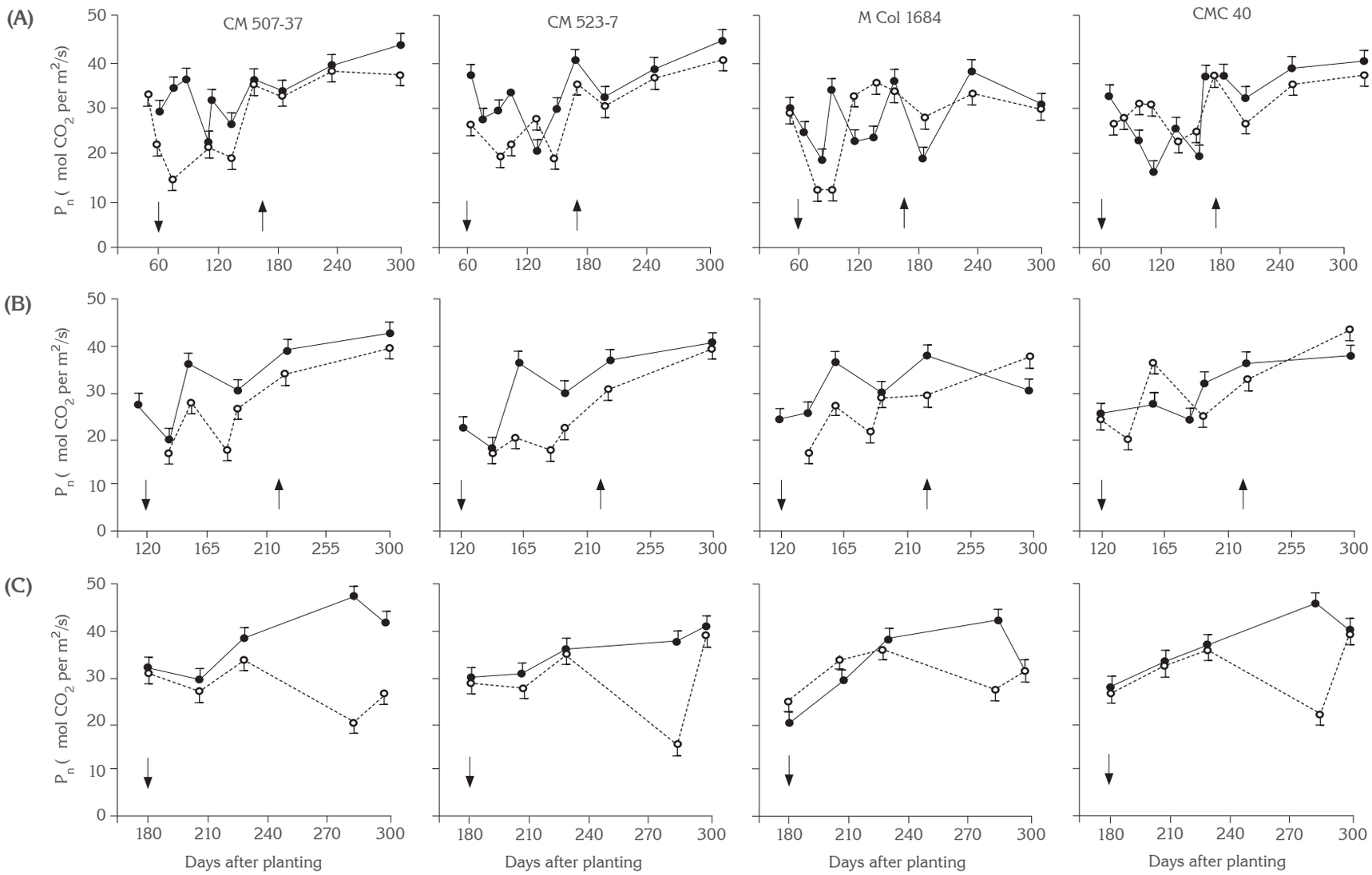

Figure 3-20. Photosynthetic rate (P_n) of upper-canopy leaves of four cassava cultivars affected by early water stress **(A)**; mid-season water stress **(B)**; and terminal water stress **(C)**. ● refers to stress; ○ to control; ↓ to stress starting; ↑ to recovery. Values are averages of four leaves ± SD. (CIAT Report 1992; El-Sharkawy 2006.)

Table 3-12. Effect of prolonged water stress on cassava at different growth stages on dry root yield, total harvestable biomass (t/ha), and nutrient-use efficiency (kg dry matter/kg total nutrient uptake) at final harvest, using an average of four clones. Note the higher nutrient-use efficiency under prolonged early and mid-season water stresses, compared with well-watered crops. The higher nutrient-use efficiency was mainly due to larger reductions in top biomass (and hence less nutrient uptake), compared with the smaller reductions in storage roots (and hence higher harvest indices).

Trait[a]	Stress treatment[b]							
	Control		Early (2–6 MAP)		Mid-season (4–8 MAP)		Terminal (6–11 MAP)	
	Roots	Total biomass	Roots	Total biomass	Roots	Total biomass	Roots	Total biomass
Root yield	11.5	—	11.9	—	13.1	—	11.0	—
Total biomass	—	15.8	—	15.5	—	18.2	—	14.5
N-UE	93	144	126	159	97	139	85	127
P-UE	615	947	971	1232	777	1109	645	966
K-UE	112	172	173	219	146	210	115	173
Ca-UE	270	414	408	519	300	433	274	413
Mg-UE	379	577	616	780	504	722	473	712
% change due to stress								
Root yield			+3		+14		-4	
Total biomass				-2		+15		-8
N-UE			+35	+10	+4	-3	-9	-12
P-UE			+58	+30	+26	+17	+5	+2
K-UE			+54	+27	+30	+22	+3	0
Ca-UE			+51	+25	+11	+5	+1	0
Mg-UE			+63	+35	+33	+25	+25	+23

a. N-UE, P-UE, K-UE, Ca-UE, and Mg-UE refer to use efficiency of nitrogen, phosphorus, potassium, calcium, and magnesium, respectively.
b. MAP refers to months after planting.

\SOURCES: CIAT Report (1993); El-Sharkawy et al. (1998b); El-Sharkawy and Cadavid (2002).

Table 3-13. Dry root yield and top biomass (t/ha) for 15 cassava clones of differing biomass weights (higher, intermediate, and lower) grown at Santander de Quilichao, Colombia. Data are from the first cycle (1994/95). Note the early bulking (i.e., storage root-filling capacity) trends within the first 5 months in clones with medium or short stature, a trait advantageous for selecting and breeding improved materials for semi-arid ecosystems.

Biomass weight	Root yield at days after planting			Tops at 303 days after planting
	126	182	303	
Higher (mean of 5 clones)	1.63	2.64	11.32	6.6
Intermediate (mean of 5 clones)	2.32	2.80	10.90	3.7
Lower (mean of 5 clones)	2.21	3.02	10.39	2.6
$LSD_{0.05}$	0.55	NS	NS	0.95

SOURCE: El-Sharkawy et al. (1998a).

The higher shoot biomass of tall cultivars meant higher nutrient uptake and less nutrient-use efficiency in terms of root production. In contrast, the total plant nutrient uptake in medium-statured and short cultivars was 15% to 30% less (Table 3-14; El-Sharkawy et al. 1998a). Furthermore, short cultivars had 12% higher leaf photosynthesis than tall ones (El-Sharkawy and de Tafur 2010). These data support the strategy of breeding and selecting for medium to short plant architecture, which would be advantageous for higher efficiency in the use of both native soil nutrients and applied fertilizers, particularly if crop residues are not recycled to the soil.

Short cassava would be furthermore beneficial for both increasing productivity and reducing soil erosion if it is planted at higher densities than normally used for mono- or intercropping. Current farming practices use about 5000 and 10,000 plants/ha in intercropped and monocropped cassava, respectively. The more rapidly the canopy closes during early growth, the less likely soils are prone to erosion. This breeding objective should be combined with greater leaf photosynthesis, longer leaf life, and host-plant resistance or tolerance of pests and diseases (i.e., improved leaf retention; Figure 3-21; Lenis et al. 2006), particularly for developing improved germplasm targeted to seasonally dry and semi-arid zones (Byrne et al. 1982; Cock and El-Sharkawy 1988a, 1988b; El-Sharkawy et al. 1990, 1992b; Hershey and Jennings 1992; El-Sharkawy 1993, 2004).

Table 3-14. Nutrient-use efficiency for root production at 10 months after planting (kg dry root/kg total nutrient uptake) for tall, medium-statured, or short cassava. Note the significantly higher nutrient-use efficiency in both medium-statured and short clones, compared with the tall ones. The higher values were mainly a result of both much smaller top biomass (and hence smaller nutrient uptake), as root yields were comparable, regardless of plant heights (Table 3-13). The medium-statured and short clones with higher nutrient-use efficiency are advantageous for sustainable production in low-input production systems and in low-fertility soils. Values are averages of 2 years (1994–1996).

Group of 5 cultivars	Nutrient				
	N	P	K	Ca	Mg
Tall	110	715	132	347	589
Medium-statured	133	837	149	439	686
Short	131	885	161	430	669
$LSD_{0.05}$	17	85	22	77	91

SOURCE: El-Sharkawy et al. (1998a).

The short cultivar M Col 2215 was selected in 1987–1989 for its high drought tolerance, high dry matter content in storage roots, and high PEPC activity in leaf extracts (El-Sharkawy et al. 1990, 2008). It was introduced to Ecuador and evaluated for several years in the semi-arid western coast, where it yielded better than local varieties. Farmers participated in field trials and quickly accepted it, which led to its official release under the name 'Portoviejo 650' in 1992.

Water uptake and use efficiency

Patterns of water uptake in various clones during extended water stress imposed at different stages of growth are shown in Figure 3-22. In all stress treatments, cassava withdrew more water from the deep soil layer (at 2 m deep), after the upper layer was almost depleted. Moreover, the water uptake from this deep layer increased as stress progressed, particularly in the terminal stress treatment, indicating deep rooting behavior, as previously reported (Connor et al. 1981; El-Sharkawy and Cock 1987b; CIAT Reports 1991 to 1994; El-Sharkawy et al. 1992b; de Tafur et al. 1997a; Cadavid et al. 1998).

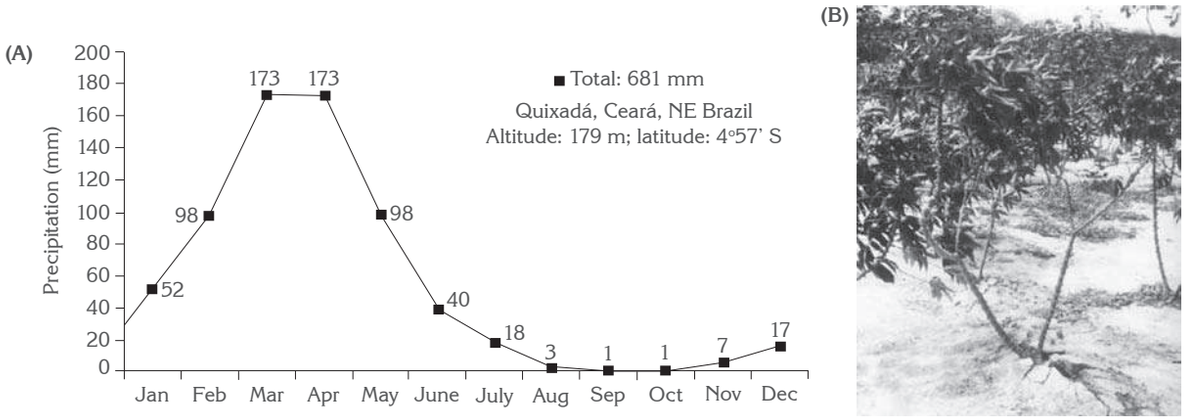

Figure 3-21. Screening for drought tolerance for the semi-arid ecosystem at Quixadá, Ceará, Northeast Brazil. **(A)** Long-term (30 years) annual precipitation; 80% occurs over 4 months and the rest of the growing season is dry, with high air temperatures, high evapotranspiration, and high solar radiation. Soils are sandy, with low water-holding capacity, and very low in nutrients. **(B)** Cassava germplasm at 8 months, showing good leaf retention and sustainable canopy. Several clones were selected with high yield potential, tolerance of drought, low HCN, and tolerance of major pests and diseases. Yield was >12 t/ha fresh root at 12 months with a dry matter content of >25% that could be greatly enhanced with a second wet cycle.
References: El-Sharkawy 1993; de Tafur et al. 1997b.

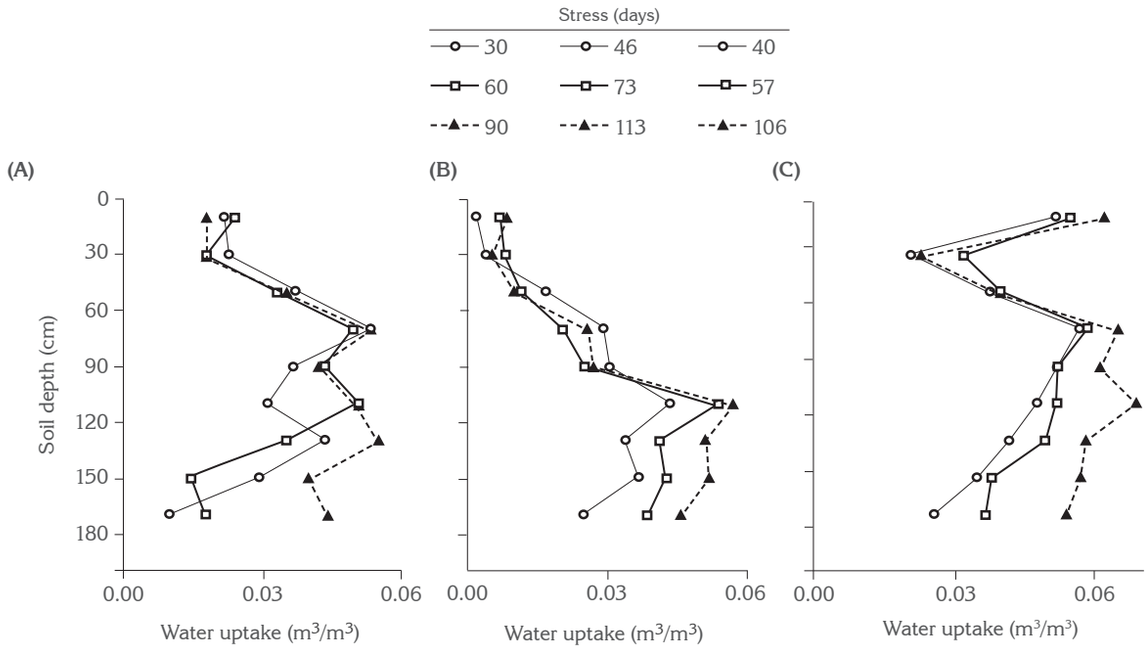

Figure 3-22. Patterns of water uptake by cassava (averages of four cultivars) during water deficits of differing lengths, Santander de Quilichao, Department of Cauca, Colombia. **(A)** Stress at 2 months after planting; **(B)** stress at 4 months after planting; and **(C)** stress at 6 months after planting. Note the greater water extraction from deeper soil layers that increased as water stress progressed over time, particularly in terminally stressed crops **(C)** (El- Sharkawy et al. 1992b; CIAT Report 1993; de Tafur et al. 1997a; El-Sharkawy 2006).

By volume, the soil water available in the first 2 m of soil ranges from 8% to 12% (Connor et al. 1981; El-Sharkawy et al. 1992b). Hence, water uptake under any of these prolonged stress treatments would probably not exceed about 200 mm (i.e., the total available water at 2 m deep). This indicates cassava's capacity to conserve and deplete deep soil water slowly over extended stress. For two cultivars (M Col 1684 and its hybrid CM 507-37) subjected to a 3-month mid-season water shortage, El-Sharkawy et al. (1992b) reported that total water uptake, as estimated from periodic sampling of soil cores at 2 m deep, was

around 100 and 160 mm during 35 and 96 days of stress, respectively. The latter value was equivalent to 70%–75% of the plant-available water at a depth of 2 m in this soil, and much less than the rate of pan evaporation of about 4.4 mm/day at the trial site.

In recent studies in Ghana, Oguntunde (2005) used the sap flow method to estimate daily cassava canopy transpiration under prolonged natural stress, which he estimated as 0.8–1.2 mm. This is equivalent to 24% of potential evapotranspiration. El-Sharkawy et al. (1992b) found that, over 43 days of peak canopy growth (117 to 160 days after planting), estimated crop water-use efficiency values were 4.4–4.8 g/kg of water in stressed crops and 3.7–4.9 g/kg in unstressed crops (clone M Col 1684 and its hybrid CM 507-37, and using total oven-dried biomass).

Because cassava has a long growth cycle and a low LAI during a significant portion of its growing season, seasonal crop water-use efficiency is reduced. A cassava crop grown in a field lysimeter was estimated as having a seasonal water-use efficiency of about 2.9 g total dry biomass/kg water. This value compares favorably with the value found for grain sorghum (C_4), which is about 3.1 g/kg, and is much higher than that found for field bean (C_3), which is about 1.7 g/kg (El-Sharkawy and Cock 1986; El-Sharkawy 2004). Because cassava has a higher HI (0.6–0.7), water-use efficiency in terms of economic yield would be even higher than for either sorghum or field bean, which have lower HI values.

A large field trial on yield, involving 16 improved cassava cultivars, was established in a seasonally dry zone (Patia Valley, Cauca, Colombia) that had less than 1000 mm of precipitation in 10 months. Average harvestable total dry biomass, excluding dropped leaves and fine roots, was more than 30 t/ha (El-Sharkawy et al. 1990). In this trial, the mean oven-dried root yield ranged among cultivars from 15 to 27.4 t/ha, with an overall mean of 20 t/ha. About 60% of rainfall occurred in months 6 and 7 of the growth cycle, with perhaps some water runoff and deep percolation into the clayey soil occurring.

However, if we assume all rainfall to have been effectively used by the crops and lost only through evapotranspiration (i.e., through plant transpiration and evaporation from exposed soils), then an evapotranspiration ratio of 270–300 is obtained. This ratio is water loss per unit of total dry matter produced. Such a ratio is comparable with values (excluding soil evaporation) observed in tropical C_4 crop species such as maize, millet, sorghum, and sudangrass. It is also much smaller than the values obtained in C_3 species that were grown in large weighable containers in a semi-arid climate almost a century ago by L.J. Briggs and H.L. Shantz at Akron, Colorado, USA (Stanhill 1986).

The actual evapotranspiration ratio of cassava might have been even lower than the estimated values here, as dropped leaves may account for 2–6 t/ha (El-Sharkawy and Cock 1987b; El-Sharkawy et al. 1992b; Pellet and El-Sharkawy 1997). In terms of economic yield, the estimated evapotranspiration ratio for cassava of about 500 in this trial is much less than the values for grain sorghum (868), proso millet (567), and maize (1405) obtained in the Briggs and Shantz trials (Stanhill 1986). If adjusted for moisture content in the grains of these cereals, the values would be much greater than in cassava. These findings indicate cassava's potentially high water-use efficiency and further strengthen its comparative advantage in water-limited zones. Under non-limiting rainfall or with irrigation, however, cassava would be more efficient in terms of annual calories production per unit land area and water consumption than most tropical C_4 grain crops (El-Sharkawy 1993, 2004).

El-Sharkawy (2005) predicted that cassava productivity would probably be enhanced further by rises in atmospheric CO_2 and temperature (i.e., as a result of global climate change), which should result in much higher water-use efficiency. This prediction was corroborated by recent findings that leaf photosynthesis and root yield of field-grown cassava under elevated levels of CO_2 in the tropics (i.e., 680 cm^3/m^3 in open-top chambers) had higher values than plants grown under ambient CO_2 (Fernández et al. 2002). These findings indicated an absence of photosynthetic down-regulation often observed in other plant species (Kramer 1981; Mooney et al. 1991; Pettersson and McDonald 1994; Webber et al. 1994; Woodrow 1994).

The absence of photosynthetic down-regulation in cassava grown under elevated CO_2 was apparently associated with the higher carboxylation efficiency of the C_3 rubisco, despite reductions in soluble protein and N contents in leaves. Thus, higher nitrogen-use efficiency in terms of CO_2 uptake is indicated. El-Sharkawy (2004) reported significant positive correlations between dry root yield and photosynthetic N-use efficiency across a wide range of field-grown cassava cultivars with no CO_2 enrichment. (Photosynthetic N-use efficiency is defined as CO_2 uptake per unit of total leaf N, as measured in normal air and with high solar irradiance in upper-canopy leaves.)

Furthermore, soluble sugars and starch contents in leaves of plants grown under elevated levels of CO_2 were reduced. These findings suggest that higher demands are made by strong sinks for assimilates, as indicated by the concomitant increases in both shoot biomass and storage root yield (Fernández et al. 2002). Ziska et al. (1991) also reported 56% increases in leaf photosynthesis of cassava plants grown for long periods under elevated levels of CO_2 under controlled conditions (at 300 cm^3/m^3 more than ambient CO_2), indicating an absence of down-regulation.

In contrast to the above findings, Gleadow et al. (2009) reported lower leaf photosynthetic rates in plants grown at higher than ambient CO_2, particularly in those grown at the highest level. They also found significant reductions in shoot and storage root biomass. However, we point out that the authors studied potted cassava grown under greenhouse conditions at different levels of CO_2, that is, at 360, 550, and 710 ppm CO_2. Obviously, these findings indicated a feedback inhibition due to the rooting systems being confined by the growth media used. Cassava is a tropical shrub that requires large volumes of soil. When grown under inappropriate conditions, it will not express either its leaf photosynthetic capacity or its potential productivity. Thus, as warned earlier in the chapter, such findings are invalid and any resulting conclusions must be questioned. In contrast, field research conducted in CIAT's sunny, hot, humid, and tropical environment, demonstrated cassava's high photosynthetic capacity and productivity.

These photosynthetic attributes, combined with a high optimal temperature for leaf photosynthesis (Figure 3-4) and elevated activity of the C_4 PEPC in cassava leaves (Tables 3-7 to 3-10), may confer adaptive advantages for cassava growth and productivity in a globally warming climate.

In seasonally dry tropical ecosystems, excess rains often recharge deeper soil layers. Cassava's deep-rooting characteristics are of paramount importance, particularly where the crop must endure several months' of prolonged drought. These characteristics, combined with partial stomatal closure in response to both soil and atmospheric water deficits; reduced leaf canopy and light interception; and adequate leaf photosynthesis make cassava a highly drought-resistant crop.

This pattern of conserved water use results in optimizing seasonal crop water-use efficiency (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 water-use efficiencies. Deep-rooting characteristics account for many differences in drought tolerance among species. These inherent mechanisms may partly explain why stressed cassava is still able to produce more adequately than cereals and grain-legume food crops. They also further strengthen the relevance of the strategy to expand cassava cultivation into drought-prone areas where severe food shortages may occur (Hershey and Jennings 1992; El-Sharkawy 1993, 2004; de Tafur et al. 1997a; Okogbenin et al. 2003).

Selecting for Tolerance of Low-Fertility Soils

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

Table 3-15 presents data on yield and root dry matter content, with and without applied K fertilizer, for a group of the screened accessions. Low levels of K in these soils were indicated by the strong responses to K applications of all clones tested. The average dry root yield for accessions receiving K applications was 10.3 t/ha. In contrast, yield for accessions not receiving K fertilizer was 5.9 t/ha. Dry matter content was, respectively, 38.1% and 36.2%. However, genetic differences were wide for yield and for tolerance levels, as estimated by the calculated low-K adaptation index (i.e., the product of yields at K levels relative to the overall mean in the trial).

Two accessions (CM 2777-2 and CM 3372-4) had high tolerance levels with an adaptation index that was 50% higher than the overall mean index (1.0). They

Table 3-15. Dry root yield (t/ha), root dry matter (DM; %), and low-K adaptation index for 15 cassava clones grown at Santander de Quilichao, Colombia. Data are from the second cycle (1994/95). Clones with high adaptation indices are good genetic sources for selecting and breeding for tolerance of low soil-fertility (El-Sharkawy and Cadavid 2000).

Clones	Zero K		100 kg K/ha		Low-K adaptation index[a]	
	Dry root	Root DM	Dry root	Root DM		
CM 2777-2	7.9	31.8	13.3	35.8	1.73	HA
CM 3372-4	7.8	38.9	12.8	40.8	1.64	
CG 402-11	7.6	22.9	11.5	27.3	1.44	
CG 1141-1	6.9	40.4	12.0	42.2	1.36	
CG 165-7	6.2	35.2	12.1	37.8	1.23	IA
CM 4777-2	5.6	40.5	13.4	42.1	1.23	
CM 4729-4	7.5	39.9	9.8	38.8	1.21	
CM 4574-7	5.7	35.9	9.8	36.9	0.92	LA
CM 3311-3	6.0	38.6	9.2	37.5	0.91	
SG 107-35	5.6	41.3	9.7	40.1	0.89	
CM 5286-3	4.9	30.6	10.7	35.9	0.86	
CM 2177-2	6.5	34.9	7.0	36.9	0.75	
CM 3306-4	3.8	41.9	10.3	43.4	0.64	
CM 2766-5	3.5	32.1	8.1	35.2	0.47	
CM 3299-4	3.3	37.9	5.1	41.2	0.28	
Mean of all clones	5.9	36.2	10.3	38.1	1.00	
$LSD_{0.05}$	1.8	3.7	2.5	2.6		

a.
$$\text{Low-K adaptation index} = \frac{\text{(yield with zero K) (yield with 100 kg K/ha)}}{\text{(mean yield with zero K) (mean yield with 100 kg K/ha)}}$$

Index of adaptation to low soil K: H = high; I = intermediate; L = low.

SOURCE: CIAT Report (1995).

therefore represent suitable genetic sources for breeding improved germplasm. Pellet and El-Sharkawy (1997) identified clones that had high yields, whether with or without fertilizer, indicating that selecting these clones, instead of landraces or other varieties, for high yield would not be detrimental to soil fertility. El-Sharkawy and Cadavid (2000) also reported on the existence of genetic variation in clones responding to K in a 5-year trial. These clones also showed high yield potential, whether with or without K application, and high K-use efficiency in terms of root production.

One clone (CM 507-37) had good levels of leaf retention and a deep fine-root system (El-Sharkawy and Cock 1987b; El-Sharkawy et al. 1992b), indicating the importance of these traits. Moreover, clone CMC 40 (i.e., M Col 1468) showed the highest nutrient-use efficiency under extended water stress at different growth stages. It had higher biomass and yield (El-Sharkawy et al. 1998b; El-Sharkawy and Cadavid 2002), but low efficiency under wet conditions (Pellet and El-Sharkawy 1997; El-Sharkawy and Cadavid 2000), highlighting that the genotype × environment interaction is important in this case.

Figure 3-23 presents data on yields, whether with or without P application, of a group of 33 accessions. Some clones had high yields, with or without P fertilizer, indicating high tolerance of low soil-P, as shown by their enhanced low-P adaptation indices. Cassava relies on vesicular arbuscular mycorrhizae (VAM) for P uptake (Howeler et al. 1982; Howeler and Sieverding 1983). However, Pellet and El-Sharkawy (1993b) reported that cultivar differences in P uptake were related more to differences in fine-root-length density than to VAM infection rates. Uptake efficiency (estimated as uptake per unit root length) did not differ among cultivars. This finding again indicates the importance of the fine-root system in cassava plant–soil relationships.

Furthermore, these authors concluded that yield response to P was regulated by the balance between the potential for shoot growth and for storage roots. Adaptation to low-P could be improved by selecting for high fine-root-length density, moderate shoot growth, and stable high HI. This conclusion was further corroborated by

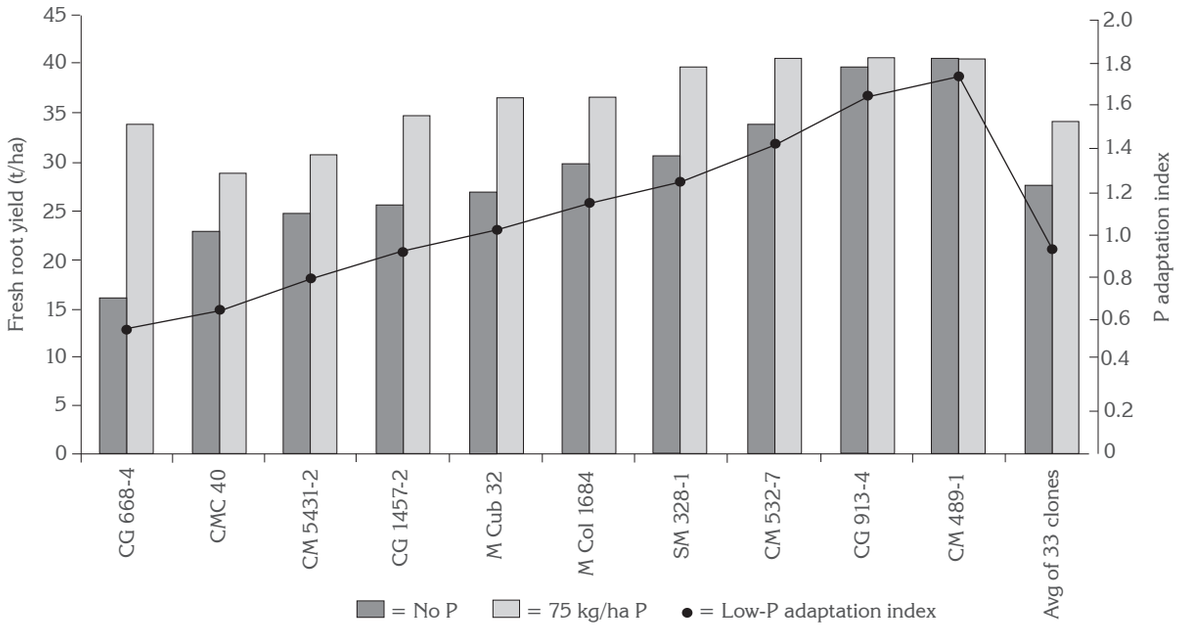

Figure 3-23. Screening cassava germplasm for tolerance of low-phosphorus soils. Note that some clones had good yield potential at low and high levels. More than 1600 accessions were screened, and clones selected for the breeding program. Low-P adaptation index was calculated as in Table 3-15. (CIAT Report 1992; El-Sharkawy 2006; Borrell 2010.)

enhanced nutrient-use efficiency under stress through higher reductions in shoot biomass than in roots (i.e., higher HI) and in terms of plant architecture (medium-statured and short versus tall cultivars) (Table 3-12; El-Sharkawy et al. 1998a, 1998b; El-Sharkawy and Cadavid 2002).

Moreover, in another group of accessions, low-P adaptation indices were correlated with standing shoot biomass at harvest (not including dropped leaves), number of harvested roots per plant, and seasonal average, upper-canopy-leaf photosynthesis (CIAT Report 1990; El-Sharkawy 2004). This finding indicated the importance of both carbon assimilation source and capacity, and of root sink capacity in selecting and breeding for tolerance of low-fertility soils. Notably, clone CM 489-1, with a high adaptation index to low-P (Figure 3-23), had the following characteristics: high photosynthetic rate at different levels of P; high efficiency in the use of nutrients and solar radiation (Pellet and El-Sharkawy 1993a, 1993b, 1997); high yields, with or without mid-season extended water deficits (Table 3-6; Figure 3-9; El-Sharkawy 1993); less reduction in leaf photosynthesis during water stress (Figure 3-17); and high PEPC activity in leaves under field conditions that correlate with photosynthesis (El-Sharkawy 2004).

These findings indicate that selecting for and assembling several desirable plant traits in one genotype is possible. Complementary multidisciplinary or, even better, multi-institutional research is crucial in this case, as it would enhance research efficiency and the benefit-to-cost ratio (El-Sharkawy 2005).

Highlights and Conclusions

The research reviewed here on cassava productivity, physiology and/or ecophysiology, and responses to environmental stresses was conducted in collaboration with a multidisciplinary team at CIAT. The Center also holds a diverse germplasm bank of the crop, which has been assembled over 40 years. The research objectives revolved around the strategy adopted for developing new technologies to enhance crop productivity in most of the edaphoclimatic zones under which cassava is cultivated, particularly stressful environments.

Under favorable environments in lowland and mid-altitude tropical zones with near-optimal climatic and edaphic conditions for the crop to realize its inherent potential, cassava is highly productive in terms of root yield and total biological biomass. For example, under trial conditions, improved germplasm

can, after 10–12 months' growth, attain >15 t/ha of oven-dried roots, containing 85% starch. The physiological mechanisms underlying such high potential productivity are:

1. High leaf photosynthetic potential, comparable with those in efficient C_4 crops (assuming the following conditions are common: high humidity, adequate soil moisture, high leaf temperature, high solar radiation, and a Pn rate that exceeds 40 μmol CO2 per m^2/s);

2. Long leaf life (>60 days), with the leaves remaining active for most of their life spans;

3. Sustainable leaf canopy that optimizes light interception during a significant portion of the growth cycle; and

4. High harvest index (>0.5), coupled with strong root sink (i.e., larger number of storage roots/plant).

Under stressful environments in seasonally dry and semi-arid tropics, productivity is reduced, with more reduction in aboveground parts (shoots) than in storage roots (i.e., higher HI). Under these conditions, the crop possesses some inherent adaptive mechanisms for tolerance. The most important one 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.

Together with this "stress avoidance mechanism" is a capacity for deep rooting that enables the plant to slowly extract water from as deep as 2 m. The crop therefore not only survives dry periods of up to 3 months long, but also produces a reasonable yield through its efficient use of water and nutrients. Moreover, leaf canopy is much reduced under prolonged stress, contributing to lower crop water consumption. When it recovers from stress, cassava rapidly forms new leaves with higher photosynthetic capacity, which compensates for yield reductions from the previous prolonged stress.

Productivity over a wide range of germplasm and in different environments correlates with upper canopy leaf photosynthesis, as measured in the field. The relationship stems mostly from non-stomatal factors, that is, from biochemical and anatomical leaf traits. Among these leaf traits is the elevated activity of the C_4 PEPC enzyme. Breeding programs could exploit the genetic variations found within cassava germplasm and wild *Manihot* for both leaf photosynthesis and enzyme activity. Leaf area duration under stress, together with host-plant resistance or tolerance of pests and diseases, is a critical trait because yield correlates with leaf retention capacity (Lenis et al. 2006). Deep rooting capacity is another important trait for selecting and breeding improved germplasm for drier zones.

In cooler zones such as higher altitudes in the tropics and lowland subtropics, cassava growth is slower and the crop stays in the ground for longer to achieve adequate yields. Under these conditions, leaf formation is slower, leaf photosynthesis is much reduced, but leaf life is longer (Irikura et al. 1979; El-Sharkawy et al. 1992a, 1993). Wide genetic variations exist for photosynthesis that may be valuable for selecting and breeding for genotypes that better tolerate cool climates. Combining enhanced leaf photosynthesis with the normally longer leaf life in cool climates may improve productivity.

Selecting for medium-statured and short cassava instead of tall cassava is advantageous for saving on nutrient uptake and ensuring higher nutrient-use efficiency for root production without sacrificing potential yield. Germplasm from the core collection was screened for tolerance of soils low in P and K, resulting in the identification of several accessions with good levels of tolerance.

Results also point to the importance of field research versus greenhouse or growth-chamber studies that do not calibrate for representative environments to account for acclimation factors (El-Sharkawy 2005). Calibration becomes even more critical when data from indoor-grown plants are used to extrapolate to the field or to develop crop models.

Much remains to be done to further improve productivity while conserving dwindling natural resources such as water and land. Developing countries, in particular, need more support to continue with maintenance research, which aims to upgrade previous findings and technologies; contribute to sustainable agricultural, economic, and social developments; and enhance food supply to meet increasing demands.

Basic research can be cost-effective, with high returns, even if slower. It can be especially successful

when conducted in collaboration with multidisciplinary and/or multi-institutional teams that follow well-planned strategies and are focused towards fulfilling a set of high priority goals and objectives.

International research and development organizations, donor agencies, and private sectors should take leading roles in financing and supporting basic research efforts, particularly those oriented towards serving the technological needs of resource-poor developing countries. Without the steady funding by only a few international donors during the 1940s and 1950s, the Green Revolution of the 1960s, which had saved many developing countries from famine, would probably never have happened. The current CGIAR policy of conducting short-term research projects, instead of team-oriented core research, is counterproductive and should be reversed.

Acknowledgments

The authors are indebted to the many colleagues, associates, students, workers, and secretaries at CIAT whose dedication and support were crucial in realizing the research achievements highlighted in this chapter.

We most appreciated the many useful reprints received from G. Bowes, V. Gutschick, P. Jarvis, Ch. Körner, R. Lösch, F. Meinzer, O. Ueno, and P. Westhoff. We thank M.B. Kirkham for the copy of her invaluable textbook on *Principles of Soil and Plant Water Relations*. Useful comments on an original version were received from D. Laing, former deputy director for research at CIAT; J.D. Hesketh, USDA/ARS and the University of Illinois, Urbana, USA; and three anonymous reviewers. Many constructive editorial changes were made by Elizabeth L. McAdam, Melbourne, Australia.

We are grateful to Farah El-Sharkawy Navarro for her highly needed help in searching the Internet for pertinent information, assembling the numerous data in tables and figures scattered in various published and unpublished documents, and typing the manuscript.

Crucial in conducting this research was the logistic and financial support from various governments and research-funding organizations, including Australia, Brazil, Colombia, Germany, Switzerland, and USA. This support allowed us to target the research towards answering the needs of some of the poorest farmers and consumers living in developing countries.

References

To save space, the acronym "CIAT" is used instead of "Centro Internaccional de Agricultura tropical".

Ackerson RC. 1980. Stomatal response of cotton to water stress and abscisic acid as affected by water stress history. Plant Physiol 65:455–459.

Ackerson RC; Hebert RR. 1981. Osmoregulation in cotton in response to water stress. Plant Physiol 67:484–488.

Allem AC. 2002. The origins and taxonomy of cassava. In: Hillocks RJ; Thresh JM; Bellotti AC, eds. Cassava: biology, production and utilization. CABI Publishing, Wallingford, UK. p 1–16.

Álvarez E; Llano GA; with the collaboration of EL McAdam. 2008. Controlling cassava root rots with the participation of Tukano communities in the Mitú area of the Colombian Amazon. Gene Conserve [online] 7(28):446–475. Available at www.geneconserve.pro.br/artigos08.htm

Alves AAC. 1998. Physiological and developmental changes in cassava (*Manihot esculenta* Crantz) under water deficit. Dissertation. Cornell University, Ithaca, NY, USA.

Alves AAC. 2002. Cassava botany and physiology. In: Hillocks RJ; Thresh JM; Bellotti AC, eds. Cassava: biology, production and utilization. CABI Publishing, Wallingford, UK.

Alves AAC; Setter TL. 2000. Response of cassava to water deficit: leaf area growth and abscisic acid. Crop Sci 40:131–137.

Angelov MN; Sun J; Byrd GT; Brown RH; Black CC. 1993. Novel characteristics of cassava, *Manihot esculenta* Crantz, a reputed C_3-C_4 intermediate photosynthesis species. Photosynth Res 38:61–72.

Appleby RF; Davies WJ. 1983. The structure and orientation of guard cells in plants showing stomatal responses to changing vapour pressure difference. Ann Bot 52:459–468.

Araus JL; Brown RH; Bouton JH; Serret MD. 1990. Leaf anatomical characteristics in *Flaveria trinervia* (C_4), *Flaveria brownii* (C_4-like), and their F_1 hybrid. Photosynth Res 26:49–57.

Aslam M; Lowe SB; Hunt LA. 1977. Effect of leaf age on photosynthesis and transpiration of cassava (*Manihot esculenta*). Can J Bot 55:2288–2295.

Aston MJ. 1976. Variation of stomatal diffusion resistance with ambient humidity in sunflower (*Helianthus annuus*). Aust J Plant Physiol 3:489–501.

Balagopalan C. 2002. Cassava utilization in food, feed and industry. In: Hillocks RJ; Thresh JM; Bellotti AC, eds. Cassava: biology, production and utilization. CABI Publishing, Wallingford, UK. p 301–318.

Beeching JR; Marmey P; Gavalda MC; Noirot M; Hayson HR; Hughes MA; Charrier A. 1993. An assessment of genetic diversity within a collection of cassava (*Manihot esculenta* Crantz) germplasm using molecular markers. Ann Bot 72:515–520.

Bellotti AC. 2002. Arthropod pests. In: Hillocks RJ; Thresh JM; Bellotti AC, eds. Cassava: biology, production and utilization. CABI Publishing, Wallingford, UK. p 209–235.

Bellotti AC; Arias V, B. 1993. The possible role of HCN in the biology and feeding behavior of the cassava burrowing bug (*Cyrtomenus bergi* Froeschner: Cydnidae: Hemiptera). In: Roca WM; Thro AM, eds. Proc First International Scientific Meeting of the Cassava Biotechnology Network. CIAT, Cali, Colombia. p 406–409.

Bellotti AC; Riis L. 1994. Cassava cyanogenic potential and resistance to pests and diseases. Acta Hortic 375:141–151.

Bellotti AC; Vargas OH; Arias B; Castaño O; García C. 1988. *Cyrtomenus bergi* Froeschner, a new pest of cassava: biology, ecology and control. In: Degras LM, ed. Proc 7th Symposium of the International Society for Tropical Root and Tuber Crops, held in Gosier (Guadeloupe), 1–6 July 1985. Institut National de la Recherche Agronomique (INRA), Paris, France. p 551–561.

Berg VS; El-Sharkawy MA; Hernández ADP; Cock JH. 1986. Leaf orientation and water relations in cassava. In: Annual Meeting of the American Society of Plant Physiologists, 8–12 June. Louisiana State University, Baton Rouge, LA, USA. p 186.

Bernal LM. 1991. Estudios sobre la actividad fosfo*enol*piruvato carboxilasa en cultivares de yuca (*Manihot esculenta* Crantz). BSc thesis. Pontificia Universidad Javeriana, Bogotá, Colombia.

Berry J; Björkman O. 1980. Photosynthetic response to temperature in higher plants. Annu Rev Plant Physiol 31:491–543.

Björkman O; Badger MR; Armond PA. 1980. Response and adaptation of photosynthesis to high temperature. In: Turner NC; Kramer PJ, eds. Adaptation of plants to water and high temperature stress. John Wiley & Sons, New York, USA. p 233–249.

Boardman NK. 1977. Comparative photosynthesis of sun and shade plants. Annu Rev Plant Physiol 28:355–377.

Bongi G; Mencuccini M; Fontanazza G. 1987. Photosynthesis of olive leaves: effect of light flux density, leaf age, temperature, peltates, and H_2O vapour pressure deficit on gas exchange. J Am Hortic Sci 112:143–148.

Borrell B. 2010. Elemental shortage. The Scientist [on line] 24(11):46. Available at www.the-scientist.com/2010/11/1/46/1/.

Boyer JS. 1996. Advances in drought tolerance in plants. Adv Agron 56:187–218.

Brekelbaum T; Bellotti A; Lozano JC, eds. 1978. Proc Cassava Protection Workshop. CIAT, Cali, Colombia.

Brown RH; Bouton JH. 1993. Physiology and genetics of interspecific hybrids between photosynthetic types. Annu Rev Plant Physiol Plant Mol Biol 44:435–456.

Brown RH; Hattersley PW. 1989. Leaf anatomy of C_3-C_4 species as related to evolution of C_4 photosynthesis. Plant Physiol 91:1543–1550.

Brown RH; Bouton JH; Evans PT; Malter HE; Rigsby LL. 1985. Photosynthesis, morphology, leaf anatomy, and cytogenetics of hybrids between C_3 and C_3/C_4 *Panicum* species. Plant Physiol 77:653–658.

Bunce JA. 1981. Comparative responses of leaf conductance to humidity in single attached leaves. J Exp Bot 32:629–634.

Bunce JA. 1982. Photosynthesis at ambient and elevated humidity over a growing season in soybean. Photosynth Res 3:307–311.

Bunce JA. 1984. Identifying soybean lines differing in gas exchange sensitivity to humidity. Ann Appl Biol 105:313–318.

Bunce JA. 1985. Effect of boundary layer conductance on the response of stomata to humidity. Plant, Cell Environ 8:55–57.

Bunce JA. 1986. Measurements and modelling of photosynthesis in field crops. CRC Crit Rev Plant Sci 4:47–77.

Burt RL. 1964. Carbohydrate utilization as a factor in plant growth. Aust J Biol Sci 17:867–877.

Byrne DH; Guerrero JM; Bellotti AC; Gracen VE. 1982. Yield and plant growth responses of *Mononychellus* mite resistant and susceptible cassava cultivars under protected vs. infested conditions. Crop Sci 22: 486–490.

Cadavid L, LF; El-Sharkawy MA; Acosta A; Sánchez T. 1998. Long-term effects of mulch fertilization and tillage on cassava grown in sandy soils in northern Colombia. Field Crops Res 57:45–56.

Caicedo JA. 1993. Respuesta de cuatro cultivares de yuca (*Manihot esculenta* Crantz) a la modificación del estado hídrico del suelo. BSc thesis. Universidad Nacional de Colombia, Palmira, Colombia.

Calatayud P-A; Barón CH; Velásquez H; Arroyave JA; Lamaze T. 2002. Wild *Manihot* species do not possess C_4 photosynthesis. Ann Bot 89:125–127.

Calvert LA; Thresh JM. 2002. The viruses and virus diseases of cassava. In: Hillocks RJ; Thresh JM; Bellotti AC, eds. Cassava: biology, production and utilization. CABI Publishing, Wallingford, UK. p 237–260.

Cayón MG; El-Sharkawy MA; Cadavid L, LF. 1997. Leaf gas exchange of cassava as affected by quality of planting material and water stress. Photosynthetica 34:409–418.

Chabot BF; Hicks DJ. 1982. The ecology of leaf life span. Ann Rev Ecol Syst 13:229–259.

Chapin III, FS. 1991. Integrated responses of plants to stress. BioScience 41:29–36.

Chu C; Dai Z; Ku MSB; Edwards GE. 1990. Induction of Crassulacean acid metabolism in the facultative halophyte *Mesembryanthemum crystallinum* by abscisic acid. Plant Physiol 93:1253–1260.

CIAT Reports. 1983 to 1998. Cassava Program annual reports. CIAT, Cali, Colombia.

Cock JH. 1985. Cassava: new potential for a neglected crop. Westview Press, Boulder, CO, USA.

Cock JH; El-Sharkawy MA. 1988a. Physiological characteristics for cassava selection. Exp Agric 24:443–448.

Cock JH; El-Sharkawy MA. 1988b. The physiological response of cassava to stress. In: Degras LM, ed. Proc 7th Symposium of the International Society for Tropical Root and Tuber Crops, held in Gosier (Guadeloupe), 1–6 July 1985. Institut National de la Recherche Agronomique (INRA), Paris, France. p 451–462.

Cock JH; Franklin D; Sandoval G; Juri P. 1979. The ideal cassava plant for maximum yield. Crop Sci 19: 271–279.

Cock JH; Porto MCM; El-Sharkawy MA. 1985. Water use efficiency of cassava, III: Influence of air humidity and water stress on gas exchange of field grown cassava. Crop Sci 25:265–272.

Cock JH; Riaño NM; El-Sharkawy MA; López Y; Bastidas G. 1987. C_3-C_4 intermediate photosynthetic characteristics of cassava (*Manihot esculenta* Crantz), II: Initial products of $^{14}CO_2$ fixation. Photosynth Res 12:237–241.

Connor DJ; Cock JH. 1981. Response of cassava to water shortage, II: Canopy dynamics. Field Crops Res 4:285–296.

Connor DJ; Palta J. 1981. Response of cassava to water shortage, III: Stomatal control of plant water status. Field Crops Res 4:285–296.

Connor DJ; Cock JH; Parra GE. 1981. Response of cassava to water shortage, I: Growth and yield. Field Crops Res 4:181–200.

Cours G. 1951. Le manioc a Madagascar. In: Memoires, 3B. l'Institute Scientifique de Madagascar, Tananarive, Madagascar. p 203–400.

Cowan IR. 1977. Stomatal behaviour and environment. Adv Bot Res 4:117–228.

Davies WJ; Kozlowski TT. 1974. Stomatal responses of 5 woody angiosperms to light intensity and humidity. Can J Bot 52:1525–1535.

Davies WJ; Metcalfe J; Lodge TA; da Costa AR. 1986. Plant growth substances and the regulation of growth under drought. Aust J Plant Physiol 13:105–125.

de Tafur SM. 2002. Fisiología de la yuca (*Manihot esculenta* Crantz). In: Ospina B; Ceballos H, eds. Cultivo de la yuca en el tercer milenio: Sistemas modernos de producción, procesamiento, utilización y comercialización. CIAT; Consorcio Latinoamericano y del Caribe de Apoyo a la Investigación y al Desarrollo de la Yuca (CLAYUCA), Cali, Colombia. p 34–45.

de Tafur SM; El-Sharkawy MA; Cadavid LF. 1997a. Response of cassava (*Manihot esculenta* Crantz) to water stress and fertilization. Photosynthetica 34:233–239.

de Tafur SM; El-Sharkawy MA; Calle F. 1997b. Photosynthesis and yield performance of cassava in seasonally dry and semiarid environments. Photosynthetica 33:249–257.

Devi MT; Raghavendra AS. 1993. Photorespiration in C_3-C_4 intermediate species of *Alternanthera* and *Parthenium*: reduced ammonia production and increased capacity of CO_2 refixation in the light. Photosynth Res 38: 177–184.

Dufour DL. 1988. Cyanide content of cassava (*Manihot esculenta*: Euphorbiaceae) cultivars used by Tukanoan Indians in Northwest Amazonia. Econ Bot 42:255–266.

Edwards GE; Sheta E; Moore Bi; Dai Z; Franceschi VR; Cheng SH; Lin CH; Ku MSB. 1990. Photosynthetic characteristics of cassava (*Manihot esculenta* Crantz), a C_3 species with chlorenchymatous bundle sheath cells. Plant, Cell Physiol 31:1199–1206.

Ekanayake IJ; Dixon AGO; Kasele IN; Noah AND. 2007. Plant water relations and photosynthetic properties of polyploidy cassava grown in the Nigerian Savanna. In: Kapinga R; Kingamkono R; Msabaha M; Ndunguru J; Lemaga B; Tusiime G, eds. Proc 13th Triennial Symposium of the International Society for Tropical Root Crops (ISTRC). ISTRC, Arusha, Tanzania. p 210–217.

El-Sharkawy MA. 1990. Effect of humidity and wind on leaf conductance of field grown cassava. Rev Bras Fisiol Veg 2:17–22.

El-Sharkawy MA. 1993. Drought-tolerant cassava for Africa, Asia, and Latin America. BioScience 43:441–451.

El-Sharkawy MA. 2004. Cassava biology and physiology. Plant Mol Biol 56:481–501.

El-Sharkawy MA. 2005. How can calibrated research-based models be improved for use as a tool in identifying genes controlling crop tolerance to environmental stresses in the era of genomics—from an experimentalist's perspective. Photosynthetica 43:161–176.

El-Sharkawy MA. 2006. International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics. Photosynthetica 44:481–512.

El-Sharkawy MA. 2010. Cassava: physiological mechanisms and plant traits underlying tolerance to prolonged drought and their application for breeding cultivars in the seasonally dry and semiarid tropics. In: da Matta FM, ed. Ecophysiology of tropical tree crops. Nova Science Publishers, Hauppauge, NY, USA. p 71–110.

El-Sharkawy MA; Cadavid L, LF. 2000. Genetic variation within cassava germplasm in response to potassium. Exp Agric 36:323–334.

El-Sharkawy MA; Cadavid L, LF. 2002. Response of cassava to prolonged water stress imposed at different stages of growth. Exp Agric 38:333–350.

El-Sharkawy MA; Cock JH. 1984. Water use efficiency of cassava, I: Effects of air humidity and water stress on stomatal conductance and gas exchange. Crop Sci 24:497–502.

El-Sharkawy MA; Cock JH. 1986. The humidity factor in stomatal control and its effect on crop productivity. In: Marcelle R; Clijsters H; Van Poucke M, eds. Biological control of photosynthesis. Martinus Nijhoff Publishers, Dordrecht, Netherlands. p 187–198.

El-Sharkawy MA; Cock JH. 1987a. C_3-C_4 intermediate photosynthetic characteristics of cassava (*Manihot esculenta* Crantz), I: Gas exchange. Photosynth Res 12:219–235.

El-Sharkawy MA; Cock JH. 1987b. Response of cassava to water stress. Plant Soil 100:345–360.

El-Sharkawy MA; Cock JH. 1990. Photosynthesis of cassava (*Manihot esculenta* Crantz). Exp Agric 26:325–340.

El-Sharkawy MA; de Tafur SM. 2007. Genotypic and within canopy variation in leaf carbon isotope discrimination and its relation to short-term leaf gas exchange characteristics in cassava grown under rain-fed conditions in the tropics. Photosynthetica 45:515–526.

El-Sharkawy MA; de Tafur SM. 2010. Comparative photosynthesis, growth, productivity, and nutrient use efficiency among tall- and short-stemmed rain-fed cassava cultivars. Photosynthetica 48:173–188.

El-Sharkawy MA: Hesketh J. 1965. Photosynthesis among species in relation to characteristics of leaf anatomy and CO_2 diffusion resistances. Crop Sci 5:517–521.

El-Sharkawy MA; Hesketh JD. 1986. Citation classic—Photosynthesis among species in relation to characteristics of leaf anatomy and CO_2 diffusion resistances. Curr Cont/Agric Biol Environ 27:14.

El-Sharkawy MA; Cadavid L, LF; de Tafur SM. 1998a. Nutrient use efficiency of cassava differs with genotype architecture. Acta Agron Univ Nac–Palmira Colombia 48:23–32.

El-Sharkawy MA; Cadavid L, LF; de Tafur SM; Caicedo JA. 1998b. Genotypic differences in productivity and nutrient uptake and use efficiency of cassava as influenced by prolonged water stress. Acta Agron Univ Nac–Palmira Colombia 48:9–22.

El-Sharkawy MA; Cock JH; de Cadena G. 1984a. Influence of differences in leaf anatomy on net photosynthetic rates of some cultivars of cassava. Photosynth Res 5:235–242.

El-Sharkawy MA; Cock JH; de Cadena G. 1984b. Stomatal characteristics among cassava cultivars and their relation to gas exchange. Exp Agric 20:67–76.

El-Sharkawy MA; Cock JH; Held K, AA. 1984c. Photosynthetic responses of cassava cultivars (*Manihot esculenta* Crantz) from different habitats to temperature. Photosynth Res 5:243–250.

El-Sharkawy MA; Cock JH; Held K, AA. 1984d. Water use efficiency of cassava, II: Differing sensitivity of stomata to air humidity in cassava and other warm-climate species. Crop Sci 24:503–507.

El-Sharkawy MA; Cock JH; Hernández ADP. 1985. Stomatal response to air humidity and its relation to stomatal density in a wide range of warm climate species. Photosynth Res 7:137–149.

El-Sharkawy MA; Cock JH; Lynam JK; Hernández ADP; Cadavid LF. 1990. Relationships between biomass, root-yield and single-leaf photosynthesis in field-grown cassava. Field Crops Res 25:183–201.

El-Sharkawy MA; de Tafur SM; Cadavid L, LF. 1992a. Potential photosynthesis of cassava as affected by growth conditions. Crop Sci 32:1336–1342.

El-Sharkawy MA; de Tafur SM; Cadavid L, LF. 1993. Photosynthesis of cassava and its relation to crop productivity. Photosynthetica 28:431–438.

El-Sharkawy MA; Hernández ADP; Hershey C. 1992b. Yield stability of cassava during prolonged mid-season water stress. Exp Agric 28:165–174.

El-Sharkawy MA; Loomis RS; Williams WA. 1967. Apparent reassimilation of respiratory carbon dioxide by different plant species. Physiol Plant 20:171–186.

El-Sharkawy MA; Loomis RS; Williams WA. 1968. Photosynthetic and respiratory exchanges of carbon dioxide by leaves of grain amaranth. J Appl Ecol 5:243–251.

El-Sharkawy MA; López Y; Bernal LM. 2008. Genotypic variations in activities of phospho*enol*pyruvate carboxylase (PEPC) and correlations with leaf photosynthetic characteristics and crop productivity of cassava grown in lowland seasonally dry tropics. Photosynthetica 46:238–247.

Essers AJA. 1995. Removal of cyanogens from cassava roots: studies on domestic sun-drying and solid-substrate fermentation in rural Africa. Dissertation. Wageningen Agricultural University, Wageningen, Netherlands.

Evans LT. 1993. Crop evolution, adaptation and yield. Cambridge University Press, Cambridge, UK.

Fanjul L; Jones HG. 1982. Rapid stomatal response to humidity. Planta 154:135–138.

Farquhar GD. 1978. Feed-forward responses of stomata to humidity. Aust Plant Physiol 5:787–800.

Farquhar GD; Schulze E-D; Kuppers M. 1980. Response to humidity by stomata of *Nicotiana glauca* L. and *Corylus avellana* L. are consistent with the optimization of carbon dioxide uptake with respect to water loss. Aust J Plant Physiol 7:315–327.

Fermont AM. 2009. Cassava and soil fertility in intensifying smallholder farming systems of East Africa. Dissertation. Wageningen Agricultural University, Wageningen, Netherlands.

Fernández MD; Tezara W; Rengifo E; Herrera A. 2002. Lack of down-regulation of photosynthesis in a tropical root crop, cassava, grown under an elevated CO_2 concentration. Funct Plant Biol 29:805–814.

Flörchinger FA; Leihner DE; Steinmüller N; Müller-Sämann K; El-Sharkawy MA. 2000. Effects of artificial topsoil removal on sorghum, peanut and cassava yield. J Soil Water Conserv 55:334–339.

Forrester ML; Krotkov G; Nelson CD. 1966. Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves, II: Corn and other monocotyledons. Plant Physiol 41:428–431.

Giaquinta RT. 1983. Phloem loading of sucrose. Annu Rev Plant Physiol 34:347–387.

Gleadow RM; Evans JR; McCaffery S; Cavagnaro TR. 2009. Growth and nutritive value of cassava (*Manihot esculenta* Crantz) are reduced when grown in elevated CO_2. Plant Biol 11(S1):76–82. DOI:10.1111/j.1438-8677.2009.00238.x

Gollan T; Turner NC; Schulze E-D. 1985. The responses of stomata and leaf gas exchange to vapour pressure deficits and soil water content, III: In the sclerophyllous woody species *Nerium oleander*. Oecologia 65:356–362.

Gutschick VP. 1984. Photosynthesis model for C_3 leaves incorporating CO_2 transport, propagation of radiation, and biochemistry, 2: Ecological and agricultural utility. Photosynthetica 18:569–595.

Guzmán G. 1989. Aspectos ecofisiológicos en cultivares anfiestomáticos de yuca (*Manihot esculenta* Crantz). BSc thesis. Pontificia Universidad Javeriana, Bogotá, Colombia.

Haberlandt G. 1914. Physiological plant anatomy. McMillan and Co., London.

Hall AE; Hoffman GJ. 1976. Leaf conductance response to humidity and water transport in plants. Agron J 68:876–881.

Hall AE; Schulze E-D. 1980. Stomatal response to environment and a possible interrelation between stomatal effects on transpiration and CO_2 assimilation. Plant, Cell Environ 3:467–474.

Hatch MD. 1977. C_4 pathway photosynthesis: mechanism and physiological function. Trends Biochem Sci 2:199–202.

Hatch MD. 1987. C_4 photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. Biochim Biophys Acta 895:81–106.

Held K, AA. 1991. Control of canopy photosynthesis and water-use efficiency in well-watered field crops. Dissertation. University of California–Davis, USA.

Henson IE. 1984. Effects of atmospheric humidity on abscisic acid accumulation and water stress in leaves of rice (*Oryza sativa* L.). Ann Bot 54:569–582.

Hermans J; Westhoff P. 1990. Analysis of expression and evolutionary relationships of phospho*enol*pyruvate carboxylase genes in *Flaveria trinervia* (C_4) and *F. pringlei* (C_3). Mol Gen Genet 224:459–468.

Hermans J; Westhoff P. 1992. Homologous genes for the C_4 isoform of phospho*enol*pyruvate carboxylase in a C_3 and C_4 *Flaveria* species. Mol Gen Genet 234:275–284.

Herold A. 1980. Regulation of photosynthesis by sink activity—the missing link. New Phytol 86:131–144.

Hershey CH. 1984. Breeding cassava for adaptation to stress conditions: development of a methodology. Proc 6th Symposium of the International Society for Tropical Root Crops, held in Lima, Peru, 20–25 Feb 1983. Centro Internacional de la Papa (CIP), Lima, Peru. p 303–314.

Hershey CH; Jennings DL. 1992. Progress in breeding cassava for adaptation to stress. Plant Breed Abstr 62:823–831.

Hershey CH; Kawano K; Lozano JC . 1988. Breeding cassava for adaptation to a new ecosystem: a case study from the Colombian llanos. In: Degras LM, ed. Proc 7th Symposium of the International Society for Tropical Root Crops, held in Gosier (Guadeloupe), 1–6 July 1985. Institut National de la Recherche Agronomique (INRA), Paris, France. p 525–540.

Hibberd JM; Quick WP. 2002. Characteristics of C_4 photosynthesis in stems and petioles of C_3 flowering plants. Nature 415:451–454.

Hillocks RJ; Wydra K. 2002. Bacterial, fungal and nematode diseases. In: Hillocks RJ; Thresh JM; Bellotti AC, eds. 2002. Cassava: biology, production and utilization. CABI Publishing, Wallingford, UK. p 261–280.

Hillocks RJ; Thresh JM; Bellotti AC, eds. 2002. Cassava: biology, production and utilization. CABI Publishing, Wallingford, UK.

Hirasawa T; Iida Y; Ishihara K. 1988. Effect of leaf water potential and air humidity on photosynthetic rate and diffusive conductance in rice plants. Jpn J Crop Sci 57:112–118.

Ho LC. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Annu Rev Plant Physiol 39:355–378.

Hoffman GJ; Rawlins SL. 1971. Growth and water potential of root crops as influenced by salinity and relative humidity. Agron J 63:877–881.

Hoffman GJ; Rawlins SL; Garber MJ; Cullen EM. 1971. Water relations and growth of cotton as influenced by salinity and relative humidity. Agron J 63:822–826.

Howeler RH. 1985. Potassium nutrition of cassava. In: Munson RD, ed. Potassium in agriculture. ASA; CSSA; SSSA, Madison, WI, USA. p 819–841.

Howeler RH. 2002. Cassava mineral nutrition and fertilization. In: Hillocks RJ; Thresh JM; Bellotti AC, eds. Cassava: biology, production and utilization. CABI Publishing, Wallingford, UK. p 115–147.

Howeler RH; Cadavid L, LF. 1983. Accumulation and distribution of dry matter and nutrients during a 12-month growth cycle of cassava. Field Crops Res 7:123–139.

Howeler RH; Cadavid L, LF. 1990. Short- and long-term fertility trials in Colombia to determine the nutrient requirements of cassava. Fertiliz Res 26:61–80.

Howeler RH; Sieverding E. 1983. Potential and limitation of mycorrhizal inoculation illustrated by experiments with field grown cassava. Plant Soil 75:245–261.

Howeler RH; Cadavid L, LF; Burckhardt E. 1982. Response of cassava to VA mycorrhizal inoculation and phosphorus application in greenhouse and field experiments. Plant Soil 69:327–339.

Hsiao TC. 1973. Plant responses to water stress. Annu Rev Plant Physiol 24:519–570.

Hsiao TC; Acevedo E; Fereres E; Henderson DW. 1976. Water stress, growth, and osmotic adjustment. Philos Trans R Soc Lond Ser B 273:479–500.

Huber W; Sankhla N. 1976. C_4 pathway and regulation of the balance between C_4 and C_3 metabolism. In: Lange OL; Kappen L; Schulze E-D, eds. 1976. Water and plant life: problems and modern approaches. Springer-Verlag, New York, USA. p 335–386.

Humphries EC. 1967. The dependence of photosynthesis on carbohydrate sinks: current concepts. In: Proc 1st Symposium of the International Society for Tropical Root Crops, held in St. Augustine, Trinidad, 2–8 April 1967. University of the West Indies, St. Augustine, Trinidad and Tobago. p 34-45.

Hunt LA; Wholey DW; Cock JH. 1977. Growth physiology of cassava. Field Crop Abstr 30:77–91.

Hylton CM; Rawsthorne S; Smith AM; Jones DA; Woolhouse HW. 1988. Glycine decarboxylase is confined to bundle-sheath cells of leaves of C_3-C_4 intermediate species. Planta 175:452–459.

Irikura Y; Cock JH; Kawano K. 1979. The physiological basis of genotype-temperature interactions in cassava. Field Crops Res 2:227–239.

Jackson WA; Volk RJ. 1969. Oxygen uptake by illuminated maize leaves. Nature 222:269–271.

James WO. 1959. Manioc in Africa. Stanford University Press, Stanford, CA, USA.

Jarvis PG. 1980. Stomatal response to water stress in conifers. In: Turner NC; Kramer PJ, eds. Adaptation of plants to water and high temperatures stress. John Wiley & Sons, New York, USA. p 105–122.

Jarvis PG; McNaughton KG. 1986. Stomatal control of transpiration: scaling up from leaf to region. Adv Ecol Res 15:1–49.

Jennings DL; Iglesias C. 2002. Breeding for crop improvement. In: Hillocks RJ; Thresh JM; Bellotti AC, eds. Cassava: biology, production and utilization. CABI Publishing, Wallingford, UK. p 149–166.

Jones H; Leigh RA; Tomos AD; Jones RGW. 1987. The effect of abscisic acid on cell turgor pressures, solute content and growth of wheat roots. Planta 170:257–262. DOI:10.1007/BF00397896

Jones MM; Turner NC. 1978. Osmotic adjustment in leaves of sorghum in response to water deficits. Plant Physiol 61:122–126.

Jones RJ; Mansfield TA. 1972. Effects of abscisic acid and its esters on stomatal aperture and the transpiration ratio. Physiol Plant 26:321–327.

Jørgensen K; Bak S; Busk PK; Sørensen C; Olsen CK; Puonti-Kaerlas J; Møller BL. 2005. Cassava plants with a depleted cyanogenic glucoside content in leaves and tubers: distribution of cyanogenic glucosides, their site of synthesis and transport, and blockage of the biosynthesis by RNA interference technology. Plant Physiol 139:363–374. (Also available at www.plantphysiol.org)

Kappen L; Haeger S. 1991. Stomatal responses of *Tradescantia albiflora* to changing air humidity and in darkness. J Exp Bot 42:979–986.

Kaufmann MR. 1982. Leaf conductance as a function of photosynthetic photon flux density and absolute humidity difference from leaf to air. Plant Physiol 69:1018–1022.

Kawano K. 1990. Harvest index and evolution of major food crop cultivars in the tropics. Euphytica 46:195–202.

Kawano K. 2003. Thirty years of cassava breeding for productivity-biological and social factors for success. Crop Sci 43:1325–1335.

Kawano K; Daza P; Amaya A; Rios M; Gonçalves WMF. 1978. Evaluation of cassava germplasm for productivity. Crop Sci 18:377–382.

Kawano K; Narintaraporn K; Narintaraporn P; Sarakarn S; Limsila A; Limsila J; Suparhan D; Sarawat V; Watananonta W. 1998. Yield improvement in a multistage breeding program for cassava. Crop Sci 38:325–332.

Kirkham MB. 2005. Principles of soil and plant water relations. Elsevier Academic Press, Amsterdam, Netherlands.

Körner Ch. 1985. Humidity responses in forest trees: precautions in thermal scanning surveys. Arch Met Geoph Biocl Ser B 36:83–98.

Körner Ch; Bannister P. 1985. Stomatal responses to humidity in Nothofagus menziesii. NZ J Bot 23: 425–429.

Körner Ch; Cochrane PM. 1985. Stomatal responses and water relations of *Eucalyptus pauciflora* in summer along an elevation gradient. Oecologia 66:443–455.

Kramer PJ. 1981. Carbon dioxide concentration, photosynthesis, and dry matter production. BioScience 31:29–33.

Kramer PJ. 1983. Water relations of plants. Academic Press, New York, USA.

Ku MSB; Monson RK; Littlejohn Jr, RO; Nakamoto H; Fisher DB; Edwards GE. 1983. Photosynthetic characteristics of C_3-C_4 intermediate *Flaveria* species. Plant Physiol 71:944–948.

Laetsch WM. 1974. The C_4 syndrome: a structural analysis. Annu Rev Plant Physiol 25:27–52.

Lange OL; Lösch R; Schulze E-D; Kappen L. 1971. Responses of stomata to changes in humidity. Planta 100:76–86.

Lenis JI; Calle F; Jaramillo G; Pérez JC; Ceballos H; Cock JH. 2006. Leaf retention and cassava productivity. Field Crops Res 95:126–134.

Leverenz JW. 1981. Photosynthesis and transpiration in large forest-grown Douglas fir: diurnal variation. Can J Bot 59:349–356.

Long SP; Ainsworth EA; Leakey ADB; Nösberger J; Ort DR. 2006. Food for thought: lower-than-expected crop yield stimulation with rising CO_2 concentration. Science 312:1918–1921.

López Y; Vélez W; El-Sharkawy MA; Mayer JE. 1993. Biochemical characterization of PEPC from cassava: a preliminary report. In: Roca WM; Thro AM, eds. Proc First International Scientific Meeting of the Cassava Biotechnology Network. CIAT, Cali, Colombia. p 340–343.

Lösch R. 1977. Responses of stomata to environmental factors: experiments with isolated epidermal strips of *Polypodium vulgare,* I: Temperature and humidity. Oecologia 29:85–97.

Lösch R. 1979. Stomatal responses to changes in air humidity. In: Sen DN; Chawan DD; Bansal RP, eds. Structure, function, and ecology of stomata. Bishen Singh Mahendra Pal Singh (Publishers), Dehra Dun, Uttarakhand, India. p 189–216.

Lösch R; Schenk B. 1978. Humidity responses of stomata and potassium content of guard cells. J Exp Bot 29:781–787.

Lösch R; Tenhunen JD. 1981. Stomatal responses to humidity: phenomenon and mechanism. In: Jarvis PG; Mansfield TA, eds. Stomatal physiology. Cambridge University Press, Cambridge, UK. p 137–161.

Ludlow MM; Ibaraki K. 1979. Stomatal control of water loss in siratro (*Macroptilium atropurpureum* (DC) Urb.), a tropical pasture legume. Ann Bot 43:639–647.

Magnin NC; Cooley BA; Reiskind JB; Bowes G. 1997. Regulation and localization of key enzymes during the induction of kranz-less, C_4-type photosynthesis in *Hydrilla verticillata*. Plant Physiol 115:1681–1689.

Mahon JD; Lowe SB; Hunt LA. 1977a. Variation in the rate of photosynthetic CO_2 uptake in cassava cultivars and related species of *Manihot*. Photosynthetica 11:131–138.

Mahon JD; Lowe SB; Hunt LA; Thiagarajah M. 1977b. Environmental effects on photosynthesis and transpiration in attached leaves of cassava (*Manihot esculenta* Crantz). Photosynthetica 11:121–130.

Maier-Maercker U. 1979a. "Peristomatal transpiration" and stomatal movement: a controversial view, I: Additional proof of peristomatal transpiration by hygrophotography and a comprehensive discussion in the light of recent results. Z Pflanzenphysiol 91:25–43.

Maier-Maercker U. 1979b. "Peristomatal transpiration" and stomatal movement: a controversial view, II: Observation of stomatal movements under different conditions of water supply and demand. Z Pflanzenphysiol 91:157–172.

Maier-Maercker U. 1983. The role of peristomatal transpiration in the mechanism of stomatal movement. Plant, Cell Environ 6:369–380.

McCree KJ. 1986. Whole-plant carbon balance during osmotic adjustment to drought and salinity stress. Aust J Plant Physiol 13:33–43.

Meidner H. 1962. The minimum intercellular-space CO_2-concentration (Γ) of maize leaves and its influence on stomatal movements. J Exp Bot 13:284–293.

Meidner H. 1976. Water vapour loss from a physical model of a substomatal cavity. J Exp Bot 27:691–694.

Meidner H; Mansfield TA. 1968. Physiology of stomata. McGraw-Hill, London.

Meinzer FD. 1982. The effect of vapour pressure on stomatal control of gas exchange in Douglas fir (*Pseudotsuga menziesii*) saplings. Oecologia 54:236–242.

Mooney HA; Drake BG; Luxmoore RJ; Oechel WC; Pitelka LF. 1991. Predicting ecosystem responses to elevated CO_2 concentrations. BioScience 41:96–104.

Morgan JM. 1984. Osmoregulation and water stress in higher plants. Annu Rev Plant Physiol 35:299–319.

Moss DN. 1962. The limiting carbon dioxide concentration for photosynthesis. Nature 193:587.

Moss DN; Musgrave RB. 1971. Photosynthesis and crop production. Adv Agron 23:317–336.

Mott KA; O'Leary JW. 1984. Stomatal behaviour and CO_2 exchange characteristics in amphistomatous leaves. Plant Physiol 74:47–51.

Mott KA; Gibson AC; O'Leary JW. 1982. The adaptive significance of amphistomatic leaves. Plant, Cell Environ 5:455–460.

Nassar NMA. 1986. Genetic variation of wild *Manihot* species native to Brazil and its potential for cassava improvement. Field Crops Res 13:177–184.

Nassar NMA; Ortiz R. 2010. Breeding cassava to feed the poor. Sci Am 302(5):78–84. (Also available at www.ScientificAmerican.com)

Neales TF; Incolls LD. 1968. The control of leaf photosynthesis rate by the level of assimilate concentration in the leaf: a review of the hypothesis. Bot Rev 34:107–125.

Nijholt JA. 1935. Opname van voedingsstoffen uit den bodem bij cassave [*Absorption of nutrients from the soil by a cassava crop*]. Alg Proefstn Landbouw Korte Meded (Buitenzorg, Indonesia) No. 15.

Nobel PS. 1976. Photosynthetic rates of sun versus shade leaves of *Hyptis emoryi* Torr. Plant Physiol 58: 218–223.

Nobel PS. 1980. Leaf anatomy and water use efficiency. In: Turner NC; Kramer PJ, eds. Adaptation of plants to water and high temperature stress. John Wiley & Sons, New York, USA. p 43–55.

Nobel PS; Hartsock TL. 1981. Development of leaf thickness for *Plectranthus parviflorus*: influence of photosynthetically active radiation. Physiol Plant 51:163–166.

North C. 1956. A technique for measuring structural features of plant epidermis using cellulose acetate films. Nature 176:1186–1187.

Nösberger J; Humphries EC. 1965. The influence of removing tubers on dry-matter production and net assimilation rate of potato plants. Ann Bot 29: 579–588.

Oguntunde PG. 2005. Whole-plant water use and canopy conductance of cassava under limited available soil water and varying evaporative demand. Plant Soil 278:371–383.

Oguntunde PG; Alatise MO. 2007. Environmental regulation and modelling of cassava canopy conductance under drying root zone soil water. Meteorol Appl 14:245–252.

Okogbenin E; Ekanayake IJ; Porto MCM. 2003. Genotypic variability in adaptation responses of selected clones of cassava to drought stress in the Sudan Savanna zone of Nigeria. J Agron Crop Sci 189:376–389.

Osmond CB; Grace SC. 1995. Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reactions of photosynthesis? J Exp Bot 46:1351–1362.

Osmond CB; Winter K; Powles SB. 1980. Adaptive significance of carbon dioxide cycling during photosynthesis in water-stressed plants. In: Turner NC; Kramer PJ, eds. Adaptation of plants to water and high temperature stress. John Wiley & Sons, New York, USA. p 139–154.

Palta JA. 1984. Influence of water deficits on gas-exchange and the leaf area development of cassava cultivars. J Exp Bot 35:1441–1449.

Parkhurst DF. 1978. The adaptive significance of stomatal occurrence on one or both surfaces of leaves. J Ecol 66:367–383.

Paul K; Yeoh H-H. 1987. K_m values of ribulose 1,5-bisphosphate carboxylase of cassava cultivars. Phytochemistry 26:1965–1967.

Paul K; Yeoh H-H. 1988. Characteristics of ribulose 1,5-bisphosphate carboxylase from cassava leaves. Plant Physiol Biochem 26:615–618.

Pellet D; El-Sharkawy MA. 1993a. Cassava varietal response to phosphorus fertilization, I: Yield, biomass and gas exchange. Field Crops Res 35:1–11.

Pellet D; El-Sharkawy MA. 1993b. Cassava varietal response to phosphorus fertilization, II: Phosphorus uptake and use efficiency. Field Crops Res 35:13–20.

Pellet D; El-Sharkawy MA. 1994. Sink-source relations in cassava: effects of reciprocal grafting on yield and leaf photosynthesis. Exp Agric 30:359–367.

Pellet D; El-Sharkawy MA. 1997. Cassava varietal response to fertilization: growth dynamics and implications for cropping sustainability. Exp Agric 33:353–365.

Pereira JF. 1977. Fisiología de la yuca (*Manihot esculenta* Crantz). Universidad de Oriente, Jusepin, Monagas, Venezuela.

Pettersson R; McDonald JS. 1994. Effects of nitrogen supply on the acclimation of photosynthesis to elevated CO_2. Photosynth Res 39:389–400.

Pettigrew WT; Hesketh JD; Peters DB; Woolley JT. 1990. A vapor pressure deficit effect on crop canopy photosynthesis. Photosynth Res 24:27–34.

Pieruschka R; Huber G; Berry JA. 2010. Control of transpiration by radiation. PNAS 107(3):13372–13377. (Also available at www.pnas.org/cgi/doi/10.1073/pnas.0913177107.)

Pingali P. 2010. Prepared remarks presented at the World Food Prize Symposium. Bill & Melinda Gates Foundation, Seattle, WA, USA. Available at www.gatesfoundation.org/speeches-commentary/Pages/prabhu-pingali-2010-world-food-prize-symposium.aspx

Porto MCM. 1983. Physiological mechanisms of drought tolerance in cassava (*Manihot esculenta* Crantz). Dissertation. University of Arizona, Tucson, AZ, USA.

Pospisilová J; Solárová J. 1980. Environmental and biological control of diffusive conductances of adaxial and abaxial epidermis. Photosynthetica 14:90–127.

Poulton JE. 1990. Cyanogenesis in plants. Plant Physiol 94:401–405.

Radin JW. 1984. Stomatal responses to water stress and to abscisic acid in phosphorus-deficient cotton plants. Plant Physiol 76:392–394.

Radin JW; Parker LL; Guinn G. 1982. Water relations of cotton plants under nitrogen deficiency, V: Environmental control of abscisic acid accumulation and stomatal sensitivity to abscisic acid. Plant Physiol 70:1066–1070.

Rajagopalan AV; Devi MT; Raghavendra AS. 1994. Molecular biology of C_4 phospho*enol*pyruvate carboxylase: structure, regulation and genetic engineering. Photosynth Res 39:115–135.

Rawson HM; Begg JE; Woodward RG. 1977. The effect of atmospheric humidity on photosynthesis, transpiration and water use efficiency of leaves of several plant species. Planta 134:5–10.

Reinfelder JR; Kraepiel AML; Morel FMM. 2000. Unicellular C_4 photosynthesis in a marine diatom. Nature 407:996–999.

Reinfelder JR; Milligan AJ; Morel FMM. 2004. The role of the C_4 pathway in carbon accumulation and fixation in a marine diatom. Plant Physiol 135:2106–2111.

Reiskind JB; Madsen TV; Van Ginkel LC; Bowes G. 1997. Evidence that inducible C_4-type photosynthesis is a chloroplastic CO_2-concentrating mechanism in *Hydrilla,* a submersed monocot. Plant, Cell Environ 20:211–220.

Riis L. 1990. The subterranean burrowing bug *Cyrtomenus bergi* Froeschner, an increasing pest in tropical Latin America: behavioural studies, population fluctuations, botanical control, with special reference to cassava. MSc thesis. Institute of Ecological and Molecular Biology, Section of Zoology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Riis L. 1997. Behaviour and population growth of the burrower bug, *Cyrtomenus bergi* Froeschner: effects of host plants and abiotic factors. Dissertation. Royal Veterinary Agricultural University, Copenhagen, Denmark.

Riis L; Bellotti AC; Bonierbale M; O'Brien GM. 2003. Cyanogenic potential in cassava and its influence on a generalist insect herbivore *Cyrtomenus bergi* (Hemiptera: Cydnidae). J Econ Entomol 96:1905–1914.

Riis L; Bellotti AC; Vargas O. 1995. The response of a polyphagous pest (*Cyrtomenus bergi* Froeschner) to cassava cultivars with variable HCN content in root parenchyma and peel. In: Proc Second International Scientific Meeting of the Cassava Biotechnology Network. CIAT, Cali, Colombia. p 501–509.

Romanoff S; Lynam J. 1992. Cassava and African food security: some ethnographic examples. Ecol Food Nutr 27:29–41.

Rosling H. 1994. Measuring effects in humans of dietary cyanide exposure from cassava. Acta Hortic 375: 271–283.

Ruppenthal M; Leihner DE; Steinmüller N; El-Sharkawy MA. 1997. Losses of organic matter and nutrients by water erosion in cassava-based cropping systems. Exp Agric 33:487–498.

Rylott EL; Metzlaff K; Rawthorne S. 1998. Developmental and environmental effects on the expression of the C_3-C_4 intermediate phenotypes in *Moricandia arvensis*. Plant Physiol 118:1277–1284.

Salvucci ME; Bowes G. 1983. Two photosynthetic mechanisms mediating the low photorespiratory state in submersed aquatic angiosperms. Plant Physiol 73:488–496.

Schoonhoven A van. 1978. Thrips on cassava: economic importance, sources and mechanisms of resistance. In: Brekelbaum T; Bellotti AC; Lozano JC, eds. Proc Cassava Protection Workshop. CIAT, Cali, Colombia. p 177–180.

Schulze E-D. 1986. Carbon dioxide and water vapour exchange in response to drought in the atmosphere and in the soil. Annu Rev Plant Physiol 37:247–274.

Schulze E-D; Hall AE. 1982. Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. In: Lange OL; Nobel PS; Osmond CB; Ziegler H, eds. Physiological plant ecology, II: Water relations and carbon assimilation. Encyclopaedia of Plant Physiology, vol 12B. Springer-Verlag, Berlin, Germany. p 181–230.

Schulze E-D; Lange OL; Buschbom U; Kappen L; Evenari M. 1972. Stomatal response to changes in humidity in plants growing in the desert. Planta 108:259–270.

Sesták Z, ed. 1985. Photosynthesis during leaf development. Academia Publishing House of The Czech Academy of Sciences (Praha); Dr. W. Junk N.V. Publishers, Dordrecht, Netherlands.

Seybold WD. 1961/1962. Ergebnisse und probleme pflanzlicher transpirationsanalysen. Jahresh Heidelb Akad Wiss 6:5–8.

Sheen J. 1999. C_4 gene expression. Annu Rev Plant Physiol Plant Mol Biol 50:187–217.

Sheriff DW. 1977. Where is humidity sensed when stomata respond to it directly? Ann Bot 41:1083–1084.

Sheriff DW. 1979. Stomatal aperture and the sensing of the environment by guard cells. Plant, Cell Environ 2:15–22.

Sheriff DW. 1984. Epidermal transpiration and stomatal responses to humidity: some hypotheses explored. Plant, Cell Environ 7:669–677.

Sheriff DW; Kaye PE. 1977. Responses of diffusive conductance to humidity in a drought-avoiding and a drought-resistant (in terms of stomatal response) legume. Ann Bot 41:653–655.

Siritunga D; Sayre RT. 2003. Generation of cyanogen-free transgenic cassava. Planta 217:367–373.

Slávik B. 1971. Determination of stomatal aperture. In: Sesták Z; Cátsky J; Jarvis PG, eds. Plant photosynthetic production: manual of methods. Dr. W. Junk N.V. Publishers, The Hague, Netherlands. p 556–563.

Solárová J; Pospisilová J. 1979. Diffusive conductances of adaxial and abaxial epidermis, 1: Response to photon flux density during development of water stress in primary bean leaves. Biol Plant 21:446–451.

Stanhill G. 1986. Water use efficiency. Adv Agron 39:53–85.

Taybi T; Cushman JC. 1999. Signaling events leading to Crassulacean acid metabolism induction in the common ice plant. Plant Physiol 121:545–555.

Tazaki TK; Ishihara K; Ushijima T. 1980. Influence of water stress on the photosynthesis and productivity of plants in humid areas. In: Turner NC; Kramer PJ, eds. Adaptation of plants to water and high temperature stress. John Wiley & Sons, New York, USA. p 309–321.

Tenjo FA; Mayer JE; El-Sharkawy MA. 1993. Cloning and sequence analysis of PEP-carboxylase from cassava. In: Roca WM; Thro AM, eds. Proc First International Scientific Meeting of the Cassava Biotechnology Network. CIAT, Cali, Colombia. p 331–334.

Thoday D. 1938. Stomatal movement and epidermal water-content. Nature 141:164.

Thorne GN; Evans AF. 1964. Influence of tops and roots on net assimilation rate of sugar-beet and spinach beet and grafts between them. Ann Bot 28:499–508.

Tibbitts TW. 1979. Humidity and plants. BioScience 29:358–363.

Tichá I. 1982. Photosynthetic characteristics during ontogenesis of leaves, 7: Stomata density and size. Photosynthetica 16:375–471.

Tinoco-Ojanguren C; Pearcy RW. 1993. Stomatal dynamics and its importance to carbon gain in two rainforest *Piper* species. Oecologia 94:388–394.

Tregunna EB; Krotkov G; Nelson CD. 1964. Further evidence on the effects of light on respiration during photosynthesis. Can J Bot 42:989–997.

Tscherning K; Leihner DE; Hilger TH; Müller-Sämann KM; El-Sharkawy MA. 1995. Grass barriers in cassava hillside cultivation: rooting patterns and root growth dynamics. Field Crops Res 43:131–140.

Turner NC. 1986. Adaptation to water deficits: a changing perspective. Aust J Plant Physiol 13:175–190.

Turner NC; Begg JE; Tonnet ML. 1978. Osmotic adjustment of sorghum and sunflower crops in response to water deficits and its influence on the water potential at which stomata close. Aust J Plant Physiol 5:597–608.

Tyree MT; Yianoulis P. 1980. The site of water evaporation from sub-stomatal cavities, liquid path resistance and hydroactive stomatal closure. Ann Bot 46:175–193.

Ueno O. 1996. Immunocytochemical localization of enzymes involved in the C_3 and C_4 pathways in the photosynthetic cells of an amphibious sedge, *Eleocharis vivipara*. Planta 199:394–403.

Ueno O. 2001. Environmental regulation of C_3 and C_4 diffferentiation in the amphibious sedge *Eleocharis vivipara*. Plant Physiol 127:1524–1532.

Ueno O; Agarie S. 1997. The intercellular distribution of glycine decarboxylase in leaves of cassava in relation to the photosynthetic mode and leaf anatomy. Jpn J Crop Sci 66:268–278.

Ueno O; Bang SW; Wada Y; Kondo A; Ishihara K; Kaneko Y; Matsuzawa Y. 2003. Structural and biochemical dissection of photorespiration in hybrids differing in genome constitution between *Diplotaxis tenuifolia* (C_3-C_4) and radish (C_3). Plant Physiol 132:1550–1559.

van Oirschot QEA; O'Brien GM; Dufour DL; El-Sharkawy MA; Mesa E. 2000. The effect of pre-harvest pruning of cassava upon root deterioration and quality characteristics. J Sci Food Agric 80:1866–1873.

Veltkamp HJ. 1985. Physiological causes of yield variation in cassava (*Manihot esculenta* Crantz). Dissertation. Wageningen Agricultural University, Wageningen, Netherlands.

Verteuil J de. 1917. Cassava experiments. Bull Dep Agric Trinidad and Tobago 16:18–21.

Verteuil J de. 1918. Cassava experiments 1916–1918. Bull Dep Agric Trinidad and Tobago 17:193–198.

Volk RJ; Jackson WA. 1972. Photorespiratory phenomena in maize: oxygen uptake, isotope discrimination, and carbon dioxide efflux. Plant Physiol 49:218–223.

Voznesenskaya EV; Franceschi VR; Kiirats O; Artyusheva EG; Freitag H; Edwards GE. 2002. Proof of C_4 photosynthesis without kranz anatomy in *Bienertia cycloptera* (Chenopodiaceae). Plant J 31:649–662.

Voznesenskaya EV; Franceschi VR; Kiirats O; Freitag H; Edwards GE. 2001. Kranz anatomy is not essential for terrestrial C_4 plant photosynthesis. Nature 414:543–546.

Walton DC. 1980. Biochemistry and physiology of abscisic acid. Annu Rev Plant Physiol 31:453–489.

Ward DA; Bunce JA. 1986. Novel evidence for lack of water vapour saturation within the intercellular airspace of turgid leaves of mesophytic species. J Exp Bot 37:504–516.

Wardlaw IF. 1990. The control of carbon partitioning in plants. New Phytol 116:341–381.

Webber AN; Nie G-Y; Long SP. 1994. Acclimation of photosynthetic proteins to rising atmospheric CO_2. Photosynth Res 39:413–425.

Westby A. 2002. Cassava utilization, storage and small-scale processing. In: Hillocks RJ; Thresh JM; Bellotti AC, eds. Cassava: biology, production and utilization. CABI Publishing, Wallingford, UK. p 281–300.

Westhoff P; Gowik U. 2004. Evolution of C_4 phospho*enol*pyruvate carboxylase—genes and proteins: a case study with the genus *Flaveria*. Ann Bot 93:13–23.

Westhoff P; Svensson P; Ernst K; Blasing O; Burscheidt J; Stockhaus J. 1997. Molecular evolution of C_4 phospho*enol*pyruvate carboxylase in the genus *Flaveria*. Aust J Plant Physiol 24:429–436.

Wilson WM. 2003. Cassava (*Manihot esculenta* Crantz), cyanogenic potential, and predation in northwestern Amazonia: the Tukanoan perspective. Human Ecol 31:403–416.

Wilson WM; Dufour DL. 2002. Why bitter cassava? Productivity of bitter and sweet cassava in a Tukanoan Indian settlement in Northwest Amazon. J Econ Bot 56:49–57.

Woodrow IE. 1994. Optimal acclimation of the C_3 photosynthetic system under enhanced CO_2. Photosynth Res 39:401–412.

Wortman S. 1981. Beyond the bottom line. The Rockefeller Foundation, New York, USA.

Zeevaart JAD; Creelman RA. 1988. Metabolism and physiology of abscisic acid. Annu Rev Plant Physiol Plant Mol Biol 39:439–473.

Zeiger E. 1983. The biology of stomatal guard cells. Annu Rev Plant Physiol 34:441–475.

Zelitch I. 1962. Biochemical control of stomatal opening in leaves. Proc Natl Acad Sci USA 47:1423–1433.

Zhang J; Davies WJ. 1989. Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. Plant, Cell Environ 12:73–81.

Ziska LH; Hogan KP; Smith AP; Drake BG. 1991. Growth and photosynthesis response of nine tropical species with long-term exposure to elevated carbon dioxide. Oecologia 86:383–389.