# Cassava biology and physiology

Mabrouk A. El-Sharkawy
*Centro Internacional de Agricultura Tropical (CIAT), A.A. 6713, Cali, Valle, Colombia; present address: A.A. (P.O. Box) 26360, Cali, Valle, Colombia; e-mail elsharkawy@telesat.com.co*

## Abstract

Cassava or manioc (*Manihot esculenta* Crantz), a perennial shrub of the New World, currently is the sixth world food crop for more than 500 million people in tropical and sub-tropical Africa, Asia and Latin America. It is cultivated mainly by resource-limited small farmers for its starchy roots, which are used as human food either fresh when low in cyanogens or in many processed forms and products, mostly starch, flour, and for animal feed. Because of its inherent tolerance to stressful environments, where other food crops would fail, it is often considered a food-security source against famine, requiring minimal care. Under optimal environmental conditions, it compares favorably in production of energy with most other major staple food crops due to its high yield potential. Recent research at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia has demonstrated the ability of cassava to assimilate carbon at very high rates under high levels of humidity, temperature and solar radiation, which correlates with productivity across all environments whether dry or humid. When grown on very poor soils under prolonged drought for more than 6 months, the crop reduce both its leaf canopy and transpiration water loss, but its attached leaves remain photosynthetically active, though at greatly reduced rates. The main physiological mechanism underlying such a remarkable tolerance to drought was rapid stomatal closure under both atmospheric and edaphic water stress, protecting the leaf against dehydration while the plant depletes available soil water slowly during long dry periods. This drought tolerance mechanism leads to high crop water use efficiency values. Although the cassava fine root system is sparse, compared to other crops, it can penetrate below 2 m soil, thus enabling the crop to exploit deep water if available. Leaves of cassava and wild *Manihot* possess elevated activities of the $C_4$ enzyme PEP carboxylase but lack the leaf Kranz anatomy typical of $C_4$ species, pointing to the need for further research on cultivated and wild *Manihot* to further improve its photosynthetic potential and yield, particularly under stressful environments. Moreover, a wide range in values of $K_m$ ($CO_2$) for the $C_3$ photosynthetic enzyme Rubisco was found among cassava cultivars indicating the possibility of selection for higher affinity to $CO_2$, and consequently higher leaf photosynthesis. Several plant traits that may be of value in crop breeding and improvement have been identified, such as an extensive fine root system, long leaf life, strong root sink and high leaf photosynthesis. Selection of parental materials for tolerance to drought and infertile soils under representative field conditions have resulted in developing improved cultivars that have high yields in favorable environments while producing reasonable and stable yields under stress.

## Cassava: a crop for sustainable agriculture and food security in developing countries

Cassava or manioc (*Manihot esculenta* Crantz, Euphorbiaceae) was originally a perennial shrub of the New World. It is an outbreeding species possessing $2n = 36$ chromosomes, and is considered to be an amphidiploid or sequential allopolyploids. The crop is widely grown as a staple food and animal feed in countries of tropical and sub-tropical Africa, Asia and Latin America between 30° N and 30° S with a total cultivated area over 13 million hectares, more than 70% of it being in Africa and Asia (El-Sharkawy, 1993). It is currently the most important food source for carbo-

hydrate, after rice, sugarcane and maize, for over 500 million people in the developing countries of the tropics and sub-tropics. Its main value is in its storage roots with dry matter containing more than 80% starch. Due to the very low protein content in storage roots (values range among cultivars from 5 to 19 g per kg dry matter, based on an average conservative Kjeldahl nitrogen-to-protein conversion factor of 2.49–3.67; Yeoh and Truong, 1996), human requirements for protein and other essential nutrients are commonly fulfilled by other food sources.

In some areas where the crop is grown, particularly in Africa, young leaves are also harvested and processed for human consumption as a vegetable or as a constituent in a form of sauce eaten along with main staple meals (Lancaster and Brooks, 1982). Also, cassava leaves have value as a protein supplement (leaf crude protein content on a dry basis ranges among cultivars from 21% to 39%; Yeoh and Chew, 1976; Ravindran, 1993) in animal nutrition either in feed formulations for monogastric animals or as a fresh forage to supplement low-quality roughages in ruminant feeds (Ravindran, 1993). Worldwide, the crop occupies the sixth place as a source of energy; it is not generally consumed outside the tropics and subtropics where it is produced. About 70% of world cassava root production (which is estimated to be over 45 million metric tons of dry root annually) is used for human consumption either directly after cooking or in processed forms; the remaining 30% is used for animal feed and other industrial products such as starch, glucose, and alcohol.

In areas where cassava is commonly used directly for human consumption, particularly in Africa and Latin America, cultivars low in cyanogens (sweet) are preferably used to avoid health hazards. In cultivars high in cyanogens (bitter), much of the hydrocyanic acid is removed from cassava roots and leaves by using a mix of complex traditional methods and modern technologies during food processing and preparation (Essers, 1995).

Cassava is produced mainly by resource-limited small farmers, virtually without purchased inputs, by virtue of its remarkable tolerance to abiotic stresses and adverse environments, in contrast with the capital-intensive and input-demanding Green Revolution cereal crops such as wheat, rice and maize. It is grown in marginal, low-fertility acidic soils under variable rain-fed conditions ranging from less than 600 mm per year in semi-arid tropics (De Tafur *et al.*, 1997) to more than 1000 mm in the sub-humid and humid trop-

ics (Pellet and El-Sharkawy, 1997). The most common production system is intercropping with other staple food crops such as maize, sorghum and grain legumes (Leihner, 1983), but a significant proportion of production occurs in single-crop system. Although cassava requires a warm climate ($>20$ °C mean day temperature) for optimum growth and production, and for maximum leaf photosynthesis (with an optimum leaf temperature of 25–35 °C; El-Sharkawy *et al.*, 1992a), it is often cultivated in the high-altitude tropics (up to 1800 m above sea level) and in the sub-tropics with a lower mean annual temperature where crop growth is slower (Irikura *et al.*, 1979), leaf photosynthetic activities are reduced and storage roots bulking and harvesting time are much delayed compared to what occurs in the warmer climates of the lowland tropics.

The crop is vegetatively propagated by mature woody stem cuttings (or stakes, 15–30 cm long) planted horizontally, vertically, or inclined on flat or ridged soils at densities ranging from 5000 to 20 000 cuttings per hectare, depending on the cropping system and purpose of production (Keating *et al.*, 1988). Seeds are used mainly in breeding programs, though its use in commercial cassava production is a promising option to obviate constraints, particularly diseases, associated with vegetative propagation (Iglesias *et al.*, 1994). Storage roots are generally harvested 7–24 months after planting, depending on cultivar, purpose of use and growing conditions. Due to root perishability and rapid deterioration after harvest, fresh roots have to be used immediately after harvesting, either eaten on the farm, marketed for consumption, processed for starch extraction, dried for flour production, roasted for food products and/or used for animal feed. Some of the processed food products are commonly known as *farinha da mandioca* in Brazil and bordering countries, *gablek* in Indonesia, and *gari* and *foufou* in West Africa. However, pre-harvest pruning in the three weeks before harvest decreases root deterioration because of increases in the total sugar/starch ratio in the roots (van Oirschot *et al.* 2000). Combining fresh cassava markets with those of its processed products should increase marketing flexibility and crop profitability, hence reducing the many risks often encountered by the producers.

## Botanical, geographical and agricultural origin of cassava

Uncertainty shrouds the botanical origin of cassava as it had been generally believed that it had no known wild ancestry or progenitor outside of cultivation (Rogers and Appan, 1973). Furthermore, it has been argued that the crop evolved through indiscriminate introgression involving about a dozen species of wild relatives within the genus *Manihot*. In contrast to this view, Allem (2002) reported that a wild population of *Manihot*, morphologically similar to the cultivated species, was found in the Central Brazilian State of Goiás, leading to the conclusion that cassava indeed grows in the wild. Also, it was suggested that the wild species, *Manihot flabellifolia* Pohl, is the likely progenitor of modern commercial cultivars and of landraces of cassava that are no longer considered cultigens (i.e. *M. esculenta* Crantz subspecies *esculenta*, as proposed by Allem, 2002).

Based on these recent findings, Allem (2002) further presented a hypothesis for geographical origin, i.e. the area where evolution and divergence of the wild ancestors occurred. Because most of the biological diversity of the genus *Manihot* occurs near the state of Goiás, it was believed that the wild progenitor of cassava and related species had evolved in the lax forests of the Central Brazilian Savanna and then the subspecies *Manihot esculenta* Crantz ssp. *flabellifolia* (Pohl) Cifferi, the postulated progenitor of cassava, migrated to and colonized the Amazonian humid rainforests.

As far as the agricultural origin or place of domestication of cassava is concerned, there has been more than one center proposed. It is believed that domestication of low-cyanogen cultivars of cassava (sweet cultivars) occurred firstly as early as 6000 B.C. in the moist Amazonian rainforests (Gibbons, 1990). Also, it was suggested that cassava might had been domesticated and cultivated before 4000 B.C. in the Casma Valley on the western Peruvian coast (Ugent *et al.*, 1986). On the other hand, Renvoize (1972) was of the opinion that sweet cultivars may have been domesticated in Mesoamerica while bitter ones were domesticated in northeastern South America.
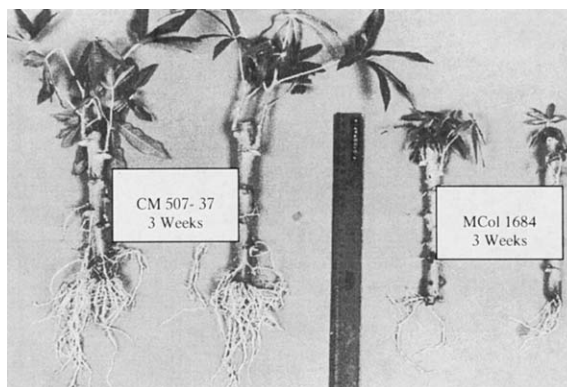


*Figure 1.* Rooting characteristics at three weeks after planting of stem cuttings of the hybrid CM 507-37 and the parent M Col 1684. Source: El-Sharkawy and Cock (1987a).

## Regeneration, development and growth of the cassava plant

### Propagation, sprouting, and quality of planting materials

In commercial production, the cassava plant is established by planting hardwood stem cuttings that contain sufficient carbohydrate reserves and mineral elements essential for the initial phase of the regeneration and the development of both roots and leaves (Figure 1). The adventitious roots are formed first from the nodes at the base of more than one axillary bud (nodal roots) 5–7 days after planting, which is then followed by the formation of rootlets from a recently formed callus at the base of the cutting (basal roots). The buds also begin to sprout and enlarge 5–8 days after planting, with the first leaves appearing by days 10–13. Sprouting is faster at a soil temperature around 28–30 °C but ceases at temperatures higher than 37 °C and lower than 17 °C (Keating and Evenson, 1979).

Genetic differences in capacity for sprouting and in rootlets formation exist within cassava germplasm (Figure 1) which persist throughout the production cycle (El-Sharkawy and Cock, 1987a), indicating the possibility of screening large number of clones at early stages of growth for the evaluation and selection for better sprouting and rooting potential. This evaluation process could be carried out either in greenhouses or in the field by sampling sprouting stakes. Alternatively, stakes could be planted in small plastic bags in the field and evaluated later by carefully washing away the soil in the bags and then quantifying rootlet traits such as length, diameter and fresh or dry weight.

*Table 1.* Effects of the nutritional status of stakes on subsequent crops leaf area, and fresh weight of foliage, stem and storage roots of cassava cultivar MCol 1684, CIAT experimental Station, Santander de Quilichao, Colombia. Source: Molina and El-Sharkawy, 1995.

| Planting material treatment (kg/ha) | | | Subsequent crop treatment | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | unfertilized | | | | fertilized[a] | | | |
| N | P | K | LAI[b] | foliage | stem | roots | LAI[b] | foliage | stem | roots |
| | | | (t/ha, fresh weight) | | | | (t/ha, fresh weight) | | | |
| 0 | 0 | 0 | 0.4 | 1.13 | 2.02 | 13.47 | 0.7 | 1.67 | 4.49 | 19.05 |
| 0 | 100 | 100 | 0.5 | 0.97 | 2.63 | 17.45 | 0.8 | 1.58 | 3.64 | 24.65 |
| 100 | 0 | 100 | 0.4 | 1.06 | 2.98 | 14.91 | 0.7 | 1.50 | 4.38 | 23.51 |
| 100 | 100 | 0 | 0.3 | 1.07 | 2.25 | 15.79 | 0.6 | 1.64 | 4.53 | 24.71 |
| 100 | 100 | 100 | 0.9 | 1.67 | 3.10 | 24.16 | 1.3 | 2.10 | 6.22 | 30.17 |
| Avg. | | | 0.5 | 1.18 | 2.60 | 17.16 | 0.8 | 1.70 | 4.65 | 24.42 |
| LSD 5% (Tukey) for comparison between subsequent crop treatments | | | | | | | | | | |
| | | | 0.2 | 0.25 | 0.33 | 2.15 | | | | |
| LSD 5% (Tukey) for comparison among planting material treatments | | | | | | | | | | |
| | | | 0.4 | 0.31 | 0.52 | 4.5 | 0.3 | 0.35 | 1.46 | 4.2 |

[a] Application at planting of 50, 43 and 83 kg/ha of each N, P and K.
[b] Leaf area index (LAI) measured at 3 months after planting.

Molina and El-Sharkawy (1995) reported that the nutritional content of cassava stems as influenced by soil fertility status affected the regenerative ability of collected stakes which, in turn, affected yields of subsequent crops irrespective of their soil fertility status (Table 1). They found that sprouting rate and root formation were strongly influenced by the nitrogen, phosphorus and potassium contents in the planting materials, with the lowest sprouting rate and the smallest amount of rootlets occurring in materials from mother plants receiving no K fertilizer in soil very low in potassium, but moderate levels of nitrogen and phosphorus. Sprouting potential was not affected by planting the cuttings in fertilized or unfertilized soils, indicating that nutritional reserves contained in stem cuttings were more important. Moreover, cuttings from mother plants growing under adequate levels of nitrogen, phosphorus and potassium resulted in plants with the greatest leaf area, and the highest production of commercial roots/plant in both fertilized and unfertilized soils (Table 1); however, leaf photosynthesis in subsequent crops was not affected by the nutritional status of cuttings (Cayón *et al.*, 1997).

Keating *et al.* (1988), studied the effect of length, thickness, orientation, and population density of cassava stakes on subsequent growth and yield in Queensland, Australia. They reported that stake orientation had little or no effect on yield, but the use of thin stakes from upper parts of the mother plant reduced yield slightly, as compared to heavy thick stakes. Similar findings were reported from Thailand (Oka *et al.*, 1987) where stakes with a high volume density (weight per unit volume), taken from the lower or middle part of the mother plant, gave better sprouting (germination) and a higher survival rate in both dry and wet seasons.

These findings highlight the paramount importance of the quality of planting materials in commercial cassava production systems.

*Plant development and growth under field conditions*

Figure 2 illustrates the patterns and amounts of dry matter accumulation and partitioning between storage roots, stems, leaves and petioles during 12 months of a growing cycle for unfertilized plants of the vigorous and early-branching cv. M Mex 59 and the less vigorous and late-branching cv. M Col 22. Both cultivars were grown at a population density of 15 625 plants/ha at CIAT-Quilichao station (3°06′ N, 76°31′ W; 990 m above sea level; 24 °C mean daily temperature), Cauca Dept., Colombia (Howeler and Cadavid, 1983). The soil was classified as Dystropept and has the following chemical characteristics pH 4.6; organic matter 8.5%; Bray II phosphorus 2.3 mg/kg; and in meq per 100 g dry soil: Al 3.1, Ca 0.91, Mg 0.58, K 0.40 and Al saturation 62%; and in mg/kg: B 0.14, Zn 1.4, Mn 26. The soil has had volcanic ash influence, is high in
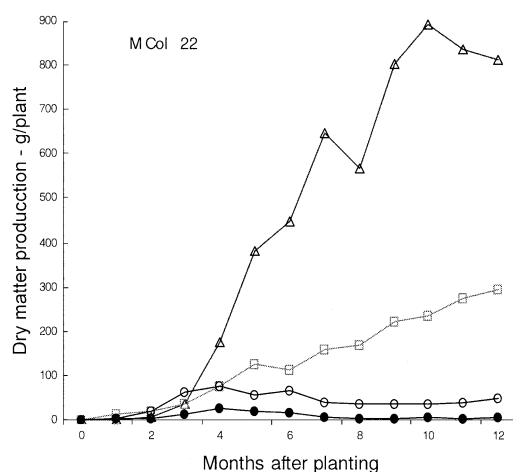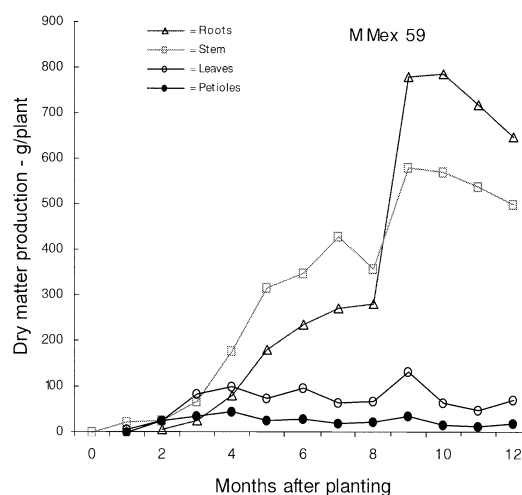
*Figure 3*. Cumulative number of leaves formed per apex at three different temperatures. Source: Irikura *et al.* (1979).



*Figure 4*. Schematic representation showing relation between whole plant growth, partitioning of growth between stems (and leaves) or roots, and the leaf area index (LAI). The vertical arrow indicates optimum LAI for root growth. Source: Cock (1984).

*Figure 2*. Distribution of dry matter among roots, stem, leaves and petioles of cv. M Mex 59 and M Col 22 during a 12-month growth cycle without applied fertilizers. Source: Howeler and Cadavid (1983).

organic matter, quite acidic and high in Al and Mn, but very low in P, B and Z. Monthly precipitations in mm were: October (initiation of planting) 100, November 160, December 360, January 60, February 52, March 80, April 60, May 160, June 100, July 20, August 100 and September (final harvest) 280. At that station mean daily pan evaporation was about 4.4 mm, thus the crop received excess water in December, the third month after planting, and in September, the last month in growth cycle. The crop suffered from moderate to severe shortage of water during the rest of the growth period.
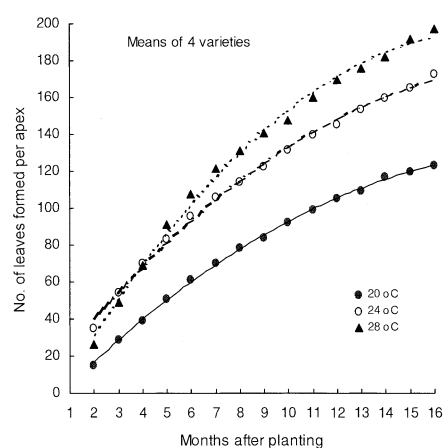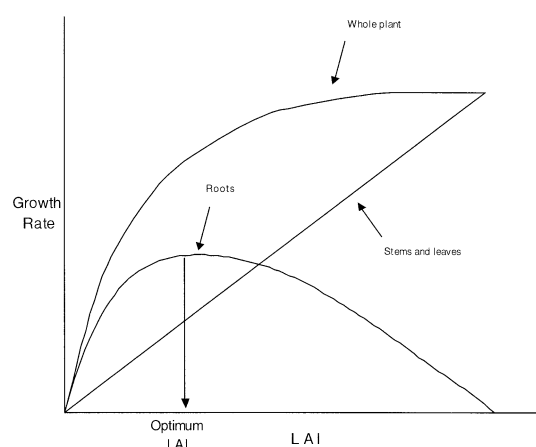
During the first two months, the cassava plant mainly develops shoots (stems and leaves) and a fine root system. It is known that the development of storage roots begins with secondary growth in several fibrous roots (ranging from as little as 3 to over 15 storage roots/plant, depending on cultivars and growing conditions) and starch deposition has been observed to occur at about 25–40 days after planting in many cultivars (Cock, 1984), but visible root bulking is noticeable only when root thickness is about 5 mm or more normally reached 2–4 months after planting. It has been suggested that root thickening starts when the supply of photoassimilates exceeds the requirements
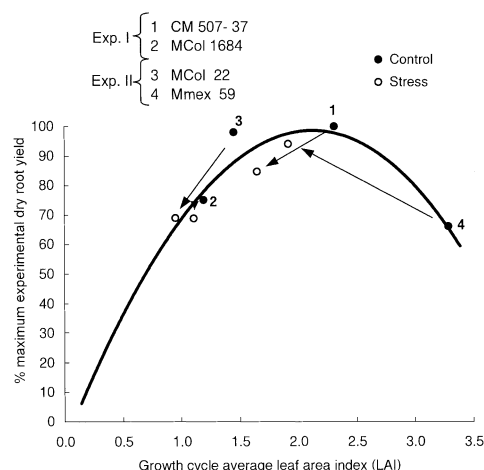
*Figure 5.* Dry root yield as a function of growth cycle average leaf area index (LAI) under non-stress and mid-term water stress conditions for 4 cultivars with different vigor. Data of experiment I for CM 507-37 and M Col 1684 from El-Sharkawy an Cock (1987a); data of experiment II for M Col 22 and M Mex 59 from Connor *et al.* (1981). Source: El-Sharkawy and Cock (1987a).

for the growth of shoot (leaves and stems) (Cock *et al.*, 1979; Tan and Cock, 1979).

The distribution patterns of dry matter among the different organs of the cassava plant change markedly during the growth cycle, with shoot having a dominance in the first 3–5 months while storage roots become the major sink for photoassimilates during the rest of the growth cycle (Figure 2). However, patterns of dry matter partitioning among plant organs are affected by growth conditions and could show different trends, particularly with changes in soil nutrient level, water regime, solar radiation, length of the day (photoperiod) and temperature (Irikura *et al.*, 1979; Connor *et al.*, 1981; Keating, 1981; Porto, 1983; Fukai *et al.*, 1984; Veltkamp, 1985; Pellet and El-Sharkawy,1997; Alves, 2002; El-Sharkawy and Cadavid, 2002). Dry matter in leaves and petioles increased only until the fourth month, after which it remained constant or decreased as new leaf formation was offset by leaf fall. The two cultivars differed mainly in the relative partitioning of dry matter between shoots and storage roots. The vigorous M Mex 59 cultivar produced greater shoots while storage root production was slow, becoming dominant only after the eighth month. In contrast, the less vigorous M Col 22 cultivar produced small shoots but partitioned greater amounts of photoassimilates to storage roots as early as the third month after planting.
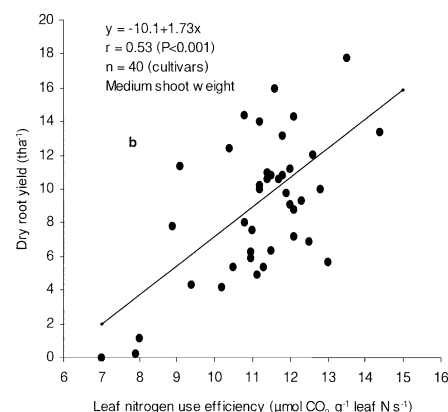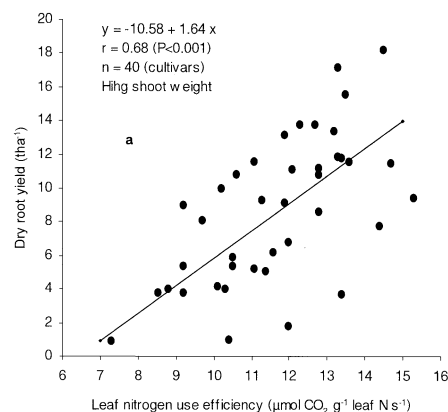


*Figure 6.* Relationship between dry root yield and leaf nitrogen use efficiency in field-grown cassava. Leaf nitrogen use efficiency values were calculated from leaf $CO_2$ exchange measurements and total leaf nitrogen concentrations. a. Cultivars with high shoot weight and large LAI. b. Cultivars with medium shoot weight and medium LAI. Figure constructed from data of El-Sharkawy *et al.* (1990).

## Fine (fibrous) root systems of field-grown cassava

At the CIAT-Quilichao experimental station, research was conducted to determine crop growth and development of various clones from the cassava core germplasm. Among the various aspects of crop growth and development studied was the fine root system of the crop, as it is a plant characteristic of paramount importance in soil-water-plant relationships (Connor *et al.*, 1981; El-Sharkawy and Cock, 1987a; Tscherning *et al.*, 1995).

Table 2 (El-Sharkawy and Cock, 1987a) summarizes data concerning the characteristics of the fine root system and soil water content within 2 m depth, sampled at 6 months after planting, for the hybrid CM

*Table 2.* Characteristics of fibrous root system and soil water profile of 6-month old field-grown plants of cassava cultivars MCol 1684 and CM 507-37; planted on 15 April and sampled on 15 October 1983 at Santander de Quilichao, Colombia. Soil cores (390 $cm^3$) were taken by hand auger for determination of root characteristics and soil water content. Values are means of 4 profiles. Source: El-Sharkawy and Cock (1987a).

| Soil layer (m) | MCol 1684 | | | CM 507- 37 | | |
|---|---|---|---|---|---|---|
| | root length density (cm/cm$^3$) | root diameter (mm) | soil water (% v/v) | root length density (cm/cm$^3$) | root diameter (mm) | soil water (% v/v) |
| 0.2 | 0.454 | 0.970 | 25.6 | 0.715 | 0.520 | 26.9 |
| 0.4 | 0.189 | 0.923 | 39.2 | 0.195 | 0.576 | 37.3 |
| 0.6 | 0.128 | 0.688 | 50.2 | 0.129 | 0.727 | 48.8 |
| 0.8 | 0.069 | 0.670 | 54.7 | 0.068 | 0.553 | 55.5 |
| 1.0 | 0.051 | 0.715 | 58.7 | 0.068 | 0.481 | 58.6 |
| 1.2 | 0.051 | 0.634 | 61.5 | 0.057 | 0.488 | 60.5 |
| 1.4 | – | – | 65.2 | 0.026 | 0.504 | 62.7 |
| 1.6 | – | – | 69.0 | 0.051 | 0.532 | 63.3 |
| 1.8 | – | – | 69.0 | 0.054 | 0.554 | 63.3 |
| 2.0 | – | – | – | 0.059 | 0.536 | – |

507-37 and the parent M Col 1684. The crops (planted on 15 April 1983) were grown under rain-fed conditions that were much less than pan evaporation from June to the time of sampling (1 October 1983).The hybrid CM 507-37 had finer roots, a greater density of root length in the upper layer of soil and its root system penetrated into deeper soil layers (perhaps beyond the 2 m depth studied) than the parent M Col 1684 did. The more intensive and extensive root system of CM 507-37 was advantageous in terms of its ability to withdraw more water (and perhaps more nutrients) from larger and deeper volumes of soil. It is noteworthy that differences between the hybrid and its parent in rooting capacities at sprouting were apparent (Figure 1), indicating the effectiveness of early screening and selection for fine root characteristics among large number of breeding materials.

Connor *et al.* (1981) reported similar findings for two other cultivars, M Mex 59 and M Col 22, but with much less rooting densities within 2.6 m soil depth. These authors also noted that in both cultivars root length density was greater in crops subjected to water shortage of two months than in well-watered crops. Moreover, Aresta and Fukai (1984) studied the effects of solar radiation on the growth of fibrous roots of cv. M Aus 7 grown in field at Queensland, Australia. These authors found that within 50 days of planting, root length density in the 0–20 cm soil layer was 1.6 cm/cm$^3$ and by 84 days it had increased to 3.6 cm/cm$^3$, with the rooting depth increasing rapidly to beyond 1.2 m by day 72. Shading the plants for 35 days at 32% of solar radiation input reduced the rate of increase in root length density (about 50% decrease in elongation rate), decreasing total root length .

Cassava fine root systems are sparse compared to those of some tropical grasses, with which it is often intercropped, indicating possible root interaction that might lead to competition in exploring shared soils for nutrient and water uptake (Tscherning *et al.*, 1995). These findings are of paramount importance in intercropping systems of cassava that involve other species with more aggressive rooting systems and growth habits that may result in partial shading of the cassava canopy. Cassava growth rates, storage root development and yield are depressed under shading (Fukai *et al.*, 1984).

*Shoot (stems and leaves) growth and its implications for storage root yield*

The dynamics of shoot growth, branching habit and patterns of leaf formation and leaf characteristics, such as size, duration of activity, and fall as affected by genotype and environment, have been extensively studied (Cock *et al.*, 1979; Irikura *et al.*, 1979; Tan and Cock, 1979; Connor and Cock, 1981; Keating, 1981; Porto, 1983; Veltkamp, 1985).

*Stem and leaf formation*

Upon sprouting, one or more axillary buds on a cutting develop rapidly and form, in sequence, nodal units consisting of a node, a bud, a palmate leaf blade

subtended by a long petiole, and an internode whose length and mass depend on the genotype, age of the plant and environment.

Due to apical dominance and the indeterminate habit of the cassava plant, new leaves are formed continuously in a spiral manner but with rates that vary with genotype, age of the plant and environmental conditions prevailing during growth. In addition to environmental influence, the total number of leaves produced per plant depends on the genotype, number of vegetative stems per plant and number of symbodial branches. The latter are formed from the topmost axillary buds once apical dominance ceases and flowering takes place. This process is called branching or forking in cassava. The rate of nodal unit formation decreases from about once per day in the early stage of growth to once per week in later stages (Figure 3; Irikura *et al.*, 1979).

At temperatures lower than 24 °C, rates of leaf formation decrease (Irikura *et al.*, 1979; Keating, 1981). Similarly, extended water shortage reduces the rate of leaf formation in all cultivars (Connor and Cock, 1981; Porto, 1983). At high temperatures, a leaf is fully expanded in two weeks and the size increases with plant age up to about four months and then declines; however, at low temperatures the maximum size decreases and is achieved at late growth stages. Leaves produced under prolonged water stress are also smaller, but leaf life or duration of leaf activity increases (Connor and Cock, 1981).

At about 24 °C leaf life varies by genotype and plant age, being about 60–80 days during the first four months of growth and increasing to about 120 days at later plant age; however, at lower temperature leaf life may extend up to 200 days in some cultivars (Irikura *et al.*, 1979).

The branching or forking behavior of stems (after the top buds turn into reproductive ones) varies widely among cultivars, ranging from zero to about four branches per branching point (initiated from the axillary buds below the point of branching) (Tan and Cock, 1979). Timing and height of the first branching point also varies among cultivars, with some branching earlier than others. The branching process continues also in the newly formed sympodial branches once apical dominance ceases, thus forming several branching points, in series, in each branch. The final outcome of shoot development and growth is the formation of crop canopy which in turn determines, in interaction with the environment, total biological productivity as well as economic yield.

## Crop canopy as yield determinant

Figure 4 illustrates, schematically, total biomass, shoot and storage root production in cassava as functions of leaf canopy area measured by the ratio of leaf surface area (one side only) to land area, or the leaf area index (LAI) (Cock, 1984). In contrast to the biologically known two-phase crop development pattern in grain crops, where vegetative and reproductive phases are separated in time (with little or no competition for current photoassimilates), in cassava shoots (stems and leaves) and roots develop simultaneously and hence photoassimilates are partitioned between growth of shoots and roots. This pattern of dry matter partitioning leads to the existence of an optimum LAI value for storage root growth at which there is a balance in dry matter distribution between the two competing plant organs. If more photoassimilates are allocated to form more stems and leaves, or a larger LAI value, less dry matter will be available for storage root growth and filling.

## Simulated ideal plant type for maximum yield

Building on the knowledge and insight gained about the physiological mechanisms underlying patterns of dry matter partitioning in cassava, Cock *et al.* (1979) developed a computer-based simulation model to determine the ideal plant type for maximum yield under favorable growth conditions. The simulated ideal plant type had the following characteristics: late branching at 6–9 months from planting with no vegetative suckers, maximum leaf size near 500 cm$^2$ per leaf blade at 4 months from planting, long leaf life of ca. 100 days, LAI between 2.5 and 3.5 during most of the growth cycle, a harvest index (the ratio of storage root mass to total plant biomass) greater than 0.5, nine or more storage roots per plant at a population density of 10 000 plants/ha, and each plant having two vegetative shoots originating from the original cuttings. If this simulated ideal plant ever existed in cassava germplasm or has been genetically bred for, then it should yield in a year, according to the model predictions, about 90 t/ha of fresh roots (about 30 t/ha dry matter), provided that the growing environment is optimal (no stress).

## Performance of the simulated ideal plant under stress

In a stressful environment, however, the ideal plant type will suffer more than a leafier type, i.e. with LAI values greater than the simulated optimum LAI (El-Sharkawy and Cock, 1987a). The hypothesis and

the mechanism underlying the response of cassava to water stress in relation to changes in LAI are shown in Figure 5 with contrasting cultivars. A cultivar with slightly higher than optimum LAI (e.g. CM 507-37) will reduce its LAI under stress with a minimal effect on yield, while an ideal plant type for non-stress conditions (e.g. M Col 22) will yield only very slightly more under non-stressed conditions, but yields will be very sensitive to stress. On the other hand, a cultivar with a very large LAI (e.g. M Mex 59) will produce less under optimal conditions than under stress. Based on this hypothesis and the apparent reasonable fitness of experimental data to it, it would appear that for stable high yields in both stress and non-stress environments, a cultivar with higher than optimal leafiness under favorable conditions such as hybrid CM 507-37 would be advantageous. Moreover, it has been suggested (El-Sharkawy and Cock, 1987a) that cultivars with higher leaf area ratio (LAR, the ratio of leaf area per unit total shoot biomass) such as that found with CM 507-37 will produce higher than optimal LAI values under non-stress conditions and reasonable LAI values under stress, with less competition with storage roots for dry matter due to thinner stems and/or shorter internodes. In addition to the advantage of producing cultivars with higher LAR, longer leaf life (i.e. better leaf retention) is another desirable leaf trait to select and breed for, as this should result in dry matter saving that could be partitioned to storage root filling (El-Sharkawy et al., 1992b).

## Physiological processes underlying potential productivity, tolerance to water stress and plant-related limiting factors

### Potential productivity in cassava

Observed yield potential of cassava, in the absence of production constraints, compares favorably with other major staple food crops in the tropics, with cassava having the greatest potential for energy production except for sugarcane (Table 3; de Vries et al., 1967; El-Sharkawy,1993). Based on the highest reported annual experimental yields, cassava produces more energy per hectare than maize, sorghum, and rice. El-Sharkawy et al. (1990) reported an average experimental yield for several improved cassava cultivars (over an experimental area of 1 ha) as high as 90 tons of fresh roots per hectare (equivalent to about 27 tons of oven-dried matter) in 10 months. This high productivity took place in Patia Valley, Cauca, Colombia

(altitude 600 m, 2°09′ N, 77°04′ W) with annual precipitation of 900–1000 mm, 60% of which occurred in the first three months of crop establishment, and with a pronounced dry period of three months before harvest. The climate at Patia Valley is characterized by high solar radiation (about 22 MJ m$^{-2}$ day$^{-1}$), a high mean day temperature (28 °C), and high atmospheric humidity (70%). These climatic factors appear to be near-optimal for high cassava productivity.

Such an observed productivity indicates that cassava has high yield potential when grown under near-optimum conditions and confirms the validity of the ideal plant type simulated by Cock et al. (1979). Furthermore, it agrees with the previously observed productivity on a small plot at the CIAT experimental station, Palmira, Valle, Colombia, of about 28 t/ha dry root per year (CIAT, 1979), and with more recently reported high yields of more than 80 t/ha fresh root per year for improved cultivars grown on several hectares by CIAT researchers (H. Ceballos, personal communication). In India, similar experimental high yields were realized with irrigation (Ramanujam, 1990).

However, the gap between the potential yield and the actual yields on farmers' fields is six-fold, suggesting that the real potential of cassava is far from being realized. Current yields of 8–16 tons/ha on a fresh weight basis, with the largest yields occurring in India, are normally attained with local, traditional varieties on marginal soils without application of agrochemicals (El-Sharkawy,1993). Thus, if the many socioeconomic constraints on cassava production, utilization, and marketing would be alleviated, farmers' yields could be easily doubled by using improved technologies.

### Photosynthesis in relation to crop productivity

The maximum leaf net photosynthetic rates in cassava grown under favorable field conditions were between 40 and 50 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ with high solar radiation (>1800 $\mu$mol m$^{-2}$ s$^{-1}$ in the range of photosynthetically active radiation) and with an optimum leaf temperature around 30–35 °C (El-Sharkawy et al., 1984d, 1992a; El-Sharkawy and Cock, 1990). These high maximum photosynthetic rates may explain, in part, the observed high productivity in cassava in the field trials conducted in Patia Valley. Also in seasonally dry and semi-arid environments (De Tafur et al., 1997) or in humid ones (El-Sharkawy et al., 1993; Pellet and El-Sharkawy, 1993a) it was found that both total biomass and storage dry root yield correlated

*Table 3.* Maximum recorded yield and food energy of some important tropical food crops. Source: de Vries *et al.* (1967).

| Crop | Annual yield (t/ha) | Daily energy production (kJ/ha) |
|---|---|---|
| Cassava (fresh root) | 71 | 1045 |
| Maize (dry grain) | 20 | 836 |
| Sweet potato (fresh root) | 65 | 752 |
| Rice (dry grain) | 26 | 652 |
| Sorghum (dry grain) | 13 | 477 |
| Wheat (dry grain) | 12 | 460 |
| Banana (fruit) | 39 | 334 |

*Table 4.* Comparative water use efficiency of cassava, grain sorghum and field bean. Source: El-Sharkawy and Cock (1986).

| Species | Water use efficiency | | |
|---|---|---|---|
| | single-leaf gas exchange $\mu$mol $CO_2$ per mmol $H_2O$ | total biomass of field-grown crops g dry weight per kg water | economic yield g dry weight per kg water |
| Cassava | 5.3 | 2.9 | 1.7 (HI 60%)* |
| Sorghum | 6.2 | 3.1 | 1.2 (HI 40%) |
| Bean | 3.5 | 1.7 | 0.7 (HI 40%) |
| Cassava/sorghum (%) | 85 | 94 | 140 |
| Cassava/bean (%) | 150 | 170 | 240 |

*HI: Harvest index = (dry grain or dry root)/(total dry weight) $\times$ 100.

significantly with mean seasonal upper canopy leaf photosynthetic rate, and generally the relations were due to non-stomatal (biochemical and anatomical) factors (El-Sharkawy and Cock, 1990; El-Sharkawy *et al.*, 1990). In the absence of limitations on the crop to maximize light interception (i.e. crop canopy near optimum LAI for maximum light interception), storage root yield correlated significantly with leaf nitrogen use efficiency (as measured in terms of rate of photosynthesis on the basis of total leaf nitrogen content) (Figure 6). This indicates that the association between yield and leaf photosynthesis was mainly due to biochemical factors controlling carbon assimilation in cassava. Thus, selection for high leaf photosynthesis, particularly under field conditions in which cassava is normally grown, should be considered in cassava breeding programs as a factor that may contribute to yield improvement when combined with other yield determinants such as appropriate LAI values and strong storage root sink (i.e. greater number of storage roots /plant).

Another physiologically important plant trait that may contribute to yield is long leaf life and better leaf retention, particularly under stressful environments (El-Sharkawy *et al.*, 1992b; El-Sharkawy, 1993). Because cassava leaves were found to remain photosynthetically active under prolonged water stress in the field (more than two months), though at a reduced rate (water-stressed leaves had on average about 40% of their observed maximum rate under favorable conditions), and because the stressed leaves were also capable of partially recovering from stress once water becomes available again, their retention, coupled with resistance to pests and diseases, may represent a significant saving in biomass invested in leaf formation.
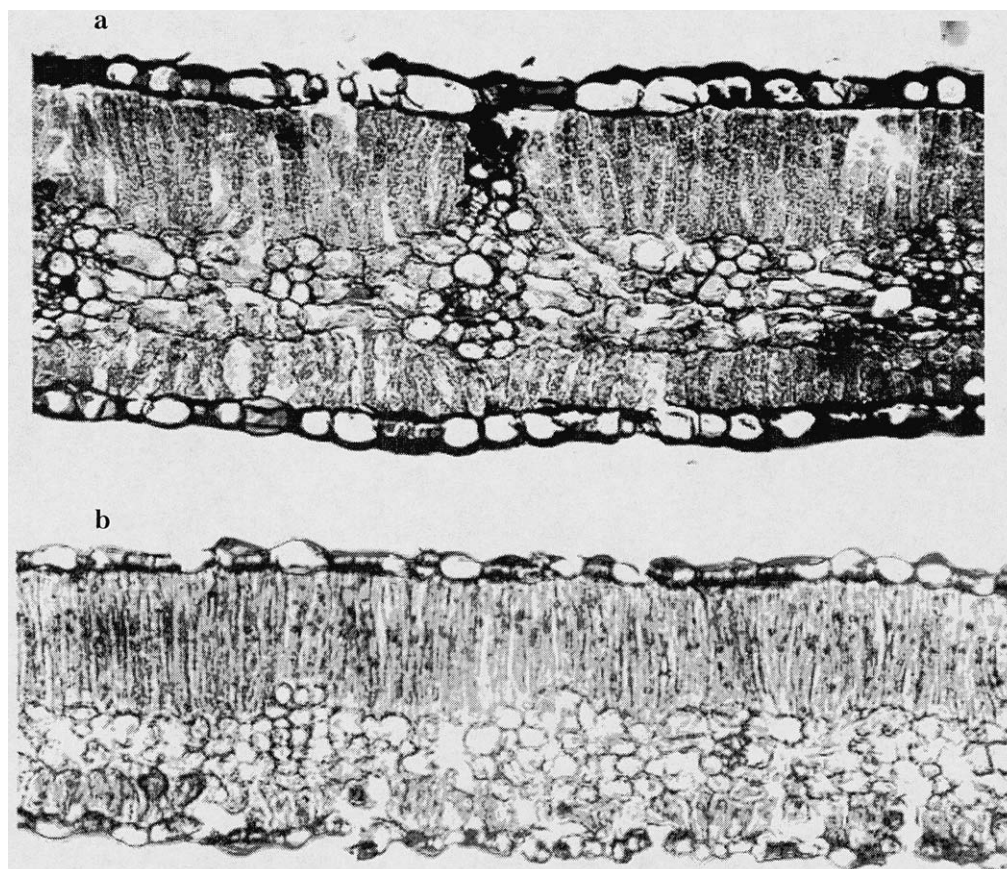
*Figure 7.* Cross sections in leaves of wild *Manihot* species showing the development of a second palisade layer at lower surface of the leaf. Note: the chloroplast-containing vascular bundle sheath between the two palisade layers, and the stomata on the upper epidermis. a, *M. rubricaulis*; b, *M. grahami*. Sources: CIAT Cassava Program Annual Report, 1994, Cassava Physiology Section, and M.A. El-Sharkawy, unpublished).

### Role of wild species in improving cassava photosynthesis

Recent research within the very limited wild germplasm of *Manihot* grown at the CIAT station, Palmira, Valle, Colombia, has revealed the existence of few species with amphistomatous leaf and with a second short palisade layer at the lower side of the leaf, in addition to the long palisade layer on the upper side (CIAT, 1994). Two species, *M. rubricaulis* and *M. grahami*, were found to possess amphistomatous leaves, a second short palisade layer, and in between conspicuous vascular bundle sheaths, though not fully developed, with chloroplasts (Figure 7) and, most notably, an elevated activity in leaf extracts of the $C_4$ photosynthetic enzyme PEP carboxylase (from 15% to over 25% of activity in leaves of the $C_4$ species sorghum and maize). PEP carboxylase activity in leaf extracts of wild *Manihot* ranged from 1.5 to 5.5 $\mu$mol per mg chlorophyll per minute, compared to 6–12 in sorghum, a $C_4$ species, and 0.2–0.4 in beans, a $C_3$ species (CIAT, 1994; Calatayud *et al.*, 2002). Moreover, these wild *Manihot* species had very high leaf net photosynthetic rates in normal air and under high photon flux density (>50 $\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$, Figure 8) and relatively low photorespiration ($CO_2$ compensation point around 25–35 ppm at 30–35 °C and high photon flux density). The observed characteristics of leaf anatomy, high photosynthetic rates, low photorespiration and elevated PEP carboxylase activities in leaves of some wild *Manihot* species might indicate that cassava and wild manihot species represent an intermediate photosynthesis between the typical $C_3$ and $C_4$ species (El-Sharkawy and Cock, 1987b). It is known that within the family Euphorbiaceae there are species representing the CAM, $C_3$ and $C_4$ photosynthetic pathways. It is possible, therefore, that the genus *Manihot* represents an evolutionary step to-

*Table 5.* Average net photosynthetic rate, $P_N$ ($\mu$mol($CO_2$)m$^{-2}$ s$^{-1}$), stomatal conductance, $g_s$ (mol($H_2O$) m$^{-2}$ s$^{-1}$), intercellular $CO_2$ concentration, $c_i$($\mu$mol/mol) and dry root yield (kg/m$^2$) of field-grown cassava under rain-fed conditions at Santo Tomás, Atlantic Department (seasonally dry environment) and Riohacha, Guajira (semi-arid environment). 1992–1993. Source: De Tafur *et al.* (1997).

| Locality and cultivar | Dry root yield | $P_N$ | $g_s$ | $c_i$ |
|---|---|---|---|---|
| Santo Tomás | | | | |
| CG 1141-1 | 0.76 | 31 | 0.38 | 179 |
| SG 536-1 | 0.72 | 29 | 0.39 | 234 |
| MCol 1505 | 0.60 | 29 | 0.43 | 215 |
| CM 3306-4 | 0.74 | 28 | 0.38 | 195 |
| MBra 191 | 0.65 | 27 | 0.38 | 196 |
| CM 4013-1 | 0.72 | 26 | 0.38 | 224 |
| MBra 12 | 0.70 | 25 | 0.36 | 196 |
| CM 3555-6 | 0.58 | 25 | 0.37 | 243 |
| CM 4063-6 | 0.62 | 25 | 0.37 | 207 |
| MCol 2215 | 0.65 | 25 | 0.44 | 209 |
| | | | | |
| Mean of all cultivars | 0.67 | 27 | 0.38 | 209 |
| LSD (0.05) | 0.15 | 3.5 | 0.064 | 35 |
| | | | | |
| Riohacha | | | | |
| CM 4013-1 | 0.33 | 20 | 0.46 | 271 |
| MCol 2215 | 0.30 | 20 | 0.47 | 233 |
| MCol 1505 | 0.23 | 15 | 0.49 | 295 |
| MCol 1734 | 0.32 | 15 | 0.43 | 257 |
| CG 1141-1 | 0.28 | 13 | 0.44 | 281 |
| MCol 1684 | 0.23 | 13 | 0.47 | 295 |
| SG 536-1 | 0.26 | 12 | 0.44 | 341 |
| CM 4063-6 | 0.32 | 12 | 0.45 | 305 |
| MCol 1468 | 0.18 | 9 | 0.47 | 310 |
| MCol 22 | 0.20 | 8 | 0.50 | 312 |
| MBra 12 | 0.12 | 7 | 0.59 | 330 |
| CM 3306-4 | 0.22 | 7 | 0.42 | 310 |
| MVen 77 | 0.04 | 7 | 0.37 | 307 |
| Mean of all cultivars | 0.23 | 12 | 0.46 | 295 |
| LSD (0.05) | 0.08 | 3.8 | 0.096 | 58 |



*Figure 8.* Leaf net photosynthetic rate, $P_N$. a. $P_N$ of *M. rubricaulis* as a function of irradiance; b. $P_N$ of *M. rubricaulis* as a function of internal $CO_2$ in comparison with maize ($C_4$) and soybean ($C_3$). Estimate of internal $CO_2$ comes from the standard Gastra equations for gas fluxes in and out of the leaf. Source: CIAT Cassava Program Annual Report, 1995, Cassava Physiology Section).

wards the $C_4$ pathway. Contrary to this hypothesis, others (Edwards *et al.*, 1990; Angelov *et al.*, 1993; Calatayud *et al.*, 2002) have concluded that neither cassava nor wild relatives possess $C_4$ photosynthesis, based on the dynamics of $^{14}C$ labeling, $CO_2$ compensation point and the natural abundance of $^{13}C$. Such discrepancies could be attributed to the lack of leaf Kranz anatomy, typical of $C_4$ species, in cassava and wild *Manihot* species. Kranz anatomy is essential, at least in $C_4$ higher-plant species, for the separation and
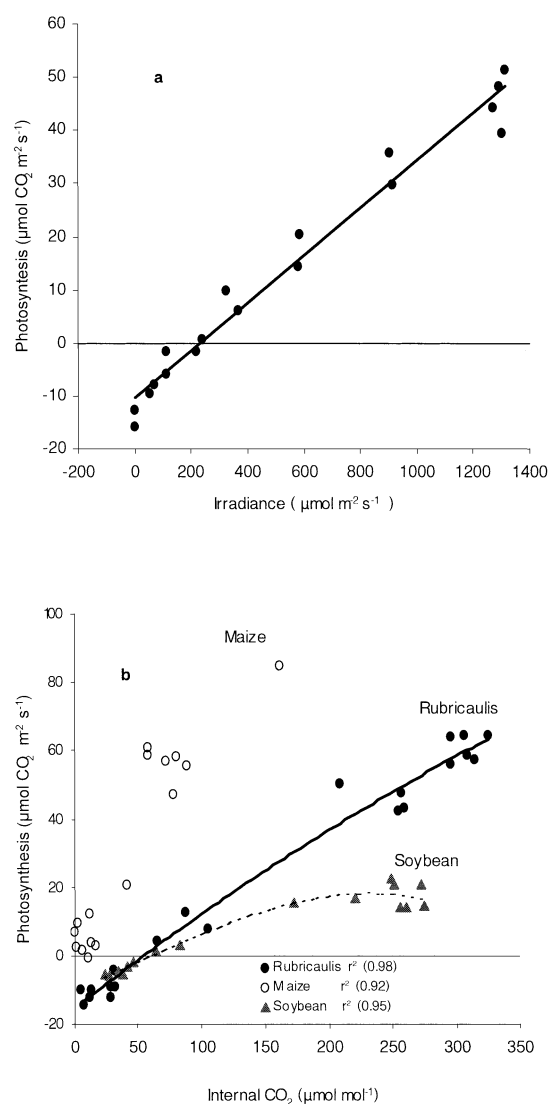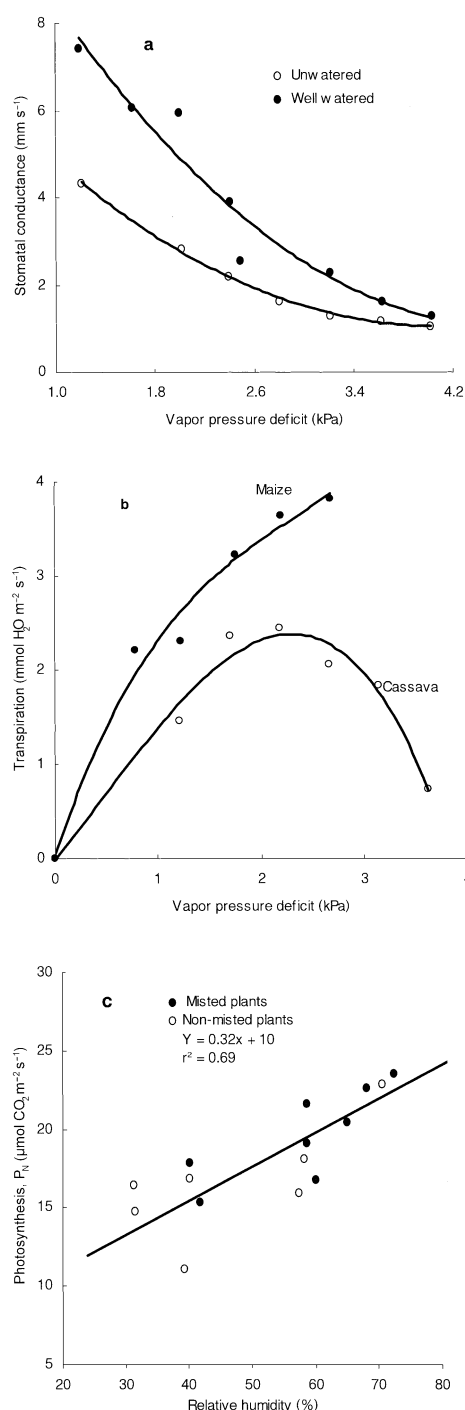
compartmentalization of the $C_3$ and $C_4$ main enzymes (Laetsch, 1974). Moreover, the kinetics of the $C_3$ photosynthetic enzyme Rubisco differ between $C_3$ and $C_4$ species, with the latter having higher $K_m$ ($CO_2$) values due to the adaptation of the enzyme to higher concentrations of $CO_2$ that normally exist at its site in the bundle sheath chloroplasts of $C_4$ leaves with Kranz anatomy. In cassava and other $C_3$-$C_4$ intermediate species, $K_m$ ($CO_2$) values and other kinetic parameters of the $C_3$ photosynthetic enzyme Rubisco are similar to

those in $C_3$ species (Yeoh *et al.*, 1980, 1981; Paul and Yeoh, 1987). Nevertheless, from an evolutionary point of view it is possible that cassava and wild relatives are evolving biochemically towards $C_4$ photosynthesis and may represent photosynthetic characteristics intermediate between $C_3$ and $C_4$ species. Moreover, it had been reported recently that biochemical characteristics of $C_4$ photosynthesis (i.e. high activities of $C_4$ enzymes) and the controlling genes already exist in stems and petioles of $C_3$ flowering plants, thus indicating that these features might represent the first step in $C_4$ evolution (Hibberd and Quick, 2002).

Cassava was previously reported to have low photorespiration rates ($CO_2$ compensation point around 25–30 ppm at 30 °C and high photon flux density), a high percentage (40–60%) of leaf-fed ^{14}C incorporated in $C_4$ acids after 5–10 s in the light and elevated activities of PEP carboxylase (10–25% of activity in $C_4$ species), but lacks the leaf Kranz anatomy characteristics of typical $C_4$ species (El-Sharkawy *et al.*, 1984b, 1992a; Cock *et al.*, 1987; El-Sharkawy and Cock, 1987b, 1990).

In view of these findings, it is of interest, therefore, to continue searching for more wild species within the genus *Manihot* that might have evolved, anatomically and/or biochemically, toward acquiring more components of the efficient $C_4$ photosynthetic pathway in order to be used in crosses with cultivated cassava in future research targeted toward crop improvement, particularly for marginal stressful environments. To speed up the search for and discovery of the sought after leaf traits and components of the efficient $C_4$ photosynthetic pathway in cassava and its wild relatives, molecular biology should play a significant role.
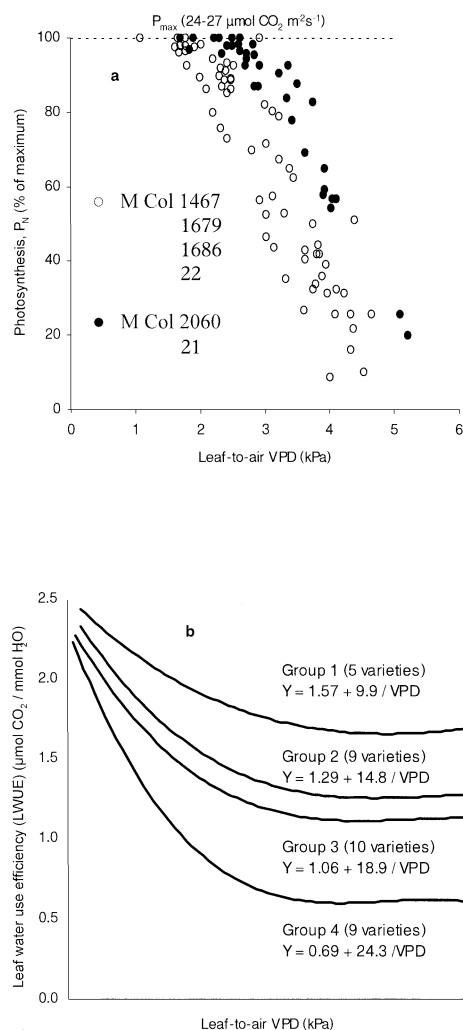
*Figure 11.* Relationship between dry root yield and intercellular $CO_2$ concentration ($C_i$) for two groups of cassava cultivars grown under rain-fed conditions at Riohacha (semi-arid) and Santo Tomas (seasonally dry). The $C_i$ values were calculated from leaf gas fluxes via standard Gastra equations: the higher photosynthetic rates, the lower $C_i$ values. Regression equation: yield $= 1.52 - 0.004C_i$; $r^2 = 0.82$ ($P < 0.001$). Source: De Tafur *et al.* (1997).



*Figure 10.* Response of cassava gas exchange to leaf-to-air vapor pressure deficit (VPD). a. Response of leaf net photosynthesis to VPD in potted cassava. Source: M.A. El-Sharkawy (unpublished). b. Response of leaf water use efficiency (LWUE) to leaf-to-air vapor pressure deficit (VPD) in field-grown cassava. LWUE is the ratio between net photosynthetic and transpiration rates. Group 1 represents varieties for humid environment; Group 2 represents varieties for sub-humid environment; Group 3 represents varieties for seasonally dry environment; Group 4 represents varieties for semi-arid environment. Note: cassava sensitivity to VPD increases from Group 1 to Group 4. Source: M.A. El-Sharkawy (unpublished).

*Figure 12.* Relationship between leaf net photosynthetic rates and PEP carboxylase activities of field-grown cassava cultivars under prolonged water stress (3 months) at CIAT-Quilichao, 1988. Both photosynthetic rates and PEP carboxylase activities were determined on the same leaves of 6-month old plants subjected to water stress at 3 months after planting. Values are means of three fully expanded upper canopy leaves per cultivar in two replications. Source: M.A. El-Sharkawy, L. Bernal and Y. Lopez (unpublished).

## Stomatal response to atmospheric humidity and its implications for tolerance to water stress and crop water use efficiency

The physiological mechanisms underlying cassava tolerance to water stress were found to be related to the remarkable sensitivity of cassava stomata to both atm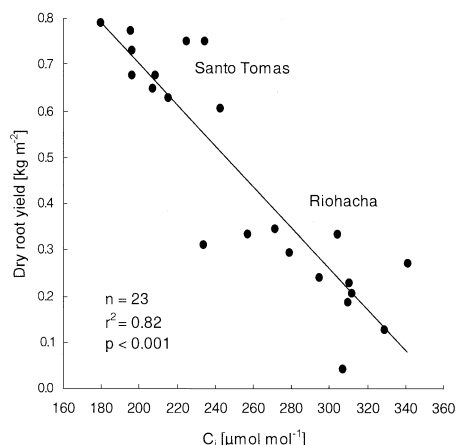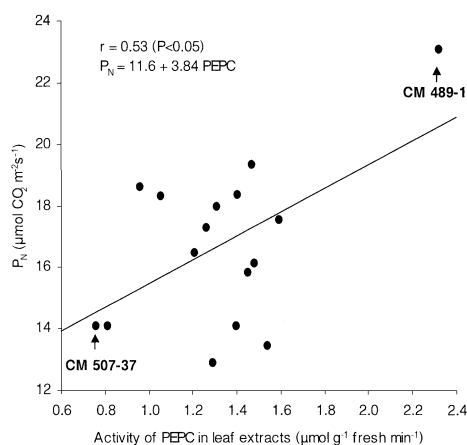ospheric and edaphic water stress (El-Sharkawy and Cock, 1984, 1986; El-Sharkawy *et al.*, 1984c, 1985; Cock *et al.*, 1985; El-Sharkawy,1993). Irrespective of soil water status, cassava stomata were found to partially close in low air humidity with virtually no changes in leaf water potential, and also in response to soil water shortage, hence protecting the leaf from severe dehydration and at the same time the leaf remains photosynthetically active (Figure 9). Genetic

differences in stomatal response to changes in air humidity were observed in potted outdoor-grown plants as well as in field-grown crops (Figure 10). Moreover, stomatal density was found to correlate positively with the degree of sensitivity to changes in air humidity across a wide range of tropical plant species including cassava (El-Sharkawy *et al.*, 1985). Since the great majority of cassava cultivars have a very high stomatal density only on the abaxial surface of the leaf blade (300–500 per mm$^2$ of leaf surface) with virtually none on the adaxial side, except a few stomata adjacent to leaf veins (El-Sharkawy *et al.*, 1984a), it was argued that in order to decrease stomatal sensitivity to air humidity, and thus to maximize photosynthesis in environments where water is not limiting, selection for amphistomatous leaves with the same total leaf conductance to gas exchange as hypostomatous ones will be advantageous (El-Sharkawy *et al.*, 1985; El-Sharkawy and Cock, 1987b). The very few clones (about 2% of 1500 clones screened) found with significant stomata on the adaxial surface might be used in crossing programs to manipulate stomatal distribution on both surfaces of the leaf .

The strong stomatal response to changes in leaf-to-air vapor pressure deficit (VPD) observed in cassava is particularly important for crops that have to endure a long dry season (3–7 months) commonly encountered during the production cycle. Under these stressful conditions, with stomata less sensitive to VPD, both $CO_2$ uptake and $HO_2$ loss will occur at relatively high rates until available soil water is rapidly depleted. The leaf water potential will then decrease to the level required to induce stomatal closure, at which time both photosynthesis and transpiration will approach zero. A greater proportion of daily transpiration loss will take place at periods of peak VPD near midday. In such a case water use efficiency (WUE) either of a single leaf or of a crop canopy will be low. On the other hand, with a larger and more direct stomatal response to VPD as that observed in cassava, the limited amount of available soil water during the dry season will be consumed slowly, as transpiration loss will occur mainly in periods of lower VPD and higher WUE early in the morning and late afternoon. This, in turn, will lead to a greater total carbon gain over the stress period and a greater seasonal crop WUE (El-Sharkawy and Cock, 1984, 1986; El-Sharkawy *et al.*, 1984c).

The water use efficiency of cassava is indeed much greater than that of beans (a $C_3$ crop) which are less sensitive to VPD (Table 4; El-Sharkawy *et al.*, 1984c; El-Sharkawy and Cock, 1986). Moreover, WUE of cassava compares favorably with that of grain sorghum, a $C_4$ crop known for its high level of tolerance to drought among tropical grasses. Due to the higher harvest index of cassava, the dry matter of economic yield produced per unit water transpired in many cases exceeds that of grain sorghum. However, with a non-limiting water supply or with brief soil water shortages, maximizing total harvestable yield would be of greater value than optimizing WUE. Under these conditions, cultivars less responsive to changes in VPD would be more useful (El-Sharkawy and Cock, 1984, 1986; El-Sharkawy *et al.*, 1984c). Therefore, it is highly desirable to select and breed for cassava cultivars with amphistomatous leaves which will be less sensitive to changes in air humidity. Such cultivars should be targeted to regions with high precipitation and atmospheric humidity, such as in Amazonian regions.

*Leaf movement in cassava and its implication for plant-water relation*

The long petiole of cassava leaf (which constitutes 20–30% of leaf weight and varies in length from 10 to 25 cm) plays a pivotal role in orientating the leaf blade towards the sun during the day, thus maximizing light interception early in the morning and in late afternoon when angles of incident solar radiation are large (i.e. heliotropism or sun tracking) (El-Sharkawy and Cock, 1984; Hozyo *et al.*, 1984), and perhaps in intercepting sunflakes that penetrate into the shaded lower part of the crop canopy. Moreover, the midribs of leaf lobes were found to be responsible for the daily changes in another type of leaf movement called 'leaf drooping or folding' (i.e. leaf bending away from the horizontal position it holds early in the morning by folding downward at midday), irrespective of soil water and leaf turgor pressure (El-Sharkawy and Cock, 1984; Berg *et al.*, 1986).

In the field, it was found that the phenomenon of leaf folding decreased light interception by 50%, compared with adjacent horizontally supported leaves, increased the angle of light incidence at midday (being about 61° in folding leaves as compared with 10° in horizontal leaves), decreased leaf temperature by 6–7 °C, decreased leaf-to-air vapor pressure deficit by about 1.3 kPa, and increased leaf conductance to gas exchange to more than double the conductance in horizontal leaf (Berg *et al.*, 1986). The outcome of leaf folding is a reduction in transpiration water loss while the leaf maintains a reasonable photosynthetic rate at

a higher leaf conductance. Thus, the phenomenon of leaf folding or drooping at midday observed in both well-watered and stressed plants (when atmospheric evaporative demand is greatest in the tropics), may act as a water stress avoidance mechanism, and hence underlies, at least partly, cassava tolerance to prolonged drought .

## Selection under field conditions for tolerance to abiotic stresses

### The rationale

Early efforts in cassava breeding focused on developing high yielding cultivars for favorable environments, where biotic and abiotic stresses are either minimal or obviated through high-input cultural practices (Kawano et al., 1978; Cock et al., 1979). Due to the fact that cassava is grown by small resource-limited farmers in adverse environments under uncertain rainfall and mostly on infertile soils, virtually without agrochemical input, goals of recent breeding strategies have shifted toward developing cultivars with reasonable and stable yield potentials under the various and complex stresses (Hershey and Jennings, 1992). In view of this environmentally sound breeding strategy, research on cassava physiology at CIAT in the past two decades has focused on both basic and applied aspects of crop behavior in order to elucidate some of its fundamental characteristics and mechanisms that might underlie and better explain the high level of cassava tolerance to adverse environments where the crop is commonly grown, as well as to identify plant traits that might be of value in breeding efforts (El-Sharkawy, 1993).

### Selection for high photosynthesis in relation to productivity under prolonged drought

Research efforts on cassava basic physiology have yielded some useful information about the crop and have led to a better understanding of the response of cassava to various abiotic stresses. Cassava is endowed with a high photosynthetic potential that positively correlates with crop productivity in both humid and dry environments (El-Sharkawy and Cock, 1990; El-Sharkawy et al., 1990, 1993; Pellet and El-Sharkawy, 1993a; De Tafur et al., 1997), and field screening under these environments have identified clones with good yields and high leaf photosynthesis that could be used as parental materials in cassava breeding for yield improvement.

Table 5 (De Tafur et al.,1997) presents data on storage root yields and leaf gas exchange for a group of cultivars from the core breeding germplasm that were grown under seasonally dry and semi-arid environments at two locations on the north coast of Colombia. Soils of both locations are sandy and extremely low in all nutrients where both trials were planted without fertilization and no irrigation. Planting took place between 20 and 25 September 1992 on the onset of rainfall and harvesting took place in the first week of August 1993. Leaf gas exchange was measured on upper canopy leaves with a portable infrared gas analyzer during several days in February/March. All gas exchange measurements were made between 08:00 and 12:00 local time with a solar light intensity in the range of photosynthetically active radiation higher than 1000 $\mu$mol m$^{-2}$ s$^{-1}$ (De Tafur et al., 1997). At the seasonally dry location (Santo Tomás, Atlantic Department), total annual precipitation was 829 mm with a 7-month dry period when monthly rainfalls oscillated from less than 5 to 80 mm; annual pan evaporation 1647 mm; mean monthly solar radiation around 560 MJ/m$^2$; mean day temperature 27.5 °C. At the semi-arid location (Rioacha, Guajira Department), total annual precipitation was 550 mm with a 8-month dry period during which monthly rainfalls oscillated between zero to 80 mm, and total annual pan evaporation was 2293 mm, but with mean daily temperature and mean monthly solar radiation being slightly higher than at Santo Tomás.

Under these stressful conditions, average cassava yield was 6.7 tons/ha oven-dried storage roots at the seasonally dry location (yields ranged among cultivars from 5.8 to 7.6 tons/ha), and 2.3 tons/ha at the semi-arid one (yields ranged from 0.4 to 3.3 tons/ha). Nevertheless, due to severe shortage of water in the semi-arid location, dry matter content in the harvested roots was lower (<30%) than for the seasonally dry one where rainfall was much higher and potential evapotranspiration was lower. Thus, for a better yield and a higher dry matter content in cassava grown in the semi-arid region, the crop growth cycle has to be extended for a second rainy season, which is common practice in northeast Brazil, where annual rainfall is less than 700 mm (El-Sharkawy, 1993). Early-bulking cultivars might also have a better yield potential in semi-arid zones.

Differences in yield among cultivars were significant at both locations. Average leaf photosynthesis

rates ranged among cultivars from 25 to 31 $\mu$mol $CO_2$ m$^{-2}$ s$^{-1}$ at the seasonally dry location, while rates ranged from 7 to 20 $\mu$mol $CO_2$ m$^{-2}$ s$^{-1}$ at the semi-arid location. Nevertheless, these rates of photosynthesis were measured on plants 4–5 months old during the driest months in both locations, i.e. February and March, when monthly rainfall was less than 5 mm including January. Thus, they represent the lowest possible rates in the field and indicate the comparative advantage of cassava over other crops in these harsh environments. Differences among cultivars were significant in all leaf gas exchange parameters measured. Dry root yield correlated positively and highly significantly with average leaf photosynthesis rate across locations ($r^2 = 0.90$, $P < 0.001$), and the association was mainly due to non-stomatal factors (De Tafur *et al.*, 1997). The yield correlated also negatively with intercellular $CO_2$ concentration ($C_i$) (Figure 11, $r^2 = 0.82$, $P < 0.001$), hence indicating that the relation was mainly due to cultivars differences in biochemical factors controlling $CO_2$ fixation such as enzymes activities.

*Activities of photosynthetic enzymes as affected by water stress*

When field-grown cassava was subjected to more than 2 months of water stress, imposed at 3 months from planting at CIAT-Quilichao station, activities of the $C_3$ photosynthetic enzyme Rubisco in leaves that developed under stress decreased by about 40% at 8 weeks after initiation of water stress, as compared to activities in unstressed plants, with significant differences among cultivars (CIAT, 1993; Table 6). On the other hand, the average activities of the $C_4$ enzyme PEP carboxylase in leaves of the stressed plants increased by about 13% above those of unstressed ones, with significant differences among cultivars. This implies that under extended water stress the activity of the $C_4$ PEP carboxylase in cassava was favored over the $C_3$ Rubisco. Moreover, in a separate field trial, it was found that net leaf photosynthesis rates in several cultivars subjected to prolonged water stress correlated significantly with the activity of PEP carboxylase of the same leaves ($r = 0.53$, $P < 0.05$, Figure 12). The $C_3$ enzyme is known to have much less affinity to $CO_2$, particularly at temperatures higher than 25 °C, compared to the $C_4$ enzyme, and it controls both carboxylation (fixing $CO_2$) and oxygenation (releasing $CO_2$) reactions simultaneously under light. On the other hand, the $C_4$ enzyme, besides having very high

affinity to $CO_2$ under higher optimum temperature for activity, can scavenge or recycle internal respiratory $CO_2$, and hence reduce the net loss of carbon during photosynthesis (i.e. less loss of $CO_2$ from both photorespiration and mitochondrial dark respiration). Furthermore, it is possible that the PEP carboxylase located in mesophyll cytoplasm plays a role in fixing atmospheric $CO_2$ in forms of $C_4$ acids which could be then mass transported to the chloroplasts where the Rubisco is located, hence obviating or reducing the normally much higher mesophyll resistance to $CO_2$ diffusion from cell wall to chloroplast. However, the $C_4$ acids must be decarboxylated in order to provide the Rubisco with $CO_2$.

It is hypothesized, therefore, that in cassava that lacks the leaf Kranz anatomy typical of $C_4$ species (where both the $C_3$ and $C_4$ main enzymes are compartmentalized in the vascular bundle sheath and in the mesophyll tissue, respectively; Laetsch, 1974), the observed higher PEP carboxylase/Rubisco ratio in water-stressed plants (Table 6) may underlie, at least partly, the relatively high leaf net photosynthesis and, in turn, the reasonable yields observed in seasonally dry and semi-arid environments where most other crops would fail. However, to be effective, selection for high photosynthesis should be combined with other yield determinants such as high harvest index (preferably >0.5), long leaf area duration (i.e. longer leaf life and better leaf retention), strong storage root sink (i.e. high storage root number per plant) and appropriate canopy architecture (i.e. maintaining a near-optimum LAI for root production during most of the growth cycle). With the availability of modern techniques that allow screening large populations of breeding materials in the field it is, therefore, recommended that cassava breeding programs include selection for leaf photosynthesis whether for identifying parental materials or in evaluating breeding lines.

*Selection for tolerance to low-fertility soils*

As most of cassava production occurs on acidic soils low in nutrients, particularly phosphorus, with little or no external input, breeding for efficient cultivars in exploiting native nutrients, i.e. higher yield per unit nutrient extracted, was warranted to develop environmentally sound technologies that helps sustain reasonable and stable productivity while alleviating pressures on natural resources in marginal lands (Hershey and Jennings, 1992; El-Sharkawy, 1993). In view of this strategy, both the cassava nutrition and physiology

*Table 6.* Activities of some photosynthetic enzymes in leaf extracts of field-grown cassava as affected by 8 weeks of water stress commencing at 92 days after pollination at Santander de Quilichao, 1993. Values are means ± SD. Activities in $\mu$mol per mg chlorophyll per minute. Source: CIAT Annual Report, 1993, Physiology Section.

| Clone | Unstressed | | | Stressed | | |
|---|---|---|---|---|---|---|
| | 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 |
| MCol 1505 | 1.09 ± 0.10 | 0.57 ± 0.13 | 1.90 | 0.96 ± 0.16 | 0.89 ± 0.14 | 1.1 |
| Avg. | 1.08 | 0.90 | 2.2 | 1.22 | 0.52 | 3.2 |
| % Average change due to stress | | | | +13 | −42 | +45 |

*Table 7.* Dry root yield of clones with high adaptation to low phosphorus, CIAT, Santander de Quilichao, Cauca, Colombia. Source: CIAT Annual Report, 1990, Physiology Section.

| Clone | Dry root yield (t/ha) | | Low-P index[1] |
|---|---|---|---|
| | 0 P | 75 kg/ha P | |
| CG 996- 6 | 13 | 17 | 2.0 |
| CM 305- 41 | 14 | 15 | 1.9 |
| CG 1374- 2 | 12 | 16 | 1.8 |
| MBra 383 | 11 | 17 | 1.7 |
| MBra 191 | 13 | 14 | 1.6 |
| Average of 33 clones | 9 | 12 | 1.0 |
| LSD (5%) | 1.9 | 2.1 | 0.43 |

[1]Index = [(yield at zero P) × (yield at 75 kg/ha P)]/[(average yield at zero P) × (average yield at 75 kg/ha Pa)].

sections at CIAT conducted large field screening trials at Quilichao station on soils very low in P (<5 mg/kg) where more than 1600 clones of cassava were tested during the past two decades at zero P and 75 kg P/ha in order to identify parental materials that are good yielders at low P while responding to fertilizer.

Table 7 (CIAT, 1990) summarizes results of some of the clones tested where a calculated adaptation index to low P, which takes into account yields of a given clone in relation to the overall mean of the trial at both low and adequate levels of applied P fertilizer, indicates a good level of adaptation when a clone exceeds the overall trial mean (i.e. exceeding 1.0). Out of 33 clones, there were 6 with very high adaptation indices ranging from 1.6 for the Brazilian landrace M Br 191 to 2.0 for the CIAT hybrid CG 996-6, a high yielder at both levels of P. Moreover, the low-P adaptation indices in this group of clones were significantly correlated with standing shoot biomass at harvest, ex-

cluding fallen leaves ($r = 0.58, P<0.001$), with the number of harvested storage roots/plant ($r = 0.67, P < 0.001$), and with seasonal average upper canopy leaf net photosynthesis ($r = 0.51, P < 0.01$). These associations highlight the importance of selections under field conditions for efficient sources of carbon (i.e. high photosynthesis), appropriate canopy architecture using shoot biomass as a proxy for leaf canopy formation, and for root sink strength using number of harvested storage roots/plant as an indicator.

In a separate two-year trial carried out with 4 cultivars on plots adjacent to this large-scale screening trial, detailed crop growth analysis (using bimonthly harvests throughout the entire growth cycle), P uptake and use efficiency and leaf gas exchange characteristics were studied at three different levels of P, but with adequate N and K fertilizers, and were compared with similar values from an unfertilized check plot (Pellet and El-Sharkawy, 1993a, b). All cultivars showed a significant positive response in total and shoot biomass, number of storage roots/plant as well as mean leaf area index to P fertilization, with significant differences among cultivars. Yield also responded to P application, except for one cultivar, M Col 1684. Dry root yield across cultivars and P levels correlated significantly in both growth cycles with seasonal mean LAI values ($r = 0.89, P < 0.001$), with number of storage roots/plant at final harvest ($r = 0.76, P < 0.001$), and with average leaf net photosynthetic rates measured between 2 and 6 months after planting ($r = 0.52, P < 0.001$), but negatively with reproductive organ weight (flowers and fruits) ($r = −0.57, P < 0.001$) (Pellet and El-Sharkawy, 1993a). In all cultivars P uptake increased with P fertilization, and cultivars differences in P uptake were related to differences in fine root length density more than to infection rates with vesi-

cular arbuscular mycorrhiza (VAM). Uptake efficiency (expressed as uptake per unit root length) did not differ among cultivars. Moreover, phosphorus use efficiency (determined as root yield per accumulated P in the whole plant) and patterns of dry matter partitioning to roots and shoots differed significantly among cultivars (Pellet and El-Sharkawy, 1993b). It was concluded that yield response to P level depended on the balance between shoot growth potential of cultivars and storage root sink strength. Moreover, adaptation to low-P soils could be enhanced by selection of clones with high fine-root length density, moderate shoot growth and stable harvest indices.

## Acknowledgements

## References

Allem, A.C. 2002. The origin and taxonomy of cassava. In: R.J. Hillocks, J.M. Thresh and A.C. Belloti (Eds.)Cassava: Biology, Production and Utilization, CABI Publishing, New York, pp. 1–16.

Alves, A.A.C. 2002. Cassava botany and physiology. In: R.J. Hillocks, J.M. Thresh and A.C. Bellotti (Eds.) Cassava: Biology, Production and Utilization. CABI Publishing, New York, pp. 67–89.

Angelov, M.N., Sun, J., Byrd, G.T., Brown, R.H. and Black, C.C. 1993. Novel characteristics of cassava, *Manihot esculenta* Crantz, a reputed $C_3$-$C_4$intermediate photosynthesis species. Photosynth. Res. 38: 61–72.

Aresta, R.B. and Fukai, S. 1984. Effects of solar radiation on growth of cassava (*Manihot esculenta* Crantz). II. Fibrous root length. Field Crops Res. 9: 361–371.

Berg, V.S., El-Sharkawy, M.A., Hernandez, A.D.P. and Cock, J.H. 1986. Leaf orientation and water relations in cassava. Annual Meeting of the American Society of Plant Physiologists, 8–12 June, Louisiana State University, Baton Rouge, LA, p. 186.

Calatayud, P.A., Barón, C.H., Velásquez, J.A., Arroyave, J.A. and Lamaze, T. 2002. Wild *Manihot* species do not possess $C_4$photosynthesis. Ann. Bot. 89: 125–127.

Cayón, M.G., El-Sharkawy, M.A. and Cadavid, L.F. 1997. Leaf gas exchange of cassava as affected by quality of planting material and water stress. Photosynthetica 34: 409–418.

CIAT. 1979. Cassava Program Annual Report for 1978. Centro Internacional de Agricultura Tropical, Cali, Colombia.

CIAT. 1990. Cassava Program Annual Report for 1990. Centro Internacional de Agricultura Tropical, Cali, Colombia.

CIAT. 1993. Cassava Program Annual Report for 1993. Centro Internacional de Agricultura Tropical, Cali, Colombia.

CIAT. 1994. Cassava Program Annual Report for 1994. Centro Internacional de Agricultura Tropical, Cali, Colombia.

CIAT. 1995. Cassava Program Annual Report for 1995. Centro Internacional de Agricultura Tropical, Cali, Colombia.

Cock, J.H. 1984. Cassava. In: P.R. Goldsworthy and N.M. Fisher (Eds.) The Physiology of Tropical Field Crops, Wiley, New York, pp. 529–549.

Cock, J.H., Franklin, D., Sandoval, G. and Juri, P. 1979. The ideal cassava plant for maximum yield. Crop Sci. 19: 271–279.

Cock, J.H., Porto, M.C.M. and El-Sharkawy, M.A. 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, J.H., Rianño, N.M., El-Sharkawy, M.A., Lopez, Y. and 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, D.J. and Cock, J.H. 1981. Response of cassava to water shortage II. Canopy dynamics. Field Crops Res. 4: 285–296.

Connor, D.J., Cock, J.H. and Parra, G.E. 1981. Response of cassava to water shortage. I. Growth and yield. Field Crops Res. 4: 181–200.

De Tafur, S.M., El-Sharkawy, M.A. and Calle, F. 1997. Photosynthesis and yield performance of cassava in seasonally dry and semiarid environments. Photosynthetica 33: 229–257.

de Vries, C.A., Ferwerda, J.D. and Flach, M. 1967. Choice of food crop in relation to actual and potential production in the tropics. Neth. J. Agric. Sci. 19: 241–248.

Edwards, G.E., Sheta, E., Moore, B., Dai, Z., Fransceschi, V.R., Cheng, S.H., Lin, C.H. and Ku, M.S.B. 1990. Photosynthetic characteristics of cassava (*Manihot esculenta* Crantz), a $C_3$ species with chlorenchymatous bundle sheath cells. Plant Cell Physiol. 31: 1199–1206.

El-Sharkawy, M.A. 1993. Drought-tolerant cassava for Africa, Asia, and Latin America: breeding projects work to stabilize productivity without increasing pressures on limited natural resources. Bioscience 43: 441–451.

El-Sharkawy, M.A. and Cadavid, L.F. 2002. Response of cassava to prolonged water stress imposed at different stages of growth. Exp. Agric. 38: 333–350.

El-Sharkawy, M.A. and Cock, J.H. 1984. Water use efficiency of cassava. I. Effects of air humidity and water stress on stomatal conductance and gas exchange. Crop Sci. 24: 297–502.

El-Sharkawy, M.A. and Cock, J.H. 1986. The humidity factor in stomatal control and its effects on crop productivity. In: R. Marcelle, H. Clijsters and M. Van Poucke (Eds.) Biological Control of Photosynthesis, Martinus Nijhoff Publishers, Dordrecht, Netherlands, pp. 187–198.

El-Sharkawy, M.A. and Cock, J.H. 1987a. Response of cassava to water stress. Plant Soil 100: 345–360.

El-Sharkawy, M.A. and Cock, J.H. 1987b. $C_3$–$C_4$ intermediate photosynthetic characteristics of cassava (*Manihot esculenta* Crantz). I. Gas exchange. Photosynth. Res. 12: 219–235.

El-Sharkawy, M.A. and Cock, J.H. 1990. Photosynthesis of cassava (*Manihot esculenta*). Exp. Agric. 26: 325–340.

El-Sharkawy, M.A., Cock, J.H. and De Cadena, G. 1984a. Stomatal characteristics among cassava cultivars and their relation to gas exchange. Exp. Agric. 20: 67–76.

El-Sharkawy, M.A., Cock, J.H. and De Cadena, G. 1984b. Influence of differences in leaf anatomy on net photosynthetic rates of some cultivars of cassava. Photosynth. Res. 5: 235–242.

El-Sharkawy, M.A., Cock, J.H. and Held, K.A.A. 1984c. 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, M.A., Cock, J.H. and Held, K.A.A. 1984d. Photosynthetic response of cassava cultivars (*Manihot esculenta* Crantz) from different habitats to temperature. Photosynth. Res. 5: 243–250.

El-Sharkawy, M.A., Cock, J.H. and Hernandez, A.D.P. 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, M.A., Cock, J.H., Lynam, J.K., Hernandez, A.d.P. and Cadavid, L.F. 1990. Relationships between biomass, root-yield and single-leaf photosynthesis in field-grown cassava. Field Crops Res. 25: 183–201.

El-Sharkawy, M.A., De Tafur, S.M. and Cadavid, L.F. 1992a. Potential photosynthesis of cassava as affected by growth conditions. Crop Sci. 32: 1336–1342.

El-Sharkawy, M.A., Hernandez, A.D.P. and Hershey, C. 1992b. Yield stability of cassava during prolonged mid-season water stress. Exp. Agric. 28:165–174.

El-Sharkawy, M.A., De Tafur, S.M. and Cadavid, L.F. 1993. Photosynthesis of cassava and its relation to crop productivity. Photosynthetica 28: 431–438.

Essers, A.J.A. 1995. Removal of Cyanogens from Cassava Roots: Studies on Domestic Sun-drying and Solid-substrate Fermentation in Rural Africa. PhD thesis, Wageningen Agricultural University, Netherlands.

Fukai, S., Alcoy, A.B., Llamelo, A.B. and Patterson, R.D. 1984. Effects of solar radiation on growth of cassava (*Manihot esculenta* Crantz). I. Canopy development and dry matter growth. Field Crops Res. 9: 347–360.

Gibbons, A. 1990. New view of early Amazonia. Science 248: 1488–1490.

Hershey, C.H. and Jennings, D.L. 1992. Progress in breeding cassava for adaptation to stress. Plant Breed. Abstr. 62: 823–831.

Hibberd, J.M. and Quick, W.P. 2002. Characteristics of $C_4$ photosynthesis in stems and petioles of $C_3$ flowering plants. Nature 415: 451–454.

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

Hozyo, Y., Megawati, M. and Wargiono, J. 1984. Plant production and potential productivity of cassava (*Manihot esculenta* Crantz). In: D. M. Tantera, Subandi, M. Ismunadji, S. Kusumo, J. Sujitno and Ph. Sutjipto (Eds.) Contributions of the Central Research Institute for Food Crops at Bogor, No 73, Central Research Institute for Food Crops, Bogor, Indonesia, pp. 1–20.

Iglesias, C., Hershey, C., Calle, F. and Bolaños, A.1994. Propagating cassava (*Manihot esculenta*) by sexual seed. Exp. Agric. 30: 283–290.

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

Kawano, K., Daza, P., Amaya, A., Rios, M. and Goncalves, W.M.F. 1978. Evaluation of cassava germplasm for productivity. Crop Sci. 18: 377–382.

Keating, B.A. 1981. Environmental Effects on Growth and Development of Cassava (*Manihot esculenta* Crantz) with Special Reference to Photoperiod and Temperature. PhD thesis, Department of Agriculture, University of Queensland, Australia.

Keating, B.A. and Evenson, J.B. 1979. Effect of soil temperature on sprouting and sprout elongation of stem cuttings of cassava. Field Crops Res. 2: 241–252.

Keating, B.A., Wilson, G.L. and Evenson, J.P. 1988. Effects of length, thickness, orientation, and planting density of cassava (*Manihot esculenta* Crantz) planting material on subsequent establishment, growth and yield. E. Afr. Agric. For. J. 53: 145–149.

Laetsch, W.M. 1974. The $C_4$ syndrome: a structural analysis. Annu. Rev. Plant Physiol. 25: 27–52.

Lancaster, P.A. and Brooks, J.E. 1983. Cassava leaves as human food. Econ. Bot. 37: 331–348.

Leihner, D. 1983. Management and Evaluation of Intercropping Systems with Cassava. Centro Internacional de Agricultura Tropical, Cali, Colombia.

Molina, J.L. and El-Sharkawy, M.A. 1995. Increasing crop productivity in cassava by fertilizing production of planting material. Field Crops Res. 44: 151–157.

Oka, M., Limsila, J. and Sarakarn, S. 1987. Relationship between characteristics and germination ability of cuttings in cassava (*Manihot esculenta* Crantz). Jpn. Agric. Res. Q. 21: 70–75.

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

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

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

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

Porto, M.C.M. 1983. Physiological Mechanisms of Drought Tolerance in Cassava (*Manihot esculenta* Crantz). PhD thesis, University of Arizona, USA.

Ramanujam, T. 1990. Effect of moisture stress on photosynthesis and productivity of cassava. Photosynthetica 24: 217–224.

Ravindra, V. 1993. Cassava leaves as animal feed: potential and limitations. J. Sci. Food Agric. 61: 141–150.

Renvoize, B.S. 1972. The area of origin of *Manihot esculenta*. Econ. Bot. 26: 352–360.

Rogers, D.J. and Appan, S.G. 1973. *Manihot* and *Manihotoides* (Euphorbiacea), a computer-assisted study. Flora Newtropica, Monograph 13. Hafner Press, New York.

Tan, S.L. and Cock, J.H. 1979. Branching habit as a yield determinant in cassava. Field Crops Res. 2: 281–289.

Tscherning, K., Leihner, D.E., Hilger, T.H., Müller-Sämann and El-Sharkawy, M.A. 1995. Grass barriers in cassava hillside cultivation: rooting patterns and root growth dynamics. Field Crops Res. 43: 131–140.

Van Oirschot, Q.E.A., O'Brian, G.M., Dufour, D., El-Sharkawy, M.A. and 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, H.J. 1985. Physiological Causes of Yield Variation in Cassava (*Manihot esculenta* Crantz). PhD thesis, Agricultural University, Wageningen, Netherlands.

Ugent, D., Pozorski, S. and Pozorski, T. 1986. Archaeological manioc (*Manihot*) from coastal Peru. Econ. Bot. 40: 78–102.

Yeoh, H.H. and Chew, M.Y. 1976. Protein content and amino acid composition of cassava leaf. Phytochemistry 15: 1597–1599.

Yeoh, H.H. and Truong, V.D. 1996. Protein contents, amino acid compositions and nitrogen-to-protein conversion factors for cassava roots. J. Sci. Food Agric. 70: 51–54.

Yeoh, H.H., Badger, M.R. and Watson, L. 1980. Variation in $K_m$ ($CO_2$) of ribulose-1,5- bisphosphate carboxylase among grasses. Plant Physiol. 66: 1110–1112.

Yeoh, H.H., Badger, M.R. and Watson, L. 1981. Variations in kinetic properties of ribulose-1,5-bisphosphate carboxylase among plants. Plant Physiol. 67: 1151–1155.