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REVIEW

Prospects of photosynthetic research for increasing agricultural productivity, with emphasis on the tropical C_4 *Amaranthus* and the cassava C_3-C_4 crops

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Abstract

Productivity of most improved major food crops showed stagnation in the past decades. As human population is projected to reach 9−10 billion by the end of the 21st century, agricultural productivity must be increased to ensure their demands. Photosynthetic capacity is the basic process underlying primary biological productivity in green plants and enhancing it might lead to increasing potential of the crop yields. Several approaches may improve the photosynthetic capacity, including integrated systems management, in order to close wide gaps between actual farmer's and the optimum obtainable yield. Conventional and molecular genetic improvement to increase leaf net photosynthesis (P_N) are viable approaches, which have been recently shown in few crops. Bioengineering the more efficient C_4 into C_3 system is another ambitious approach that is currently being applied to the C_3 rice crop. Two under-researched, yet old important crops native to the tropic Americas (*i.e.*, the C_4 amaranths and the C_3-C_4 intermediate cassava), have shown high potential P_N, high productivity, high water use efficiency, and tolerance to heat and drought stresses. These physiological traits make them suitable for future agricultural systems, particularly in a globally warming climate. Work on crop canopy photosynthesis included that on flowering genes, which control formation and decline of the canopy photosynthetic activity, have contributed to the climate change research effort. The plant breeders need to select for higher P_N to enhance the yield and crop tolerance to environmental stresses. The plant science instructors, and researchers, for various reasons, need to focus more on tropical species and to use the research, highlighted here, as an example of how to increase their yields.

Additional key words: agricultural sustainability; breeding; C_3, C_4, C_3-C_4 species; canopy; climate change; crop modeling; environmental stress; enzyme; food security; genetic engineering; grain; leaf Kranz anatomy; photorespiration; photosynthetic pathway; protein; starch; storage-root; water; yield.

Introduction

Today the human population exceeds 7 billion and current projections suggest that by the end of the 21st century it might reach 9−10 billion, mostly from increases in developing countries (Lutz *et al.* 2014). In the next 35 years, agricultural productivity will have to increase as much as it has in the past 10,000 years. That is challenging, especially in the context of a changing climate that is going to change what we currently know about agricultural systems and food security. In order to be able to feed the increasing world population with a sufficient supply of

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Abbreviations: APAR − absorbed photosynthetically active radiation; C_a − ambient CO_2 concentration; C_i − intercellular CO_2 concentration; CA − carbonic anhydrase; g_s − stomatal conductance; GDC − glycine decarboxylase; IRGA − infra-red gas analyser; LAI – leaf area/land surface area index; K_m – Michaelis constant; NAD-ME – NAD-malic enzyme; NADP-ME – NADP-malic enzyme; P_N − net photosynthetic rate; PEP − phospho*enol*pyruvate; PEPC − phospho*enol*pyruvate carboxylase; PEPCK − PEP-carboxykinase; PER – protein efficiency ratio; PNUE – photosynthetic nitrogen-use efficiency; PPDK – pyruvate, phosphate dikinase; RUE – radiation-use efficiency; TCA − tricarboxylic acid; V_{max} –maximum carboxylation rate.

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nutritious food, feed, and energy (Sasson 1990), societies must be prepared *via* their various concerned institutions, including governance systems, policy makers, scientists, and the population at large to adjust their way of living in a changing environment within the limitations and boundaries set by the planet Earth, whose carrying capacity is not infinite. Of *ca.* 14–16 billion ha of ice-free land on the Earth, *ca.* 1.3–1.6 billion ha are used for crop cultivation (about 15–18% irrigated, and the rest is rain-fed), and about 3.0–4.0 billion ha are used for pastures and animal feed. Forests constitute about 28–30% of the ice-free land surface (El-Sharkawy 2014). Cropping systems, pastures, and forests account collectively for approximately 50–60% of the Earth's land covers (Houghton 1990, FAO 2007, Tubiello *et al.* 2007). These are precious resources for human needs, and judicious management practices are then required in order to sustain current production levels and ecosystem services, and to prevent resource degradation and losses, as well as to guarantee the needed supplies of food, feed, energy, and ecosystem services in the coming decades. According to FAO (2007), these agro-ecosystems are being progressively degraded either naturally or *via* human activities causing soil losses by desertification, erosion, flooding, and salinization due to heavy irrigation in intensive cultivation systems.

Role of agricultural research and development

The role of agricultural research, and particularly that related to selecting and breeding for improved species and cultivars with higher productivity, nutritional value, and resistance to biotic and abiotic stresses, as well as adapted to available environmental resources and limitations, is of a high priority to meet the increasing demand for food, feed, and energy supply (El-Sharkawy 2005, 2006; George *et al.* 2014). Ecophysiological research of cultivated crops plays a crucial role in this case. When it is conducted within a broader genetic improvement research program, besides increasing the basic and applied knowledge base, physiology may lead to improved crop productivity *via* elucidating mechanisms underlying biomass production and tolerance to stresses, and by identifying useful yield-related traits to be selected for in new cultivars. An illustrative example for that needed physiological research is the innovative approach in studying root development, architecture, anatomy, morphology, and metabolism, subjects that received much less attention compared to other plant organs and traits, particularly in relation to tolerance to drought and soil nutrients acquisition, factors that feedback on photosynthetic efficiency and crop productivity (Lynch 2015).

Prospects of applying photosynthetic research for increasing sustainable agricultural productivity

Until today, increasing primary biological productivity of agro-ecosystems and a potential yield (theoretically estimated yield in optimal conditions) of various crops, beyond the remarkable achievements of the "Green Revolution of 1960's" is still a challenging issue, and awaiting the application of the advances in photosynthetic research (*e.g.*, Stoy 1969, Zelitch 1982, Evans 1993, Fischer *et al.*1998, El-Sharkawy 2006, Zhu *et al.* 2010, Parry *et al.* 2011, El-Sharkawy *et al.* 2012a,b, Reynolds *et al.*2012, Gready *et al.* 2013, Yin and Struik 2015), and it needs to take into account crop responses to elevated carbon dioxide and warming climate, coupled with possible long droughts and soil-nutrient limitations (Kirkham 2011, El-Sharkawy 2014, Lynch 2015).

Yield gaps and their causes: Worldwide, a range of yield gaps has been observed, with farmers' average yield reaching from 20 to 80% of the attainable/exploitable potential yield in almost all crops (Lobell *et al.* 2009, Tittonell and Giller 2013, van Ittersum *et al.* 2013, Fischer *et al.* 2014). The wider gaps are noticeable in developing countries. As an example, the world average cassava root yield is around 4 t(dry mass, DM) ha^{-1} yr^{-1}, while the experimental yield of several improved cultivars grown under near-optimal environmental conditions (rainfall 1,000 mm, daily mean temperature 28ºC, 70% relative humidity, solar radiation 22 MJ m^{-2} day^{-1}) on the fertile soils of the Patia Valley, Cauca, Colombia, S.A., exceeded 20 t(DM) ha^{-1} in 308 d, with one cultivar reaching 27 t ha^{-1} (El-Sharkawy *et al.* 1990, 1993; El-Sharkawy and Cock 1990). Cassava has a high leaf photosynthetic potential approaching values of C$_4$ plants. P_N greater than 40 μmol(CO$_2$) m^{-2} s^{-1} was observed under normal conditions. Seasonal average P_N of upper canopy leaves was associated with biomass and yield, when measured over years, environments, and with widely diverse germplasm (El-Sharkawy *et al.* 1990, 2006, 2010, 2012a,b; de Tafur *et al.* 1997). The main causes of the very low farmer cassava yields, particularly in Africa, are marginal, poor soils, lack of fertilizer application, inadequate crop management, water shortages, and the use of local unimproved cultivars susceptible to pests and diseases (Hershey and Jennings 1992, El-Sharkawy 1993, Fermont 2009, Tittonell and Giller 2013, Rabbi *et al.* 2014). van Ittersum *et al.* (2013) outlined a quantitative methodology for estimating, globally, yield gaps and their underlying causes. Peng *et al.* (2004) observed a decreasing trend in the rice yield due to increased nighttime temperature associated with global warming. They reported that the rice yield from 1992 to 2003, at irrigated experimental farms, declined by 10% for each 1°C increase in growing-season minimum temperature in the dry season. Affholder *et al.* (2013) reported that yield gaps between potentially attainable and nonlimited water yields in the tropics were not due to weather factors, or soil water, but rather due to infertile soils and poor weed management (*e.g.*, suboptimal growth conditions).

Projections of yield increases in order to meet future food needs, *via* present crops, are variable and uncertain (Ray *et al.* 2013). Because of the existence of several biological and physical constraints in most growing regions, closing the yield gap is challenging, unless the potential yield is improved through both genetics and cropping-system management.

Estimates of potential radiation use efficiency (RUE): As a measure of photosynthetic efficiency, Loomis and Amthor (1999) estimated potential (theoretical) radiation use efficiency (RUE, based on absorbed photosynthetically active radiation, APAR, in the 400–700 nm light spectral band) in C_4 maize at about 5.00 g (total biomass) MJ^{-1} (assuming about 16 quanta per mole of CO_2), which corresponds to about 8.5% of APAR [assuming 17 kJ g^{-1}(DM)]. Later, Amthor (2010) calculated newly modified potential theoretical APAR efficiencies, for hypothetical C_3 and C_4 cereal crops, based on 'extant plant metabolic reactions and pathways', at 30ºC and atmospheric 350 μmol(CO_2) mol^{-1}, near 0.092 and 0.114 JJ^{-1}, respectively. However, at 20ºC, the estimated efficiency for C_3 cereal crop increased to 0.118 JJ^{-1}, perhaps due to lesser respiratory costs. He further suggested that field-based data indicated exceptional crops may attain a significant fraction of potential efficiency. From these potential estimates, and in a climate with higher CO_2 than the current concentrations, one may expect that C_3 crops, grown within their favorable thermal range (other factors are constant), may have similar, or even greater RUE efficiencies than C_4 crops, perhaps due to their greater P_N response to CO_2 and to suppressed photorespiration.

Based on aboveground biomass, the maximum observed maize RUE in irrigated field trials (1998–2002, Nebraska, USA) was about 3.8 g(total biomass) MJ^{-1} (about 6.45% of APAR) (Lindquist *et al.* 2005). This maximum value in maize is similar to the observed value in sugar cane (C_4), grown in Australia, for total aerial biomass, including crop trashes (Sinclair and Muchow 1999). Yin and Struik (2015), *via* modeling and theoretical analysis of constraints to the potential efficiency of converting solar radiation into phyto-energy in annual crops, estimated an achievable efficiency of APAR, through future genetic improvement, of 3.6 and 4.1% for C_3 and C_4 crops, respectively. These estimates are greater than commonly attainable efficiencies (based on intercepted PAR) in supposedly well managed C_3 (2.0–2.8%) and C_4 crops (2.8–3.6%) (*e.g.*, Kiniry *et al.* 1989). However, Sinclair and Horie (1989) and Sinclair and Muchow (1999) reported lower RUE values in several C_3 and C_4 crops, which were found to be closely associated with P_N, and with specific leaf nitrogen content. Also, RUE is sensitive to VPD, leaf canopy conductance, water stress, and to diffuse light within canopy.

Maximum measured RUE under optimal controlled conditions in C_3 and C_4 species: Hand *et al.* (1993) reported some of the highest values of light energy conversion efficiency (based on incident PAR) to biomass in cabinet-grown, well-managed plant stands (under 50% of daily solar radiation). The C_3 eggplant (*Solanum melongena* L. var. *esculentum*, aubergine) showed about 10, 13, and 14% efficiency, when grown with 400, 800, and 1,200 μmol(CO_2) mol^{-1}, respectively. The stands' photosynthetic rate greatly increased with elevated CO_2 up to 1,200 μmol(CO_2) mol^{-1}. In contrast, the C_4 grain amaranth (*Amaranthus caudatus* L. var. *edulis*) stands showed much lesser response to elevated CO_2, resulting practically in no change in light energy conversion efficiency with about 10.3–10.4 % at both 400 and 1,200 μmol(CO_2) mol^{-1}. The smaller response to elevated CO_2 in grain amaranth was due to the unique leaf Kranz anatomy (*see* Fig. 1) and the biochemical/biophysical characteristics of the C_4 pathway that concentrates CO_2 at Rubisco, *via* decarboxylation of the C_4 acids in the bundle-sheath chloroplasts, and hence, suppresses photorespiration, as well as enhancing carboxylation reactions (El-Sharkawy 1965, El-Sharkawy and Hesketh 1965, 1986, El-Sharkawy *et al.* 1967,1968; Björkman 1968, Hatch and Slack 1970, Laetsch 1974). Hand *et al.* (1993) attributed the much greater light conversion efficiency values, as compared to those obtained with field-grown crop stands, to the exceptionally favorable environment, with optimal control of CO_2 concentration, humidity, temperature, water supply, and mineral nutrition. This indoor-controlled study indicates the importance of applying improved agronomical practices in the field, beside the genetic factors, for enhancing RUE of present food, feed, and biofuel agro-ecosystems (Hatfield and Walthall 2015). It also points to the possibility of increasing RUE of C_3 crops in expected future climate change, with elevated CO_2 and higher temperatures, provided that adapted/improved cultivars can be developed.

Enhancing photosynthetic capacity through conventional breeding: It appears that improving crop photosynthetic capacity (through both light and dark reactions) using conventional research methods, and hence, greater RUE, is possible. For instance, large gas exchange measurements, by flux towers (aerodynamic method), of major grain legumes stands (soybeans, peanuts, pea, and faba bean) grown at northern America and Europe, illustrated a lower net ecosystem productivity [about 330 g(CO_2) m^{-2}(land surface area) yr^{-1}, as compared to the perennial forage legume, alfalfa, of 980 g(CO_2) m^{-2} yr^{-1}]

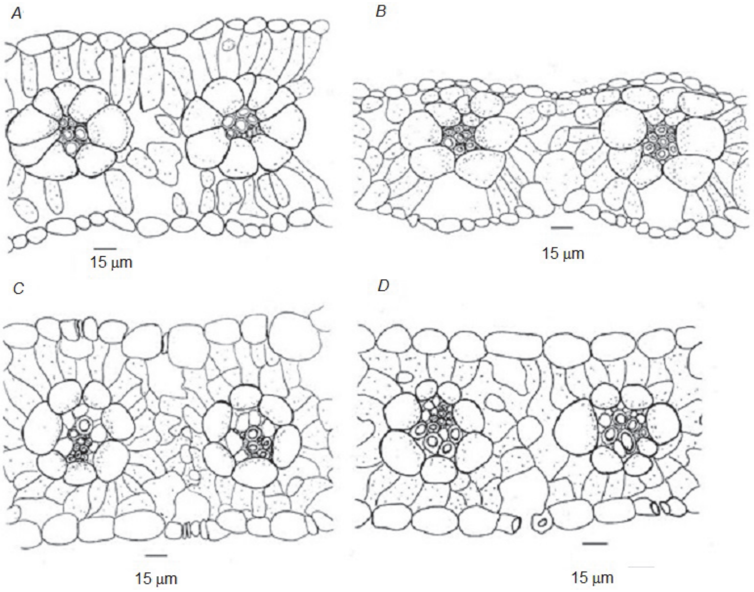

Fig. 1. Camera lucida drawings of transverse sections of leaves. (*A*) Palmer weed (*Amaranthus palmeri*); (*B*) Bermuda grass (*Cynodon dactylon*); (*C*) grain sorghum (*Sorghum bicolor*); (*D*) maize (*Zea mays*). Notice the arrangement of vascular bundles and the large compact and thick-walled cells of the bundle sheath. The black dots in the bundle sheath and the mesophyll cells represent chloroplasts. Most of the chloroplasts in bundle sheath cells are centripetally located (*i.e.*, located at the inner side of the cells). Source: El-Sharkawy (1965) (*see* also El-Sharkawy 2009a,b).

(Gilmanov *et al.* 2014). These findings indicate the moderate photosynthetic CO_2-source capacity in these protein-source crops that needs further improvement in order to cover carbon costs for their biological nitrogen fixation processes, which range, among species and growth conditions, from 2 to 20 g(C) $g^{-1}(N_2$ fixed), and hence, consume ~ 15–25% of crop net photosynthesis (Davis 1980, Phillips 1980, Vance and Heichel 1991, Zahran 1999, Liu *et al.* 2011). Additionally, increasing APAR *via* early rapid leaf canopy development, as emphasized by Milthorpe in the mid-20th century (1956) and Šestak *et al.* (1971), and confirmed by Muramoto *et al.* (1965) *via* growth analysis among several crop species (including C_4 and warm climate C_3 crops), may further increase a yield potential. Longer leaf life (stay green, Borrell *et al.* 2001) and extended growing seasons, are also important factors for increasing crop productivity.

Research at the International Center for Tropical Agriculture, (CIAT), Colombia, showed the close association between productivity and field-measured leaf P_N across large groups of accessions over years and environments. Several selectable traits were identified, including a greater harvest index, long leaf life (leaf retention), stomatal characteristics (amphistomatous leaves), plant architecture (late branching, and medium to short stemmed), extensive fine and deeper root systems, storage root characteristics (a root number per plant), high P_N, and high photosynthetic enzyme activity (both C_3 and C_4 enzymes) (Cock *et al.* 1979, 1987, El-Sharkawy *et al.* 1984b, Cock and El-Sharkawy 1988, El-Sharkawy and Cock 1987a,b, 1990; El-Sharkawy 1993, 2004, 2006, 2010; Pellet and El-Sharkawy 1993, 1994, de Tafur *et al.* 1997, El-Sharkawy *et al* 1990, 1992a,b, 1993, 2008, 2012a,b; Lenis *et al.* 2006, El-Sharkawy and de Tafur 2010). At the International Maize and Wheat Improvement Center (CIMMYT), Mexico, wheat yield progress was associated with stomatal conductance (g_s) and P_N (Fischer *et al.* 1998). The approach used in China for exploring the potential of hybrid rice (exploiting “heterosis” *via* crossing inbred lines), as a mean for the increasing yield, is a good example of innovative breeding research (Cheng *et al.* 2004, 2007; Huang *et al.* 2015). Bhatt and Rao (1981) reported that the P_N of C_3 cotton hybrids (both intraspecific *Gossypium hirsutum* hybrids, and interspecific *G. hirsutum* × *G. barbadense* hybrids) was comparable with those of the C_4 maize and sorghum and much higher than the mean parental rates. These hybrids expressed a high level of heterosis over parents in terms of the seed cotton yield. In the former USSR, Nasyrov (1978, 1981) reported a direct relation between leaf photosynthetic rate and productivity of cotton that was attributed mainly to carboxylation efficiency. Cotton hybrids had higher rates than the parents. In salt-stressed plants, activities of both phospho*enol*pyruvate carboxylase (PEPC) and PEP kinase [this enzyme catalyzes the regeneration of the CO_2 acceptor phospho*enol*pyruvate (PEP), in the C_4 pathway] were greatly enhanced, whereas activity of Rubisco decreased. Nasyrov (1981) believed that the possibility exists to improve photosynthesis genetically by selecting for higher carboxylation efficiency of both Rubisco and PEPC. Future breeding research should focus on selection for both photosynthetic capacity and for a strong sink for photoassimilate accumulation.

Bioengineering C_4 system into C_3 crops: In this direction, an effort (Sheehy *et al.* 2000, 2007) was initiated at the International Rice Research Institute (IRRI), the Philippines, with funding support from the Bill & Melinda Gates Foundation, in order to transform genetically one of the main food crops, the C_3 rice plant, to the more efficient C_4 photosynthetic system. Because of the complexity of the C_4 system (also known as “C_4 syndrome”, Laetsch 1974), and the multitudinous genes controlling C_4 unique leaf anatomy, tissue organization, biophysical, and biochemical characteristics, which are expressed and controlled/regulated in different leaf tissues and cellular organelles (*e.g.*, Nelson and Langdale 1989, 1992; Langdale and Nelson 1991, Sheen 1999, Gowick *et al.* 2011, Sage *et al.* 2012), the C_4-bioengineered rice endeavor might take a long time and large financial cost. For instance, studies with the C_4 grain amaranth suggested that the regulation of genes encoding the C_4 PEPC is complex and could involve the differential expression of divergent PEPC genes or possibly regulation at the post-transcriptional level (Wang *et al.* 1992).

This complexity was further illustrated by the early research conducted by Nobs and Björkman (Björkman *et al.* 1969) with $C_3 \times C_4$ *Atriplex* hybrids, where neither the first filial (F_1) nor the segregating F_2 plants showed a complete transfer of the entirety of the C_4 syndrome (both structure and function). In fact, F_1, F_2, and F_3 plants behaved almost like the C_3 plants in many aspects of their photosynthetic traits compared to the C_4 parent (Björkman *et al.* 1971). Similar results have been obtained recently from studies repeating this earlier research (Oakley *et al.* 2014). Oakley *et al.* (2014) concluded that with the new $C_3 \times C_4$ hybrids in *Atriplex* they have re-established an important system for investigating the genetic control and physiological function of C_4 photosynthesis. In the F_2 lines, they demonstrated a loss of an efficient C_4 function, further supporting the hypothesis that all of the components of the C_4 pathway must be in place for C_4 photosynthesis to occur. Attempts using other wild plant species, to transfer C_4 to C_3 plants through crossing among C_4, C_3, and C_3-C_4 intermediates, such as in the genus *Flaveria*, yielded inconclusive results (*e.g.*, Brown *et al.* 1993).

Nontransformed C_3 species (*i.e.*, lacking the C_4 system) may benefit from the projected rises in atmospheric CO_2, which should lead to suppression of their photorespiration and to enhancement of their P_N, thus closing the existing gap in RUE between C_3 and C_4 systems. In free-air CO_2 enrichment field trials (FACE), Long *et al.* (2006) observed much lesser enhancement of photosynthesis, biomass, and yield, due to elevated CO_2, in C_4 than in C_3 crops. Moreover, Busch *et al.* (2013) reported that wheat and rice leaves possess a continuous chloroplast layer adjacent to cell wall facing mesophyll air spaces, and hence, they re-assimilated ~ 24–38% of respired CO_2 (both from photorespiration and dark respiration in the TCA cycle) at ambient and subambient CO_2 concentration. Because of this trait, P_N was apparently enhanced in wheat and rice. A similar CO_2-reassimilation phenomenon was reported earlier in other C_3 crops, such as sunflower, sugar beet, and cotton, as they reassimilated ~ 40–70% of total respired CO_2 when exposed to CO_2-free air and light (El-Sharkawy *et al.* 1967, also *see* Figs. 2 and 10). This mechanism might be selected for in C_3 species in order to reduce losses of carbon, especially from photorespiration. Also, selection for a lesser oxygenase/carboxylase reaction ratio may improve P_N and biomass in C_3 species (Sage 2013). It appears that the increasing CO_2 concentration at Rubisco site, *via* CO_2 concentrating mechanisms, is a beneficial trait. For instance, in cassava, PEPC activity and P_N were positively correlated with the yield and biomass (Cock *et al.* 1987, El-Sharkawy and Cock 1987a, El-Sharkawy *et al.* 1990, 2008, 2012a,b; El-Sharkawy 2004, 2006). In some C_3-C_4 intermediates having 'Kranz-like' leaf anatomy, 'photosynthetic/photorespiratory C_2-cycle CO_2 pump' was suggested to be instrumental in elevating CO_2 at Rubisco site (Sage *et al.* 2012, Keerberg *et al.* 2014).

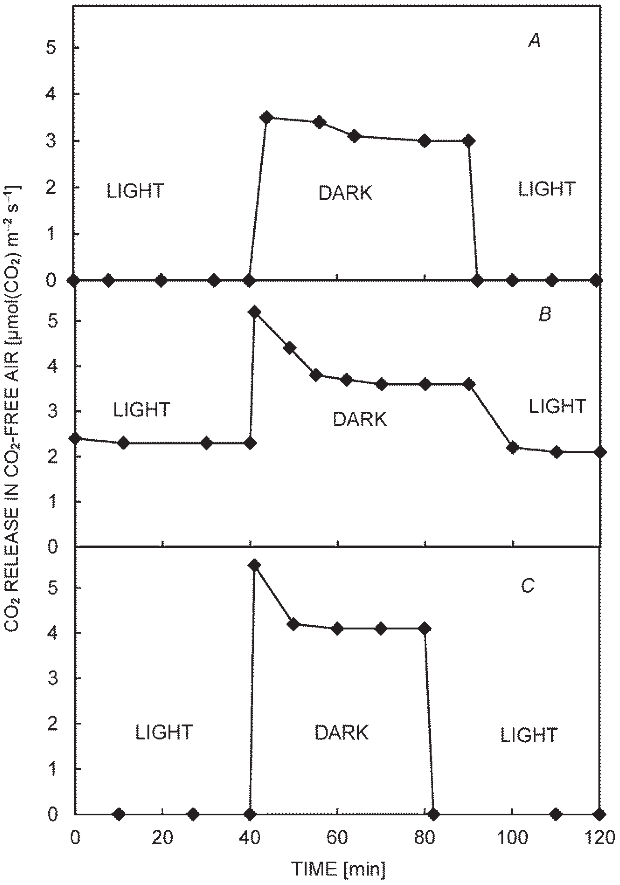

Fig 2. Time course of apparent light and dark respiration in CO_2-free air: *Zea mays* (*A*), *Helianthus annuus* (*B*), *Amaranthus edulis* (*C*). Zero time designates the change from normal air containing 310 μmol(CO_2) mol^{-1} to CO_2-free air. Note: the difference between CO_2 releases in light and dark was attributed to reassimilation/recycling, which was 100% in C_4 maize and grain amaranth, and around 45–50% in C_3 sunflower. Also note the CO_2 surge after shifting to darkness, which was more pronounced in sunflower (C_3), and in amaranthus (C_4) than in maize (C_4). Source: adapted from El-Sharkawy *et. al.* 1967.

Photosynthesis of ancient crops

Photosynthetic traits and discoveries made in two food, feed, and energy crops, *i.e.*, *Amaranthus* spp. (Amaranthaceae), a C_4 species, and *Manihot* spp. (Euphorbiaceae), a C_3-C_4 intermediate species, which have been neglected until recently, are discussed in the following sections. Both are under-researched crops (*see* Sauer 1950, NRC 1984, 1989, Tucker 1986, Baker 2006, Connor *et al.* 2011) despite their domestication and cultivation for millennia by the natives (Aztecs, Incas, and Mayas) of the New World. Most of the world now receives the bulk of its calories and proteins from a mere 20 species, notably cereals, such as wheat, rice, maize, millets, and sorghum, root crops, such as potato, sweet potato, and cassava, legumes, such as beans, peanuts (groundnuts), and soybeans, and sugarcane, sugar beet, and bananas (NRC 1984). To diversify the food base, we should not overlook lesser-known indigenous crops, such as amaranth. Some of them promise to become global resources. Most of the indigenous crops were domesticated, cultivated, and developed by ancient Indian people before the Inca Empire 1,400 BC, who had widely spread these crops in the Andean region (NRC 1989). For religious reasons, the Spanish conquest eliminated grain amaranth cultivation and its use as a food, despite its nutritious quality. Currently, it is the emerging crop that produces a reasonable seed yield (the yield ranged from 1.5 to 6 t ha^{-1} in 4–5 months, and the the higher yield occurs in low-land hot tropics with annual precipitation from 600–800 mm), having high quality proteins with balanced amino acids, high lysine, and sulfur-containing amino acids contents (NRC 1984, 1989, Downton 1973, Connor *et al.* 1980). Since 1976, Rodale Research Center (RRC), Pennsylvania, USA, gathered more than 1,000 accessions of amaranths from different countries. Breeding and agronomic research at RRC successfully developed new cultivars suitable for mechanized farming systems with desired traits, such as a short-stem, not lodging, large non-shattering seeds, plant height uniformity, and high yield.

Amaranthus species (C_4)

Discovery of the weedy C_4 amaranth (*Amaranthus palmeri*): P_N of a leaf taken from a weed amaranth that was growing in cotton-field plots was greater than that of many of the cotton leaves (of both *G. hirsutum* and *G. barbadense*) that researchers had just measured that day by an infra-red gas analyzer, housed in a nearby air-conditioned trailer at Campbell Avenue Farm, the University of Arizona, Tucson, USA (*e.g.*, El-Sharkawy 1965, 2009a,b; El-Sharkawy and Hesketh 1965, 1986). Its P_N light-response curve never reached saturation up to full sunlight (around 2,000 μmol m^{-2} s^{-1} PPFD), with a maximum P_N greater than 40 μmol(CO_2) m^{-2} s^{-1} in normal air containing 300 μmol(CO_2) mol^{-1}. Leaf sections for laboratory microscopic observations proved that the weed amaranth possessed “Kranz anatomy” (Fig. 1, El-Sharkawy 1965, 2009a,b; El-Sharkawy and Hesketh 1965, 1986). This was the first dicotyledonous species found to possess the Kranz anatomy and P_N similar to those of efficient C_4 tropical grasses, such as maize, grain sorghum, sugar cane, Bermuda grass, Sudan grass, and elephant grass (El-Sharkawy and Hesketh 1964, 1965, 1986). These ground-breaking discoveries facilitated the consequent research that established biochemical details of the C_4 system (Hatch *et al.* 1967, Hatch and Slack 1970, Laetsch 1974, *see* also Berry 2012, Lundgren *et al.* 2014).

The C_4 weedy amaranths belong among the most aggressive and competitive plants (Ehleringer 1983, Webster 2006, Schonbeck 2014). Elmore and Paul (1983) published a list of C_4 weeds, including 11 species of the genus *Amaranthus*, indicating their competitive behavior and adaptability. Because of their high P_N, rapid growth rate, large biomass production, huge quantity of small seeds, acquisition and accumulation of nutrients, particularly nitrogen, in their shoots, tolerance to heat stress, tolerance to salt and drought, some weedy C_4 amaranths might become invasive in a globally warming climate, and hence, compete for resources with cultivated crops (*e.g.*, Qin *et al.* 2013, Ziska and Dukes 2014). For instance, the green pigweed, *A. powellii*, similarly as *A. palmeri* and *A. retroflexus*, responded greatly to nitrate application. When measured at 1945 μmol(CO_2) mol^{-1} and 20% O_2, intense light, and 35ºC of leaf temperature, P_N reached a maximum at 60 μmol(CO_2) m^{-2} s^{-1} with high nitrate, compared to 10 μmol(CO_2) m^{-2} s^{-1} with low nitrate. The photosynthetic quantum yield (based on APAR), and the saturating PPFD increased with increasing nitrate applied. The same trend was observed in shoot mass, stomatal density, leaf area, specific leaf mass, and relative leaf growth rate (Hunt *et al.*1985). El-Sharkawy *et al.* (1968) reported a much lower P_N in old leaves, compared with young fully expanded ones, in grain amaranth, *A. caudatus*. var. *edulis*, and a lower saturating PPFD, presumably due to a lower nitrogen content in the old leaves. The same trend was observed with shade-grown leaves. The old leaves, and the shade-grown young leaves, showed measurable releases of CO_2 in a stream of CO_2-free air in light, indicating the presence of photorespiration, although small, which was apparently absent in the young leaves grown under high irradiance and with high nitrogen content. These findings might suggest that, in the old leaves, shaded leaves, and in low-nitrate leaves, the carboxylation activity of Rubisco in the chloroplasts of bundle sheath cells was reduced and, hence, the photorespiratory and dark respiratory CO_2 leaked out before being recycled back *via* photosynthesis. Kiirats *et al.* (2002) reported that leakiness (estimated as a ratio φ = CO_2 leak rate out of the bundle sheath/rate of CO_2 supply by C_4 cycle) can be substantial in *Amaranthus* spp. (~ 20-30%), depending on alteration in the bundle sheath cells conductance and on their CO_2 concentrations.

Sage and Seemann (1993) reported that in *A. retroflexus* the activity ratio of Rubisco (*i.e.*, carboxylase/oxygenase activity) was regulated by light intensity and declined dramatically below 500 μmol m^{-2} s^{-1} PPFD. However, in *Z. mays*, the Rubisco activity ratio was relatively insensitive to light intensity. Sawada *et al.* (2002), using single-rooted leaves of *A. cruentus*, reported a significant decreases in P_N and in Rubisco activity, which were attributed to feedback inhibition by sucrose accumulation, and hence, indicating the importance of a source-sink relationship in regulating P_N and the involved photosynthetic enzymes in amaranthus. Castrillo *et al.* (1997) used *in situ* immunofluorescent localization of Rubisco in amaranthus leaf tissues. The enzyme was apparently detected in the mesophyll cells of *A. caudatus* var. *edulis*, and in *A. dubius*, indicating incomplete compartmentalization of Rubisco in the bundle sheath cells. Hong *et al.* (2005), by using the immunogold-labeling technique in the leaf tissues of *A. tricolor*, reported that both Rubisco and Rubisco activase were localized in mesophyll cells as well as in outer vascular bundle sheath cells. This information suggests that the genes controlling amaranth Rubisco may be partially expressed in the mesophyll tissue. Amaranthaceae (including Chenopodiaceae) contains the majority of known C_4 dicot species. To my knowledge, these amaranth species are all C_4, as the genus *Amaranthu*s (containing about 60 species) has not been shown, so far, to include C_3 or C_3-C_4 intermediate species (*e.g.*, Sage *et al.* 2007).

Characteristics of Rubisco in amaranth leaves: Wu *et al.* (1990) studied purified Rubisco, isolated from C_4 *A. hypochondriacus* leaves, and compared its kinetics with the C_3 wheat Rubisco. The enzyme characteristics in amaranth (A) and in wheat (W) were for carboxylase reaction: K_{mCO2} of 9.5 (A) and 15.5 μmol L^{-1} (W); K_{mRuBP} of 25 (A) and 35 μmol L^{-1} (W); V_{max} of 1.02 (A) and 0.68 μmol(CO_2) min^{-1} mg^{-1} (W); for oxygenase reaction: K_{mRuBP} of 42 (A) and 33 μmol L^{-1} (W), V_{max} of 0.136 (A) and 0.230 μmol(O_2) min^{-1} mg^{-1} (W). It is apparent that there are striking differences between Rubisco from C_4 amaranth and C_3 wheat. The amaranth enzyme had higher affinity to CO_2, and greater carboxylation reaction activity than that from wheat, based on their K_m and V_{max} values, respectively. With regard to the enzyme oxygenase reaction, Rubisco in amaranth shows lower affinity to O_2, and lower oxygenase reaction activity, based on their respective K_m and V_{max} values. The oxygenase reaction results in losses of CO_2 in photorespiration, and hence, reduces the net carbon gain, which is much greater in C_3 than that of C_4 systems. Tolbert *et al.* (1969) and Tolbert (1971) reported that the enzymes required for the operation of the two-carbon (C_2) photorespiratory/photosynthesis cycle are present in the subcellular organelles of *A. hybridus* leaves. Thus, the photorespiration phenomena exists in C_4 species, albeit at a much lower rate than that in C_3 species (*e.g.*, Jackson and Volk 1969, 1970; Volk and Jackson 1972, Döring *et al.* 2016). Döring *et al.* (2016) reported that in the C_4 *Sorghum bicolor*, with NADP-dependent malic enzyme sub-pathway, the majority of photorespiratory gene expression, with some important exceptions, is restricted to the bundle sheath cells.

Translating these contrasting biochemical characteristics between these C_3 and C_4 species with respect to their photosynthetic pathways and leaf P_N, the superiority of the amaranth´s photosynthetic system becomes evident. Besides, amaranth is endowed with the C_4 Kranz anatomy and with the biochemical components of the C_4 system. These traits assist in fixing CO_2 rapidly in mesophyll cells *via* PEPC, at much lower g_s, and hence, lower internal CO_2 concentration, with the resulting dicarboxylic C_4 acids being mass-transported into the inner bundle sheath cells. By decarboxylating the C_4 acids, the liberated CO_2 becomes concentrated, to a greater concentration than that in the C_3 system at Rubisco sites in the bundle sheath chloroplast stroma. With higher concentrations of CO_2, Rubisco in C_4 leaves has greater activity, and its kinetics are far superior and more efficient, compared to those in the C_3 wheat enzyme. Taking into account that Rubisco constitutes ~ 45–55% of the leaf soluble protein in C_3 leaves, and ~ 25–30% in C_4 leaves, the photosynthetic nitrogen use efficiency (*i.e.*, CO_2 uptake per unit of nitrogen) of amaranth is far above that of wheat (*e.g.*, Brown 1978, Sage and Pearcy 1987, Oaks 1994, El-Sharkawy 2009a,b). Water use efficiency, both at the leaf level and the crop level, are greater in amaranth than in wheat (*e.g.*, Briggs and Shantz 1914, Shantz and Piemeisel 1927, El-Sharkawy 2009a,b). Under field rain-fed conditions at Prosper, North Dakota, USA, Johnson and Henderson (2002) reported a crop transpiration ratio of 314 kg(H_2O) kg^{-1}(DM of total biomass) in grain amaranth (*A. caudatus var. edulis*) during four seasons, which was lower than the range commonly observed in C_3 crops (from 500–900), including wheat. In comparative studies among several weedy and cultivated amaranths, dry seed yields ranged from 0.7 to 3 t ha^{-1}, with the lowest yield observed without watering and no fertilization (Hauptli and Jain 1978). These traits attest to the potential and suitability of grain amaranth (*e.g.*, "pseudocereal") to enhance food security, particularly, in drier eco-zones, where the crop can survive and produce at low precipitation of 200 mm.

C_4 enzymes: Berry *et al.* (1997), working with the grain amaranth, *A. hypochondriacus*, reported that Rubisco, and C_4 PEPC, and pyruvate, phosphate dikinase (PPDK) mRNAs are abundant in meristems and in leaf primordia, but are utilized only during specific developmental stages. This might suggest that at the certain stages of leaf development, the C_4 genes may not be expressed. According to these authors, the C_4 genes are independently regulated by multiple control mechanisms in response to developmental, environmental, and metabolic signals. Tazoe *et al.* (2006) grew *A. cruentus* under different light and nitrogen regimes. On one hand, nitrogen allocation to Rubisco was greater in leaves grown under high light and high nitrogen than in other leaves grown with low light and low nitrogen. On the other hand, the C_4 enzymes were not affected by light nor by nitrogen supply. Carbon isotope ratios, $\delta^{13}C$, in the leaf dry matter were more negative (−19.3% ‰) in the low-light leaves than that in the high-light ones (−16.0 ‰), regardless of nitrogen supply. This might suggest that changes occurred in the ratio of Rubisco carboxylase/oxygenase activity, as it was observed earlier in *A. retroflexus* by Sage and Seemann (1993), and hence, photorespiration must had increased in the leaves with more negative $\delta^{13}C$. Tazoe *et al.* (2006) concluded that *A. cruentus* leaves do not acclimate efficiently to low growth light. This conclusion may further substantiate the great reduction in P_N of both old leaves grown under high light, but shaded by upper leaves, and in young shade-grown leaves, with noticeable increase in CO_2 releases in CO_2-free air and light, which was reported earlier in *A. caudatus* var. *edulis* by El-Sharkawy *et al.* (1968), and in Fig. 4. Moreover, Lester and Goldsworthy (1973) observed high CO_2 compensation concentration in glass-house (shade conditions) grown *A. caudatus* var. *edulis*.

When grown under low CO_2, sodium-deficient *A. tricolor* plants behaved, in some aspects, like C_3, presumably due to impairment of the C_4 pathway. In that case, a greater proportion of CO_2 that was fixed by Rubisco in the bundle sheath cells might have entered *via* direct diffusion (*i.e.*, slow process), rather than being fixed first by PEPC in the mesophyll cells, to form C_4 dicarboxylic acids, which can be mass-transported (*i.e.*, rapid process) into the bundle sheath cells (Johnston *et al.* 1984). Sodium is necessary for the C_4 shunt to operate properly (Brownell and Crossland 1972) and its deficiency reduces both P_N and plant growth in C_4 species. This behavior may point to "leakiness" in the bundle sheath cells of *Amaranthus* species. Pearcy and Ehleringer (1984) emphasized the phenomena of CO_2 leakage out of the bundle sheath cells, which is greater in NAD-dependent malic enzyme (NAD-ME) and PEPCK C_4-subpathway types than in the NADP-dependent malic enzyme (NADP-ME) subpathway type, and is greater in dicots than in monocots. The genus *Amaranthus* is the NAD-ME subpathway type, whose photosynthetic system requires higher concentrations of manganese to function properly compared to other C_4 subpathways (Kering 2008).

Quality of amaranth as a future food crop: The amaranth edible seeds, commonly known as grains (with different colors), have a greater protein content (about 16–18%), lack functional gluten, and are low in leucine amino acid, with more balanced amino acids and higher lysine and sulfur-containing amino acids than other cereals crops (Downton 1973, Connor *et al.* 1980, NRC 1984, 1989). On dry mass basis, starch ranges from 50 to 70% in seeds and consists of small grains easily degradable. Fats range from 6 to 8%, crude fibers from 3 to 5%, and ash is about 3%. Also, the nutritional value of amaranth protein is high, with protein efficiency ratio (PER) ranging from 1.5 to 2.0 (casein has 2.5 PER). Amaranth protein has a biological value at 75 (on a zero to 100 scale), compared with maize 44, wheat 60, soybean 68, and cow milk 72 (NRC 1984). In the near future, grain amaranth and its other food crop relatives, collectively called "pseudocereals", such as the Incas quinoa (*Chenopodium quinoa*), also a C_4 species, should receive the same attention as that received by wheat and other traditional cereals and grain legumes. In a global warming climate, amaranth may have a better chance than many other food, feed, and energy crops. The first reported genome and transcriptome analysis of *A. hypochondriacus* has been recently published (Sunhil *et al.* 2014). The estimated genome size is around 466 Mb, which might facilitate research and development of grain amaranths. NRC (1984) states that it seems likely that this ancient grain of the Americas will return to grace modern age. Eventually, it may prove to be as rich a legacy of the American Indian as maize and beans. After four centuries of obscurity, the former Aztec crop offers modern farmers a promising alternative (Tucker 1986).

Discovery of the cultivated C_4 grain amaranth (*A. caudatus* var. *edulis*) and the weedy red-root C_4 amaranth (*A. retroflexus*): Another two amaranth species, *A. edulis* (later renamed *A. caudatus* var. *edulis*), a cultivated grain amaranth, and the red-root pigweed, *A. retroflexus*, show also high P_N (>40 $\mu mol(CO_2)\ m^{-2}\ s^{-1}$) in air containing 300 $\mu mol(CO_2)\ mol^{-1}$, a zero CO_2 compensation point, a high optimum temperature around 40°C, no light saturation, and no apparent release of respiratory CO_2 over a range of irradiances (Fig. 2*C*, El-Sharkawy *et al.* 1967, 1968). The grain amaranth (*A. caudatus* var. *edulis*) is native to Peru and other Andean countries. The seeds used in our research were from a population originally obtained from Salta, Argentina, where the species is cultivated (El-Sharkawy *et al.* 1968), and it had been cultivated for food by the natives of the New World (*i.e.*, the Aztecs and the Incas) before the time of Cortez. Another two C_4 grain amaranth species, *A. hypochondriacus* and *A. cruentus*, are native to Mexico and Guatemala, respectively, and had been cultivated for millennia by the natives (*e.g.*, Sauer 1950, 1967, NRC 1984, 1989). All three grain amaranth species are still cultivated, though on a small scale, in mountain valleys of Mexico, Central America, and South America. Several other leafy and protein-rich *Amaranthus* species, such as *A. tricolor*, are cultivated in humid tropical and subtropical

Africa, the Caribbean, South Asia, and southern China for vegetable soup and potherbs (*e.g.*, Lin and Ehleringer 1983). When grown during the warm summer (planted in June-July) in southeastern USA, P_N of *A. tricolor* reached maximum value, and the crop produced the good leaf yield (Whitehead *et al.* 2002). In the North American desert, the native "Amerindians" harvest a wild-grown "palmer weed" (*A. palmeri*) for their consumption, when the weather is too hot and dry for other vegetables to grow. During one of my early trips (1971-1973) to some central African countries, during a severe, prolonged drought period, young amaranth plants were the only green vegetable available at local food markets, where they were grown in small plots or in "house gardens", indicating their tolerance to hot dry weather. Some amaranths can also grow in cooler, tropical highlands at elevations greater than 3,500 m (*e.g.*, the Andean mountains, China, India, Nepal, Pakistan, and Tibet), but they require a longer growth period and show reduced productivity. In Europe, there is ongoing research on amaranths for food, feed, and energy. By exploiting heterosis, interspecific hybridization among *Amaranthus* spp. might be a promising breeding approach to increase biomass productivity, particularly in dry and hot environments (Lehmann 1989).

Responses of P_N in *A. caudatus* var. *edulis* to light, CO$_2$, and temperature: Fig. 3 illustrates responses of P_N to light and CO$_2$ concentration in fully expanded young leaves from indoor-grown plants in normal air containing 310 µmol(CO$_2$) mol^{-1}, as measured with an IRGA in an open-end mode, using humidified air of constant CO$_2$ concentration from gas cylinders (El-Sharkawy *et al.* 1968). In CO$_2$-free air, and over a range of light intensities, and leaf temperature around 38ºC, P_N was zero with no CO$_2$ releases (*i.e.*, no measurable apparent photorespiration, Fig. 3*A*), despite using a high flow rate (inside leaf chamber, wind speed was around 1.5 cm s^{-1} to ensure that boundary layer resistance was minimal, conditions that allow open stomata and facilitate rapid gas exchange between leaf and surrounding air). On one hand, P_N, at 50 and 180 µmol(CO$_2$) mol^{-1}, reached saturation at about 200 and 600 µmol m^{-2} s^{-1} PPFD. On the other hand, P_N at 310 and 500 µmol(CO$_2$) mol^{-1} showed increasing response up to 1,500 µmol m^{-2} s^{-1} PPFD (the maximum irradiance provided by light sources [six 300 W incandescent lamps, immersed in cooled water] in the gas-exchange laboratory experimental set up). However, at PPFD greater than 400 µmol m^{-2} s^{-1}, P_N rates were substantially greater with 500 than with 310 µmol(CO$_2$) mol^{-1}, which defy the notion that C$_4$ system is not responsive to CO$_2$ enrichment, at least in some C$_4$ species in short-time measuring studies. This conclusion was substantiated by Hand *et al.* (1993), who later used the same grain amaranth species and variety in their long-term CO$_2$ enriched cabinet-grown plant stands [P_N was greater at 1,200 than with 400 µmol(CO$_2$) mol^{-1}], although the percentage enhancement was lesser than that in C$_3$ eggplant. In contrast to indoor-grown grain amaranth, in field-grown *A. palmeri*, P_N showed no saturation, with greater rate, up to full sunlight (2000 µmol m^{-2} s^{-1} PPFD), and in normal air containing 300 µmol(CO$_2$) mol^{-1} in a sunny hot summer in Tucson, Arizona (El-Sharkawy 1965). These data support the theory that the C$_4$ system in terrestrial plants evolved from the much older, and more common, C$_3$ system during millions of years in response to a decreasing atmospheric CO$_2$, perhaps coupled with high temperature and drought.

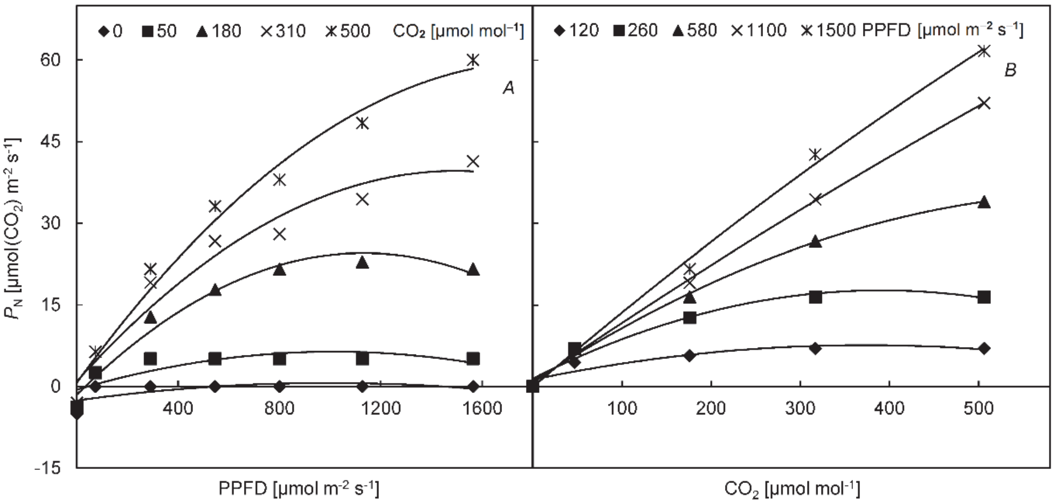

Fig. 3. Net photosynthetic rate (P_N) in recently expanded leaves of *Amaranthus edulis* as functions of PPFD and [CO$_2$] at 38ºC. Plants were cabinet-grown at 30ºC with 700–800 µmol m^{-2} s^{-1} PPFD and at 22ºC during dark period. Note: the P_N response to elevated CO$_2$. Source: adapted from El–Sharkawy *et. al.* (1968).

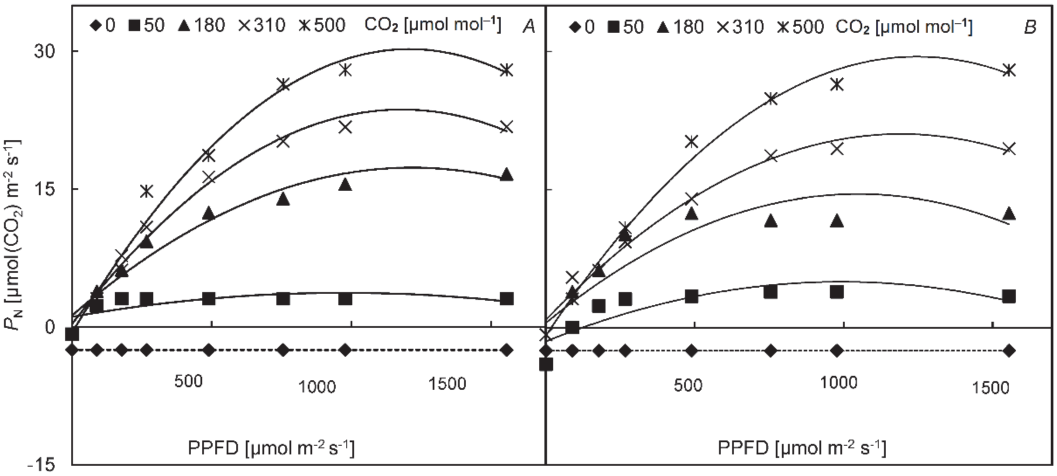

Fig. 4. Net photosynthetic rate (P_N) of *Amaranthus edulis* as a function of PPFD at different CO_2 concentration. (*A*) Old leaves (4 week old) of plants grown during June–August 1966 inside glasshouse at 1,700 µmol m^{-2} s^{-1} PPFD at solar noon; (*B*) Recently expanded leaves of shaded plants [plants were grown under the same conditions as (*A*) but shaded to near 460 µmol m^{-2} s^{-1} PPFD at solar noon]. Note: Dashed lines illustrate photorespiration at zero CO_2 [~ 2.5 µmol(CO_2) m^{-2} s^{-1}). Compare Fig. 4 with Fig. 3. Source: adapted from El - Sharkawy *el al*. (1968).

Fig. 3*B* illustrates P_N as a function of CO_2 concentration at different light intensities. As P_N increased with irradiance, the response to CO_2 became greater and more linear, with gradual elimination of P_N saturation with increasing irradiance. This response indicates that at the irradiance lesser than 50% of full sunlight, the P_N of amaranths is influenced significantly by the surrounding CO_2 supply.

Effects of leaf age and shading on P_N: Fig. 4*A* illustrates P_N in old leaves (four-week old) of grain amaranth, as a function of irradiance level at different concentrations of CO_2 for glasshouse-grown plants from June through August 1966 with solar noon irradiance around 1,600–1,800 µmol m^{-2} s^{-1} PPFD (*see*, El-Sharkawy *et al*. 1968). Compared to recently expanded young leaves (two-week old, Fig. 3*A*), P_N in the old leaves were much lower at all CO_2 concentrations, particularly under higher PPDF. The old leaves released measurable amounts of CO_2 [~ 2.5 µmol(CO_2) m^{-2} s^{-1}] into CO_2-free air at all PPDF levels (*see* the dashed line marking P_N = zero in Fig. 4*A*). Also, at 310 and 500 µmol(CO_2) mol^{-1}, P_N reached saturation at 1,000 µmol m^{-2} s^{-1} PPFD. It was apparent that leaf age significantly affected both P_N and respiration (in light and dark). It was concluded that since the effects are distinct in strong illumination only, variations in dark reactions (including carboxylation) may be responsible for such differences. It appears significant that respiratory rates in light and CO_2-free air are negatively associated with maximum rates of CO_2 assimilation in normal air (El-Sharkawy *et al*. 1968). Apparently, in the old leaves, the ratio of the carboxylase/oxygenase activity of Rubisco was reduced in this case, and hence, photorespiration was measurable due to lack of refixation of respired CO_2 (in contrast to the young leaves, Fig. 3*A*, and also El-Sharkawy *et al*. 1967), either inside the bundle sheath cells by Rubisco and/or by PEPC in the mesophyll cells. It is noteworthy to indicate that this research was conducted before the biochemical roles of the C_4 pathway enzymes (*e.g.*, Hatch *et al*. 1967, Hatch and Slack 1970, Jackson and Volk 1970, Laetsch 1974), and the dual role of Rubisco (*see* Bowes *et al*. 1971, Ogren 1984) were identified.

Fig. 4*B* illustrates P_N as a function of irradiance and CO_2 concentrations in fully expanded young leaves from glasshouse-grown plants under shading conditions for several weeks (PPFD inside the glasshouse at solar noon was around 460 µmol m^{-2} s^{-1}) (El-Sharkawy *et al*. 1968). As in the old leaves of plants grown under high irradiance, P_N in the shaded young leaves was much lesser, at all CO_2 concentrations, than that obtained with recently expanded young leaves of unshaded plants (*see* Fig. 3*A*). The effects of shading were greater at high measured irradiances at all CO_2 concentrations. At all irradiance levels, the shaded leaves released measurable respiratory CO_2 [~ 2.5 µmol (CO_2) m^{-2} s^{-1}] into CO_2-free air (*see* the dashed line marking P_N = zero, Fig. 4*B*). At 310 and 500 µmol(CO_2) mol^{-1}, the P_N of the shaded leaves was saturated at less than 1,000 µmol m^{-2} s^{-1} PPFD, similarly to the old leaves in the unshaded plants (Fig. 4*A*), and in contrast to the unshaded fully expanded young leaves (Fig. 3*A*). As discussed above with the old leaves, the smaller P_N and the apparent releases of respiratory CO_2 into CO_2-free air and light in young shaded leaves might have been a result of a reduced ratio of carboxylase/oxygenase activity of amaranth Rubisco. Also, it is known that plants grown under high irradiance possess higher photosynthetic capacity, greater leaf mass per unit of leaf area, and a higher exposed mesophyll surface area per volume than shade-grown plants (Poorter *et al*. 2009). These anatomical traits may affect the path of CO_2 diffusion within leaves, and hence, they affect P_N. El-Sharkawy and Hesketh (1965) found a positive relationship between P_N and the exposed mesophyll surface area per volume of mesophyll cells among different species of C_3 and C_4 pathways.

The effect of 'glasshouse phenomena': Reduced P_N in plants that were grown under low light intensity, hence, shade conditions, was previously observed in C_3 species, such as sunflower and cotton; both are warm climate crops (El-Sharkawy and Hesketh 1964, El-Sharkawy *et al.* 1965). The P_N in these species, when grown in glasshouses, was almost 50% the values observed in plants grown outdoors at higher light intensity. Light saturation of glasshouse-grown plants was reached at less than 1,000 μmol m^{-2} s^{-1} PPFD, whereas outdoor-grown plants never reached saturation at >1,800 μmol m^{-2} s^{-1} PPFD (*e.g.*, El-Sharkawy *et al.* 1965). These findings, and those of others (*e.g.*, Bohning and Burnside 1956, Burnside and Bohning 1957, Boardman 1977) changed the prevailing paradigms, at the time, that P_N in terrestrial plants reached light saturation at less than 50% of full sunlight. Most earlier photosynthetic research was conducted with indoor-grown plants, and hence, maximum P_N was much lower and light saturation was also much lower than that in sun-grown plants. Unless indoor research is calibrated under natural conditions outdoors, or under intense light, the findings have a limited value, particularly, when interpreted in relation to the real P_N potential, and when they are used in crop modeling (*e.g.*, El-Sharkawy 2005, Long *et al.* 2006). With the advent of modern and easy to manage photosynthetic measurement methodology and equipment, photosynthetic researchers should avoid conducting their studies under artificial and inadequate growing conditions.

Response of P_N in fully expanded leaves to temperature in interaction with light and CO_2 concentration is shown in Fig. 5*A,* which presents effects of leaf temperature on P_N in air containing 310 μmol(CO_2) mol^{-1} at different irradiances. At low PPFD (110 μmol m^{-2} s^{-1}), P_N remained unchanged over a wide range of temperatures (10–35ºC). In contrast, P_N increased gradually with increasing temperature at 280 and rapidly at 1,400 μmol m^{-2} s^{-1} PPFD, respectively. The optimum temperature at which P_N reached a maximum value, shifted from 35 to 40ºC, at 280 and 1,400 μmol m^{-2} s^{-1} PPDF, respectively. The temperature coefficients (Q_{10}) for P_N were 1.0, 1.3, and 2.0, at PPFD of 110, 280, and 1,400 μmol m^{-2} s^{-1}, respectively.

The interaction between the effects of leaf temperature and CO_2 concentration, at 1,500 μmol m^{-2} s^{-1} PPDF, on P_N are illustrated in Fig. 5*B*. At all CO_2 concentrations, P_N increased with rising temperature from 10 to 40ºC, then declined rapidly, but remained positive at 50ºC. The decline in P_N at temperatures higher than 40ºC might indicate inactivation in photosynthetic enzymes as well as increased resistance to CO_2 diffusion within the leaf (El-Sharkawy *et al.* 1968). Over the temperature range tested, P_N at 500 was greater than that in 180 and 310 μmol(CO_2) mol^{-1}, suggesting that amaranthus responded to elevated CO_2. Therefore, these data might demonstrate amaranthus' tolerance to global warming phenomena. Moreover, the apparent upward shift in optimum temperature for maximum P_N at elevated CO_2 supports this conclusion.

In conclusion, it may be said that amaranth species are endowed with several desirable physiological, anatomical, and biochemical traits, including high P_N equivalent to values in tropical C_4 grasses, such as maize, sorghum, and sugarcane, all of which point to a brighter future as a potential food, feed, and energy crop. Furthermore, the edible grain amaranth has the higher protein content compared to true cereals (*e.g.*, maize, wheat, rice), with well-balanced amino acid components (Connor *et al.* 1980).

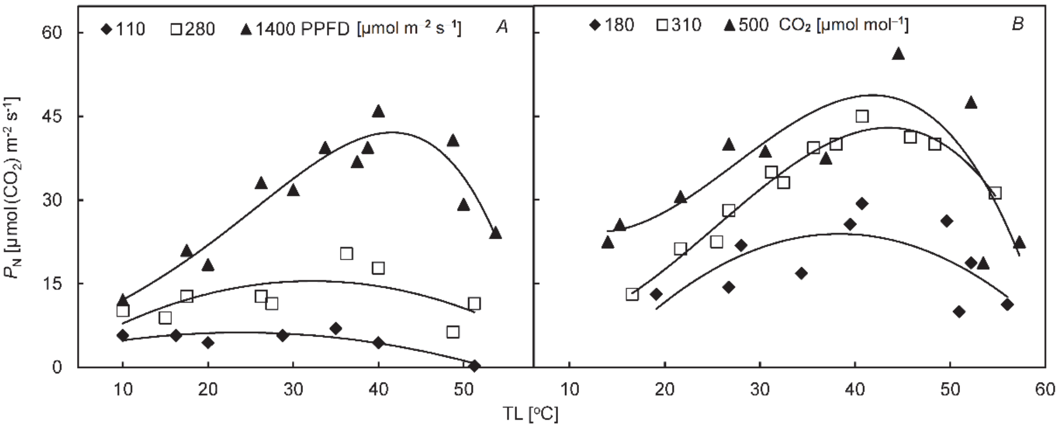

Fig. 5. Net photosynthetic rate (P_N) in recently expanded leaves as function of leaf temperature. Plants were cabinet-grown at 30°C with 700–800 μmol m^{-2} s^{-1} PPFD and at 22°C during dark. (*A*) PPFD in μmol m^{-2} s^{-1} at [CO_2] = 310 μmol mol^{-1}. (*B*) [CO_2] in μmol mol^{-1}. Note: The shift in optimum temperature with increasing PPFD (*A*); with increasing [CO_2] (*B*). Source: adapted from El-Sharkawy *et al.* (1968).

Photosynthesis and the C_3-C_4 intermediate characteristics of cassava (*Manihot esculenta*)

Origin and domestication of cassava: As with amaranth, starchy-root cassava was domesticated and cultivated by the natives of the New World in Central and South America. It is believed that the low cyanogen (sweet) cassava was domesticated as early as 6,000 B.C. in the moist Amazonian rainforests (Gibbons 1990). Ugent *et al.* (1986) suggested that cassava might had been domesticated and cultivated long before 4,000 B.C. on the Peruvian coast and in other parts of the Americas. Renvoize (1972) suggested that sweet cassava may have been domesticated in Mesoamerica while bitter ones (high cyanogen) were domesticated in northeastern South America. The crop was introduced, in the 16th century, to Africa by the Portuguese, and later to Asia in the 17th century (*see* Allem 2002, El-Sharkawy 1993, 2004).

International research on cassava: Very few national research programs gave sufficient attention to cassava as an important small-holder´s food, feed, and energy crop (*e.g.*, Brazil, India, Thailand) before the initiation of the International Research Centers in 1967 (CIAT in Colombia, and the International Institute for Tropical Agriculture, IITA, in Nigeria). At CIAT, the cassava program focused on the ecophysiology of the crop, as well as breeding, soils, crop management, and utilization. The cassava physiology section intensively studied photosynthesis of cassava, as the basic process underlying primary productivity and yield, based on the concept that efforts to improve P_N in relation to productivity might lead to the increasing crop yield *via* selection of germplasm with high P_N, in combination with other yield determinant traits (*e.g.*, Cock *et al.* 1979, Porto 1983, Veltkamp 1986, Cock and El-Sharkawy 1988, Pellet and El-Sharkawy 1993, 1994, El-Sharkawy 1993, 2004, 2006, 2007, 2009a,b, 2010; El-Sharkawy *et al.* 1984a,b,c,d, 1985, 1990, 1992a,b; 2012a,b; El-Sharkawy and Cock 1984, 1987a,b, 1990; Cayón *et al.* 1997, de Tafur *et al.* 1997). Results of this research were documented in CIAT annual reports from the early 1980's up to 1997, in students thesis, in conference meetings, in peer-reviewed journals, proceedings, and book chapters. Therefore, in the following subsections, a few important aspects of this research are highlighted. The readers are advised to consult the original publications for more information.

Potential photosynthesis in cassava: Table 1 presents data on upper canopy leaf P_N of several field-grown cultivars grown in a near optimal environment, Cauca Department, Colombia. In field experiments, air drawn from above canopy [containing 325–335 µmol(CO_2) mol^{-1}], was fed to a portable IRGA (*LCA-2* gas analyzer, *Analytical Development Co*., Hoddesdon, England) to measure P_N of attached leaves between 9:00 to 13:00 h of the local time. A small leaf chamber (*Parkison Broad Leaf Model*), connected to the portable gas analyzer, was clamped over the middle portion (6.25 cm^2) of the central lobe of the measured leaves and was held toward the sun for 30 to 60 s to obtain steady-state gas exchange (H_2O and CO_2) rates. Data were collected over several days: on 27 to 29 July, 9 to 15 August, 10 to 17 September, 8 to 15 October, and 19 to 27 November, 1990, on several cassava cultivars planted on 7 May 1990. The maximum P_N of upper canopy leaves measured in wet soils was not light-saturated at irradiance greater than 1,800 µmol m^{-2} s^{-1} PPFD, and ranged among cultivars from 39 to 50 µmol(CO_2) m^{-2} s^{-1} , with a mean of 43.5 µmol(CO_2) m^{-2} s^{-1} (Table 1, and El-Sharkawy *et al.* 1992a, 1993). Seasonal averaged P_N varied, among cultivars, from 26 to 34 µmol(CO_2) m^{-2} s^{-1}, with a mean 31.4 µmol(CO_2) m^{-2} s^{-1}. Moreover, seasonal averaged P_N was significantly correlated with biomass and the yield at final harvest (11 month after planting). The ratio of the intercellular/ ambient CO_2 (C_i/C_a) varied, among cultivars, from 0.37 to 0.43, with a mean value of 0.42. These P_N and C_i/C_a values are much greater than those commonly observed in C_3 warm-climate crops and they are approaching values obtained in tropical C_4 crops. The C_i/C_a ratio is used to differentiate among plant species with different photosynthetic pathways. For example, C_3 plants normally have the C_i/C_a ratio around 0.7, and the C_4 plants have C_i/C_a around 0.4 (Penning de Vries *et al.* 1989). From these data, it was concluded that the photosynthetic potential of cassava is high and can be fully expressed only in near-optimum environments. To express its photosynthetic potential, growth, and productivity, cassava requires high solar irradiance, high air temperature (optimum temperature range for photosynthesis is 30–40ºC), and high atmospheric humidity to allow maximum g_s. Under such environmental conditions, with annual rainfall around 1,000 mm, several cassava cultivars, selected for their high yield and high P_N, from a preliminary field trial with more than 120 accessions, had the average oven-dried storage root yield around 20 t ha^{-1}, with one cultivar reaching 27 t ha^{-1} in 10 months (El-Sharkawy *et al.* 1990, El-Sharkawy and Cock 1990). In these large field trials, the root yield and total harvested biomass (fallen leaves were excluded) in one hectare of experimental area, were significantly correlated with seasonal averaged upper canopy P_N. Moreover, the oven-dried storage root yield was significantly correlated with photosynthetic nitrogen use efficiency (PNUE), suggesting that the relation was controlled, mainly, by biochemical traits within the leaf mesophyll (Fig. 6). The same trend was observed in field trials conducted in subhumid, seasonally dry, and semiarid conditions (Fig. 7; *see* also, Pellet and El-Sharkawy 1993, 1994; de Tafur *et al.* 1997, El-Sharkawy 2006, El-Sharkawy *et al.* 2012a, b).

Table 1. Leaf net 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 a final root yield. Source: El-Sharkawy *et al.* 1992a, 1993. C_i – intercellular CO_2 concentration; C_a – atmospheric CO_2 concentration.

Cultivar	P_N [μmol(CO_2) m^{-2} s^{-1}] (n = 6)	C_i/C_a (n = 6)	Seasonal average P_N [μmol(CO_2) m^{-2} s^{-1}] (n = 30)
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 at 5%	1.70	0.08	1.80

Evolution of C_4 system: After the discovery of the C_4 system in the 1960's, much research on the evolution of the photosynthetic C_4 pathway from C_3 ancestors was carried out at many laboratories in different countries, indicating the complexity and multitudinous genes involved (*e.g.*, Monson 1989, Sheen 1999, Sage 2004, Westhoff and Gowik 2004, Gowik and Westhoff 2011, Sage *et al.* 2011a,b). It was suggested that the C_4 syndrome had evolved in terrestrial plants in response to environmental stresses, such as a decreasing atmospheric CO_2 concentration, a rising temperature, and an exposure to dry conditions. These environmental factors reduce the availability of CO_2, due to decreasing g_s, at the site of the C_3 mesophyll Rubisco; hence, there is more photorespiration and less P_N. However, under these conditions, the increased photorespiration *via* the oxygenase reaction is beneficial for protecting C_3 plants from photoinhibition caused by excesses in APAR (Kozaki and Takeba 1996). The evolved, and coordinated, unique C_4 anatomical, structural, and biochemical traits, including spatially separated enzymes in different tissues and cellular organelles, resulted in increasing CO_2 concentration at Rubisco in the bundle sheath, and hence, higher carboxylation activity and greater P_N. Another advantage of the elevated CO_2 in the bundle sheath cells of C_4 is the suppression of the Rubisco oxygenase reaction, and hence, less photorespiration. El-Sharkawy and Hesketh (1965) and El-Sharkawy *et al.* (1967, 1968) attributed, early, the superiority of P_N in C_4 species, and the lack of apparent CO_2 releases in CO_2-free air in light, to variations in leaf anatomy, and to differences in dark reactions, including faster carboxylation, which can lead to assimilation of all internal CO_2, including those originating from photorespiration, and dark mitochondrial respiration [*i.e.*, the tricarboxylic acid cycle (TCA cycle)].

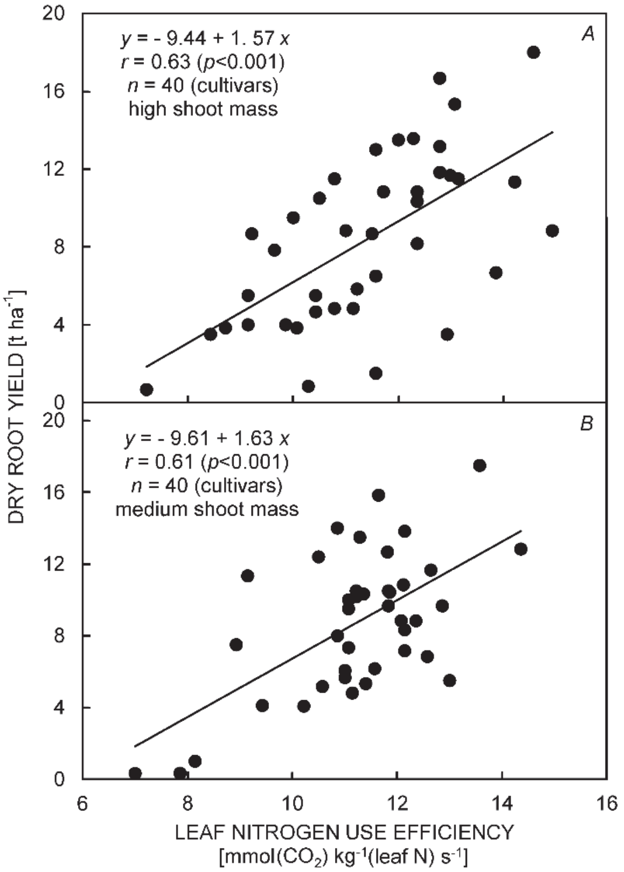

Fig. 6. Relationship between dry root yield and leaf photosynthetic nitrogen-use efficiency (PNUE) in field-grown cassava. PNUE values were calculated from leaf CO_2-exchange measurements and total leaf N contents. Cultivars with high (*A*) or medium (*B*) shoot mass and large LAI. Figure constructed from data of El-Sharkawy *et al.* (1990) – *see* El-Sharkawy (2003, 2004), El-Sharkawy *et al.* (2008).

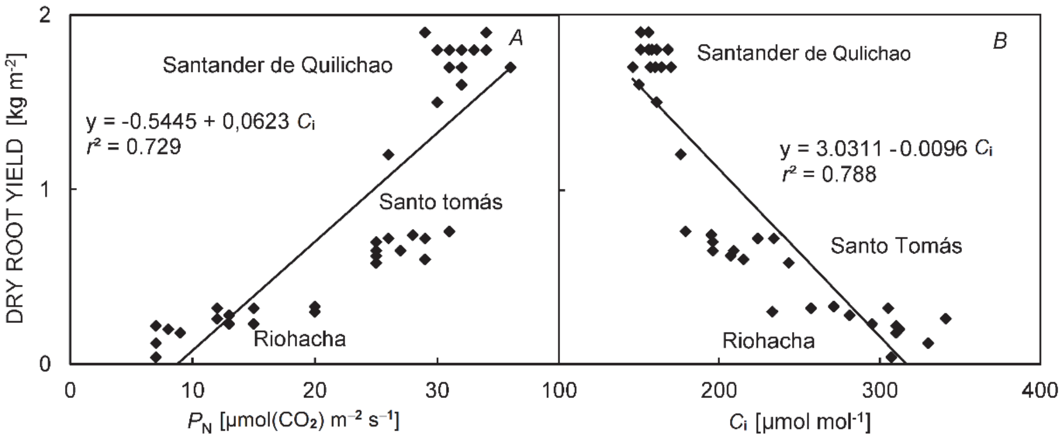

Fig. 7. Relationships between the dry root yield and upper canopy leaf net photosynthetic rate, P_N (*A*) and the intercellular CO$_2$ concentration, C_i (*B*) for a group of cassava cultivars grown at three locations in Colombia: subhumid (Santander de Quilchao, Cauca Dept.), seasonally dry (Santo Tomas, Atlantic Dept.), and semiarid (Riohacha, Guajira Dept.). P_N was made with portable infrared gas analyzers between 08:00 and 13:00 h of local time on 4–6-month-old crops during the driest period in all locations. Crops were rain-fed and harvested after 10–12 months (El-Sharkawy *et al.* 1993, De Tafur *et al.* 1997). Similar relations were previously observed between single-leaf photosynthesis, C_i and total biomass and yield across 127 clones grown in large field trials at a seasonally dry location in Patia Valle, Cauca Dept., Colombia (El-Sharkawy *et al.* 1990, El-Sharkawy and Cock 1990, El-Sharkawy 2006).

Moreover, Hesketh *et al.* (1965) found lower activities of carbonic anhydrase (CA), which catalyzes the reversible hydration reaction of CO$_2$ to HCO$_3^-$, in C$_4$ leaf extracts than that in C$_3$ plants. The same trends of lower chloroplastic CA activities in C$_4$, as compared to C$_3$ species, were reported later by Triolo *et al.* (1974). Also, lower levels of CA in C$_4$ leaves were reported to be around 10-20% of that in C$_3$ leaves (Everson and Slack 1968). As the thick-walled, and suberized, bundle sheath cells (Fig. 1) normally have very low conductance to CO$_2$ diffusion than that for bicarbonate, the low CA may lead to little, if any, 'CO$_2$ leakage' out of the bundle sheath in C$_4$ 'Kranz anatomy-leaves', which was observed by El-Sharkawy and Hesketh (1965) and by El-Sharkawy *et al.* (1967, 1968). Maintaining high CO$_2$ concentrations at the bundle sheath Rubisco, *via* low bundle sheath CA, is apparently essential for the effectively functioning C$_4$ pathway (Burnell and Hatch 1988). Tanz *et al.* (2009), in their molecular genetic analysis of CA in the genus *Flaveria*, which contains C$_3$, C$_4$, and C$_3$-C$_4$ intermediates species, concluded that all three CAs of the C$_3$, *F. pringlei*, have orthologs in the closely related C$_4$ plant, *F. bidentis*, and comparisons of ortholog sequences, expression patterns, and intracellular locations of their products indicated that *CA1* and *CA2* have maintained their ancestral role in C$_4$ plants, whereas modifications to the C$_3$ *CA3* gene led to the evolution of the CA isoform that catalyzes the first step in the C$_4$ photosynthetic pathway. Thus, CA is a central enzyme in the C$_4$ pathway, where it converts CO$_2$ to HCO$_3^-$ in the mesophyll cells, which is the substrate for PEPC (Hatch and Burnell 1990, Tanz *et al.* 2009). Also, recently, Igamberdiev and Roussel (2012) and Igamberdiev (2015) suggested that CA in the chloroplast may play a significant role in the C$_3$ system, when CO$_2$ is low, by creating a '$CO_2 \leftrightarrow HCO^-_3$' pump at the site of Rubisco in the chloroplast stroma *via* recycling CO$_2$ from photorespiration. Therefore, the evolution of CA must be an integral part within the C$_3$ and C$_4$ pathways evolution and function [*see* also Chigwidden *et al.* (2000)].

Apparently, the evolution of the C$_4$ system occurred several times across a range of families (19 families) and genera (about 3% of all terrestrial plant species), both monocot and dicot, about 25–35 million years ago. According to Sage (2004) and Sage *et al.* (2011), the most diversified C$_4$ origins occurred in dicots, with at least 36 lineages and at more recent epochs (< 5 million years), than in monocots. In monocots, there are 26 lineages, mostly in the grass family and sedges. C$_3$-C$_4$ intermediates include 21 lineages, with 9 of them are neither immediately associated with C$_4$ lineage nor sharing a related C$_4$ species with other C$_3$-C$_4$ intermediates, and hence, indicating their independency (Sage *et al.* 2011a,b). In nature, it is rare to find C$_3$-C$_4$ species growing among relatives possessing C$_3$ and C$_4$ systems. However, earlier photosynthetic researchers believed that 'C$_3$-C$_4$ intermediacy' is a necessary transitory step toward the development of the C$_4$ system (Kennedy and Laetsch 1974, Bauwe 1984, Holaday and Chollet 1984, Monson *et al.* 1984). Others (*e.g.*, Monson 1989, Rawsthorne 1992, Duvall *et al.* 2003, Bauwe 2011, Sage *et al.* 2013) restricted C$_3$-C$_4$ intermediacy to only species with photosynthetic/photorespiratory C$_2$-cycle concentrating mechanism, mostly based on the genus '*Flaveria*' as a model plant system. Accordingly, the glycine decarboxylase (GDC), and the C$_2$ cycle, must be confined within the bundle sheath cells in 'Kranz-like' C$_3$-C$_4$ leaves for decarboxylating the glycine molecules originating from mesophyll located Rubisco oxygenase. This step is essential for providing the bundle sheath Rubisco with elevated CO$_2$ concentration (*e.g.*, Sage *et al.* 2013, Keerberg *et al.* 2014).

This process (sometimes called 'glycine shuttle and/or C_2-photosynthesis CO_2 pump') is thought to be a key intermediate step in the evolution of C_4 photosynthesis (*e.g.*, Hylton *et al.* 1988, Monson 1989, Rawsthorne 1992, Monson and Rawsthorne 2000, Sage 2004, Sage *at al.* 2012, Fisher *et al.* 2015).

Nevertheless, the problem with this '*Flaveria*'-based hypothesis is that there are species with C_3-C_4 intermediate physiological and biochemical traits, such as cassava and wild *Manihot* spp., but they are lacking the bundle sheath, and the surrounding mesophyll cells organization, typical of the C_4 Kranz anatomy or the 'Kranz-like' C_3-C_4 intermediates [*see* Fisher *et al.* (2015) on C_3-C_4 'Kranz-less' leaf anatomy in the genus *Blepharis*, Acanthaceae]. Cassava and wild *Manihot* spp. possess a pronounced green bundle sheath beneath their long palisade layers (Figs. 8, 9) (*e.g.*, Cock *et al.* 1987, El-Sharkawy and Cock 1987a, 1990; Riaño *et al.* 1987a,b; Aguilar 1995, El-Sharkawy 2004, 2006, 2010; El-Sharkawy and de Tafur 2007, El-Sharkawy *et al.* 2008, 2012a,b). Furthermore, the expression and patterns of distribution of GDC within different mesophyll cells are influenced by the leaf developmental stage and the environmental conditions under which plants are grown (*e.g.*, Rylott *et al.* 1998). For instance, Ueno and Agarie (1997) reported that cabinet-grown cassava plants had a higher mitochondrial immunogold P-protein labeling density of GDC in the long palisade cells than in the spongy mesophyll cells. El-Sharkawy and Cock (1987a, 1990), El-Sharkawy (2006), and El-Sharkawy *et al.* (2012a) found that, in CO_2-free air and under intense light, cassava amphistomatous leaves recycle all its internal CO_2 (from both mitochondrial TCA cycle and photorespiratory origin) within the long palisade layer, when abaxial stomata are closed. This phenomenon was observed under a wide range of light intensities and temperatures in various cultivars (Fig. 10). Therefore, El-Sharkawy and Cock (1987a, 1990) hypothesized that cassava has, to a certain extent, the biochemical components of the C_4 system but lacks the typical C_4 leaf Kranz anatomy, which is essential for enzymes, *i.e.*, Rubisco and PEPC, separation and compartmentalization in distinct tissues. A possible CO_2 concentrating mechanism may exist in the cassava palisade layer, as indicated by the pronounced CO_2 surge in short periods in darkness (Fig. 10). Thus, the existence of GDC in the palisade cells of cassava does not contradict our hypothesis of C_3-C_4 intermediacy (*e.g.*, El-Sharkawy and Cock 1987a, 1990, Cock *et al.* 1987, El-Sharkawy 2004, 2006; El-Sharkawy *et al.* 2012a,b). Kelly and Latzko (2006) suggested that any phenotypic variation in C_3 cells, tissues, and organs, which may deviate from the typical C_3 characteristics, might be considered a 'C_3-C_4' trait. These changes, even subtle, might represent evolutionary steps toward C_4 development. Only some uncultivated species were identified during the past four decades as C_3-C_4 photosynthetic intermediates, based on anatomical, biochemical, phylogenetic, physiological criteria, as well as on evidence from molecular genetic analysis (*e.g.*, Monson *et al.* 1984, Monson 1989, von Caemmerer and Furbank 1997, Sheen 1999, Sage 2004, Westhoff and Gowik 2004, Voznesenskaya *et al.* 2010, Sage *et al.* 2011a,b, 2012). Many of these species lacked the typical C_4 Kranz leaf anatomy and all have $\delta^{13}C$ values similar to those in C_3 plants (ranging from –25 to – 35‰), whereas in several field-grown cassava cultivars, the $\delta\ ^{13}C$ values ranged from –25 to –27‰, with the less negative values (*i.e.*, lower Rubisco discrimination against ^{13}C) were in upper canopy leaves having the highest P_N (El-Sharkawy and de Tafur 2007). There were significant differences in $\delta^{13}C$ among cassava genotypes and leaf canopy layers. Therefore, neither known C_3-C_4 uncultivated species nor cassava show a $\delta\ ^{13}C$ range close to values observed in C_4 plants ($\delta^{13}C$ –8 to –17‰). The only exception in this regard

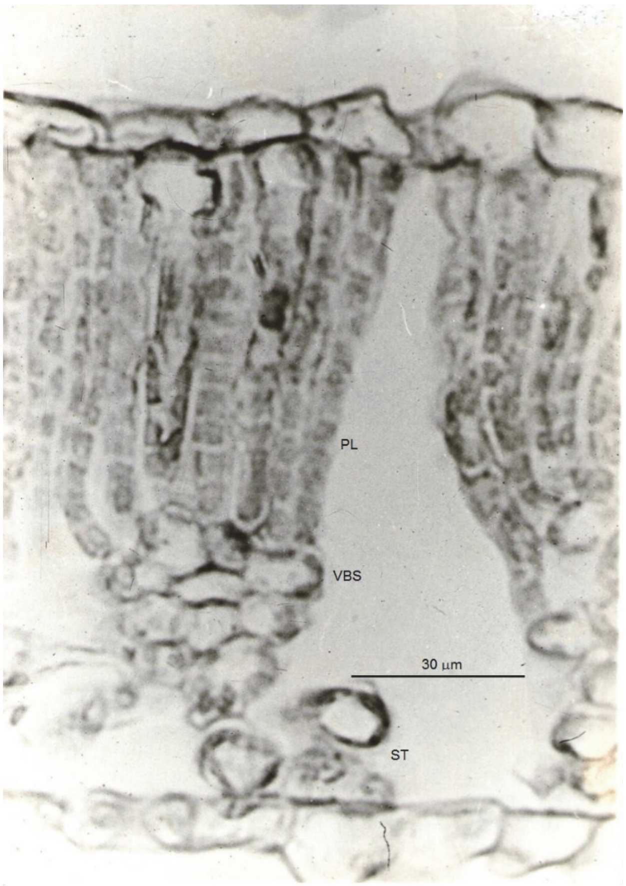

Fig. 8. Photomicrograph of cross section of amphistomatous field-grown cassava leaf (cv. M Col 90. CIAT cassava germplasm bank). Note: the long palisade layer (PL) and the conspicuous chlorophyllous vascular bundle sheath cells (VBS) situated beneath the palisade layer; the narrow spongy tissue (ST); the short distance between vascular tissue minor veins (< 50 µm); the wide airspace between upper and lower epidermis. These anatomical features were observed in many cultivated cassava varieties, improved CIAT breeding lines, and land races. Compare Fig. 8 with Fig. 1. Source: (M. A. El-Sharkawy unpublished, El-Sharkawy and Cock 1987a,1990, Riaño 1987, Aguilar 1995).

is the Kranz-like C_3-C_4 *Flaveria brownii*, that has $\delta^{13}C$ –16 to –17‰ and assimilates >75% of its carbon *via* the C_4 cycle, and whose classification status is controversial (Holaday *et al.* 1988, Monson 1989, El-Sharkawy and de Tafur 2007). The complete separation and compartmentalization of C_4 and C_3 enzymes in distinct cells and tissues of Kranz-type leaves reduce discrimination against ^{13}C by Rubisco located in the chloroplasts of bundle sheath cells. The cytosolic C_4 PEPC in mesophyll cells of C_4 plants has much less discrimination against ^{13}C.

In light of these different debatable views about C_3-C_4 evolution, and consequently, C_4, in terrestrial plants, important questions arise on the first induction step of C_4 (Sage *et al.* 2013): (*1*) Which does evolve first among several C_4-system complex components, which are controlled by multiple genes expressed in different cells and subcellular organelles? Are they biochemical traits or anatomical and structural traits?; and (*2*) is it possible that any component of the C_4 system may evolve independently of others, in response to environments *vs*. genetics interactions? *Bienertia cycloptera*, an uncultivated species of the Chenopodiaceae family, grows in salty depressions in Central Asia. This plant species has been shown recently to possess the functional C_4 pathway within a single cell, but lacks the typical C_4 Kranz anatomy, while key C_4 and C_3 enzymes are presumably located and compartmentalized within the cytosol or chloroplasts of the same mesophyll cell (Voznesenskaya *et al.* 2001, 2002). Likewise, the submersed monoct *Hydrilla verticillata*, which also lacks the C_4 Kranz anatomy, was found to possess the functional C_4 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 it is common in terrestrial C_4 species (Salvucci and Bowes 1983, Magnin *et al.* 1997, Reiskind *et al.* 1997). 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 they can have 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 incomplete compartmentalization of Rubisco, which was located in both mesophyll and bundle sheath cells (Ueno 1996). From this, as well as from our own experience in plant photosynthetic research, it is perhaps unwise to set up rigid views and rules, or formulate hypothesis, while working with limited number of species, sometimes grown under inadequate environments, and try to generalize them on the whole plant or animal kingdom. Nature is certainly wiser when it comes to the diverse biosystems structures, functions, and their evolutionary development on the planet Earth. Evolution is a dynamic process operating under natural selection for the fittest in response to changing environmental pressures interacting with complex genomes, such as that control photosynthetic capacity.

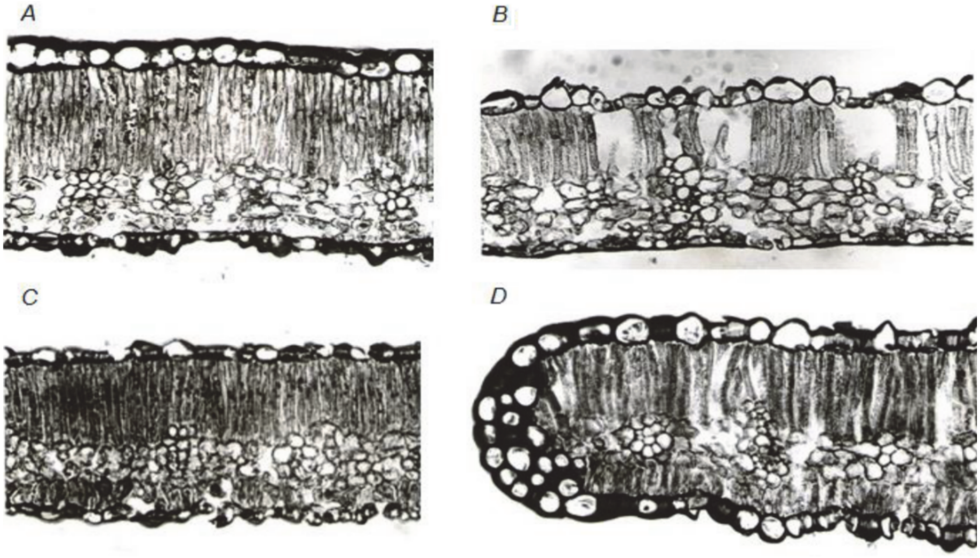

Fig. 9. Photomicrographs of leaf cross sections of field-grown wild *Manihot* spp. (CIAT germplasm bank). (*A*) *M. carthaginensis,* (*B*) *M. crassisepala,* (*C*) *M. grahami*, (*D*) *M. rubricaulis*. Note: the amphistomatous trait; the chlorophyllous vascular bundle sheaths situated beneath the upper long palisade layer; the short lower palisade layer in *M. graham* and *M. rubricaulis*; the very short distance between vascular tissue minor veins. These anatomical characteristics were observed in several wild *Manihot* spp. (El-Sharkawy unpublished, El-Sharkawy 2003, 2004). In normal air containing 345 μmol(CO_2) mol^{-1}, and under intense light and leaf temperature of 35ºC, P_N in *M. rubricaulis* was around 50 μmol(CO_2) m^{-2} s^{-1}, and responded to CO_2 up to C_i of 400–500 μmol(CO_2) mol^{-1}; both wild *Manihot* and cultivated cassava possess high PEPC activities (10–30% of activities in C_4 maize and sorghum). Source: El-Sharkawy unpublished, El-Sharkawy and Cock 1990, El-Sharkawy and de Tafur 2007, El-Sharkawy 2004, 2006, 2014, El-Sharkawy *et al.* 2008, 2012a,b. Compare Fig. 9. with Fig.1 showing typical C_4 leaf Kranz anatomy in amaranth, Bermuda grass, sorghum, and maize.

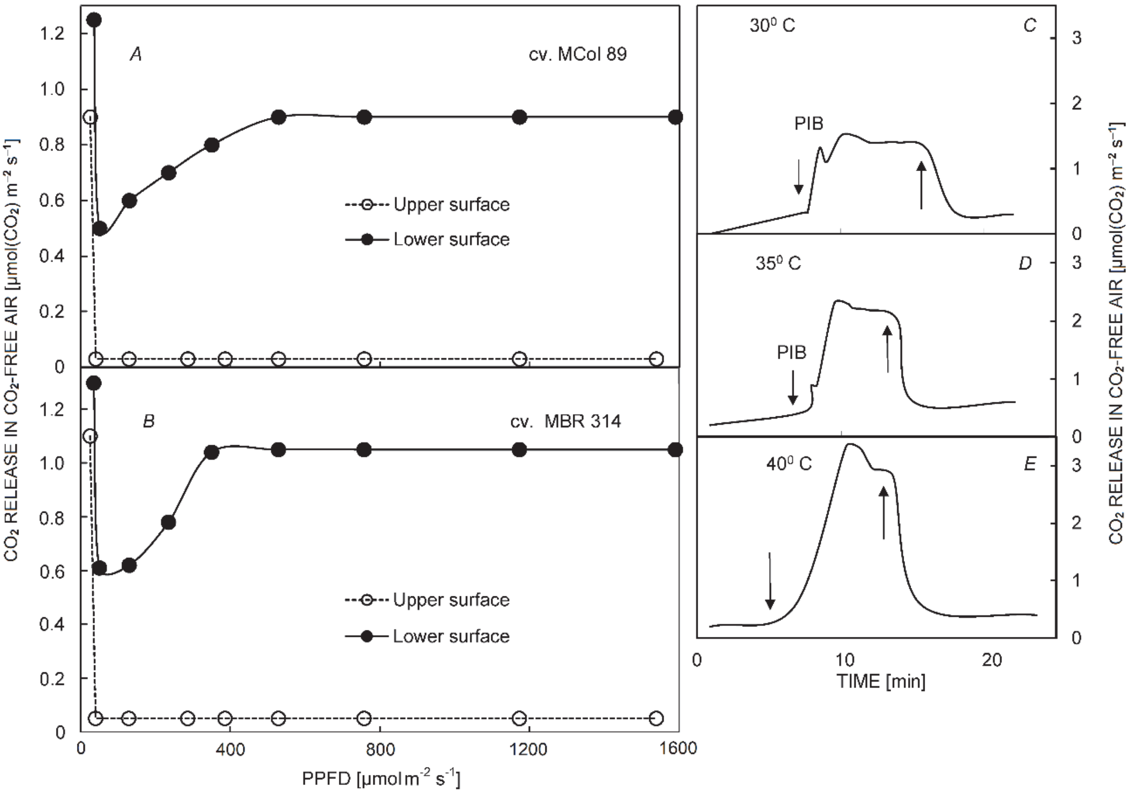

Fig. 10. Differential CO_2 releases in CO_2-free air from the upper and lower surfaces of amphistomatous cassava leaves of cv. M Col 89 (*A*) and cv. M Br 314 (*B*) as a function of photosynthetic photon flux density (PPFD) at a constant leaf temperature (27°C). Note the consistent lack of CO_2 release from the upper surface of both cultivars, when the abaxial stomata were blocked, as compared with the release from lower surface indicating the complete refixation/recycling of respiratory CO_2 (both photorespiration and dark mitochondrial) within the long palisade layer (that occupies more than 60% of leaf thickness). The spongy mesophyll in cassava is very thin, about two-cell thick layer with large air spaces. In these amphistomatous leaves rate of gas exchanges in normal air and saturating PPFD was substantial from both surfaces and in proportion to stomatal densities (El-Sharkawy *et al.* 1984b). (*C–E*) Recorder traces of CO_2 releases in CO_2-free air in light and dark from the upper surface of amphistomatous cassava leaves (cv. M Bra 314) at 30, 35, and 40°C leaf temperatures. PPFD was 1,200 µmol m^{-2} s^{-1} (↓: light off, ↑: light on). PIB: post-illumination CO_2 burst. Note the lack of CO_2 release in light which was observed in several light-dark cycles over longer time (>1 h); the decrease in the magnitude of PIB with rising leaf temperature and eventually its disappearance at 40°C; the pronounced surge of CO_2 within 3 min in darkness. The lack of CO_2 release in light was attributed, mainly, to an efficient refixation/recycling system in the palisade cells (El-Sharkawy and Cock 1987a, 1990, El-Sharkawy 2003,2004,2006, El-Sharkawy *et al.* 2012a,b).

Intermediate C_3-C_4 characteristics in cassava: Research at CIAT indicated that cassava photosynthesis behaved differently from that normally observed in both C_3 and C_4 species. The cassava family, Euphorbiaceae, is known to contain CAM, C_3, and C_4 species, and perhaps C_3-C_4 (*e.g.*, Sage *et al.* 2011a,b). Cassava is considered a C_3-C_4 intermediate species, based on various unique physiological, anatomical, and biochemical traits. Others (*e.g.*, Edwards *et al.* 1990, Angelov *et al.* 1993, Ueno and Agarie 1997, Calatayud *et al.* 2002) viewed cassava as a C_3 species, but they were working with indoor-grown cassava (*i.e.*, under shading conditions and at lower than optimum temperature, which are not adequate for expressing the photosynthetic capacity). These authors reported lower P_N, lower optimum temperature, and lower light saturation intensity than what have been observed in field-grown or outdoor-grown plants in hot, sunny, and humid tropical environments.

Traits that led us to consider cassava a C_3-C_4 intermediate are: (*1*) atypical C_3 leaf anatomy, where a pronounced compact bundle sheath exists, with thin-walled cells and numerous large granal chloroplasts, but not typical of C_4 Kranz anatomy; (*2*) close physical association of chloroplasts with numerous mitochondria and peroxisomes in both bundle sheath and mesophyll cells; (*3*) high percentage (30 to 60%) of ^{14}C incorporation into C_4 acids within 5–10 s of exposure to $^{14}CO_2$ in light; (*4*) low photorespiration [CO_2 compensation concentration (Γ) = 20–30 µmol(CO_2) mol^{-1} under high irradiance and leaf temperature of 30ºC, and small CO_2 release, less than 10% of P_N from illuminated whole leaves in CO_2-free air and rapid air flow rate]; (*5*) ability to recycle all respiratory CO_2 within the palisade cells of amphistomatous leaves at wide ranges of PPFD and temperature, when the abaxial stomata are closed; (*6*) elevated PEPC activities (from 10 to 30% of those in C_4 species, such as maize and sorghum); (*7*) the C_i/C_a ratio near 0.4, which is similar to values in C_4 species and much smaller than those (0.7) observed in C_3 species; (*8*) immunological analysis and DNA hybridization of PEPC from cassava and wild *Manihot* species against antibodies and a *ppc* probe from maize, showed variable degrees of hybridization among cultivars and land races (Cock *et al.* 1987, El-Sharkawy and Cock 1987a, 1990; Riaño *et al.* 1987a,b, El-Sharkawy *et al.* 1992a, 1993, 2008, 2012a,b; Lopez *et al.* 1993, Aguilar 1995, El-Sharkawy 2004, 2006, 2010, 2012; El-Sharkawy and de Tafur 2007).

Recent genomic analysis indicated that cassava possesses a C_4 genome with several genes responsible for C_4 photosynthesis, including the genes encoding PEPC, with activities observed at the transcriptional level (Saithong *et al.* 2013). Cassava is the only C_3-C_4 intermediate species discovered so far among important crops. These unique characteristics collectively underpinned the high photosynthetic capacity and the high productivity of cassava, which were observed in different environments (El-Sharkawy 1993, 2004, 2006, 2010, 2012; El-Sharkawy *et al.* 2012a,b). More research, however, is needed with cultivated cassava and wild *Manihot* spp. in order to further improve P_N *via* molecular genetics (*e.g.*, Rabbi *et al.* 2014), particularly for globally warming environments.

In conclusion, due to inherent cassava tolerance to heat, water stress, and poor soils, this crop is highly adaptable to warming climate. Such a trait should enhance its role in food security in the tropics and subtropics (*e.g.*, El-Sharkawy *et al.* 1984c, El-Sharkawy 1993, 2009a,b, 2010, 2012a,b, 2014; Jarvis *et al.* 2012, Rosenthal and Ort 2012, Porter *et al.* 2014).

General conclusions

This review summed up the prospects of applying photosynthetic research into improving agricultural productivity and food security by raising the potential yield of biological systems. Several approaches were suggested that may fulfill that target, depending on the time required, the possible impacts, uncertainty encountered in the involved research, and on the resources available. Emphasis was given to two crop species that have originated in the tropical New World, *i.e*, *Amaranthus* spp. (the C_4 crop, rich in good quality protein in leaves and seeds), and cassava (the C_3-C_4 intermediate crop, rich in starch of its roots and rich in leaf protein). Both are highly productive and tolerant to adverse environmental conditions. These crops were domesticated, cultivated, and developed by the natives for millennia. Yet, they are considered, so far, orphan crops, which have received limited research, compared to other traditional food, feed, and energy crops. In a globally warming climate, and in view of increasing human population, particularly in developing countries, both amaranth and cassava have a significant role to play in providing products for future increased demands for life-supporting resources. The world needs more genetic resources to diversify productivity and to sustain food, feed, and energy security.

Plant breeders need to immediately start working on increasing genetically the heat tolerance of tropical as well as temperate crop species. Soil scientists need to survey potential crop land that might become relevant at the higher latitudes on both ends of the Earth; crop modelers need to take into account the long days and lower maximum available sunlight, and perhaps a lower minimum night temperature at these latitudes.

Future photosynthetic research in relation to biological productivity

Future young scientists, armed with much better methodology, vast information, better communication systems, and having brighter innovative ideas, than we had 60 years ago, must take hold of cracking new scientific frontiers, but not loosing sight to target their research for the benefit of humanity. They should try to translate their findings into better crop production systems, hence, contributing to food security of coming generations. As a guide for that challenging mission, was the modest, and the perseverant, Barbara McClintock, the Nobel Laureate. She was the great 20th century lady scientist, who single-handedly discovered, using simple methods, the phenomenon of jumping genes (*i.e.*, transposable elements) in maize, and hence, paved the road to molecular genetics advances and the application of genetic engineering into biosystems (Fedoroff 2012). Scientific research is expensive, but when conducted properly with multidisciplinary/multi-institutional teams it might become an economically viable means serving societal needs.

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