Pioneering research on C_4 leaf anatomical, physiological, and agronomic characteristics of tropical monocot and dicot plant species: Implications for crop water relations and productivity in comparison to C_3 cropping systems[**]

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Abstract

The review is done to summarise the history of the discoveries of the many anatomical, agronomical, and physiological aspects of C_4 photosynthesis (where the first chemical products of CO_2 fixation in illuminated leaves are four-carbon dicarboxylic acids) and to document correctly the scientists at the University of Arizona and the University of California, Davis, who made these early discoveries. The findings were *milestones* in plant science that occurred shortly after the biochemical pathway of C_3 photosynthesis in green algae (where the first chemical product is a three-carbon compound) was elucidated at the University of California, Berkeley, and earned a Nobel Prize in chemistry. These remarkable achievements were the result of ground-breaking pioneering research efforts carried out by many agronomists, plant physiologists and biochemists in several laboratories, particularly in the USA. Numerous reviews and books written in the past four decades on the history of C_4 photosynthesis have focused on the biochemical aspects and give an unbalanced history of the multidisciplinary/multinstitutional nature of the achievements made by agronomists, who published much of their work in *Crop Science*. Most notable among the characteristics of the C_4 species that differentiated them from the C_3 ones are: (I) high optimum temperature and high irradiance saturation for maximum leaf photosynthetic rates; (II) apparent lack of CO_2 release in a rapid stream of CO_2-free air in illuminated leaves in varying temperatures and high irradiances; (III) a very low CO_2 compensation point; (IV) lower mesophyll resistances to CO_2 diffusion coupled with higher stomatal resistances, and, hence, higher instantaneous leaf water use efficiency; (V) the existence of the so-called "Kranz leaf anatomy" and the higher internal exposed mesophyll surface area per cell volume; and (VI) the ability to recycle respiratory CO_2 by illuminated leaves.

Additional keywords: agriculture, biochemistry, breeding, carbon assimilation, crops, drought, enzymes, gas exchange, photorespiration, photosynthesis, water use efficiency.

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Abbreviations: C_i − intercellular CO_2 concentration; DM − dry mass; NAD-ME − NAD-dependent malic enzyme; NADP-ME − NADP-dependent malic enzyme; PAR − photosynthetically active radiation; PCK − phospho*enol*pyruvate carboxykinase; PCRC − photosynthetic carbon reduction cycle − PEPC − phospho*enol*pyruvate carboxylase; PGA − 3-phosphoglycerate; PNUE − photosynthetic nitrogen use efficiency; PPDK − pyruvate orthophosphate dikinase; P_N − leaf net photosynthetic rate; r_m − mesophyll resistances to CO_2 diffusion; r_k − intracellular resistances to CO_2 diffusion; Rubisco − ribulose-1,5-bisphosphate carboxylase/ oxygenase; S − internal exposed surface area per external leaf area; S/V − internal exposed surface area per volume of cell; WUE − water use efficiency.

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[**] This review is dedicated to the memory of Dr. Zdeněk Šesták (4 August 1932 – 14 November 2008), Editor-in-Chief of Photosynthetica (1991-2008), renowned plant physiologist and world leader in the field of photosynthesis research for five decades. Photosynthetica, Dr. Šesták's inspired brainchild born in 1967, was the first international journal of its kind dedicated to the

dissemination of the findings of original research, in various languages, in all aspects of plant photosynthesis, ranging from molecular to plant community levels, basic and applied, conducted by researchers across continents without discrimination. The environment surrounding the home-base of Photosynthetica was known, at an early time, for its rich tradition in many activities and development of methodology in photosynthesis and plant physiology. Before the initiation of this journal, research findings on plant photosynthesis were normally published in a diverse net of journals that covered many areas such as chemical, biological, botanical, and agronomical research. In our case as agronomists, our early photosynthetic research reviewed here was mainly published in the then new journal *Crop Science,*USA, that was dominated by plant genetic and breeding publications. There, we felt as outlaw or off-type at the time!. Since I and my colleagues have contributed several articles to Photosynthetica in the past 20 years covering some of our research on cassava conducted at CIAT, Colombia, I became an admirer of the personal qualities of Dr. Šesták as an eminent scientist, efficient communicator, as well as an inspiration to fellow scientists, particulary those in Third World countries who are increasingly publishing in Photosynthetica. His sharp intellect, fairness, unbiased judgement and constructive criticism during the reviewing process, as Editor-in-Chief, were crucial in encouraging us publishing in Photosynthetica. The invaluable editorial inputs provided by him and his staff at Prague to our publications were fundamental in improving the clarity, readability and quality of the submitted manuscripts. I personally have lost, and surely along with me the rest of the international community in the field of photosynthesis reseacrh, a great scientist, a friend and an honest collaborator. I humbly dedicate this review to his memory. We are surely following your green footsteps, Dr. Šesták, on earth and in heaven.

Introduction

Reviews have highlighted the historical progress achieved during the past 350 years in elucidating the biophysical, botanical, and biochemical features of plant photosynthesis (Huzisige and Ke 1993). From the late 1940's to the early 1950's, details of the carbon metabolism pathway were sorted out by the use of the then new methods and techniques of paper chromatography and the ^{14}C isotopes at the University of California, Berkeley (Calvin 1989). This creative and innovative research that spanned approximately ten years was done using the unicellular green algae *Chlorella pyrenoidosa* as a plant model system, which is easily grown and manipulated under the required laboratory conditions. The biochemical pathway involved in CO_2 fixation started with the formation, under illumination in fractions of a second, of the first stable three-carbon compound phosphoglyceric acid (PGA). In only thirty seconds after exposing the algae to illumination and $^{14}CO_2$, the labeled carbon atom was found to be transferable to - and detectable in - various compounds that formed a series of intermediate metabolites leading to the formation of six-carbon sugars. The key enzyme in fixing CO_2 (located in chloroplasts) was first called 'carboxydismutase' but afterward renamed 'ribulose-1,5-bisphosphate carboxylase/oxygenase' (Rubisco), because it was later discovered by USDA/ARS scientists at the University of Illinois, Urbana IL, that both CO_2 and O_2 can be used by Rubisco in reaction with the five-carbon substrate ribulose-1,5-bisphosphate (for more information on the discovery of the dual role of Rubisco, *see*, for example, Bowes *et al.* 1971, Ogren and Bowes 1971, Ogren 1984, Andre 2006). This pathway is commonly known as "The Benson-Calvin-Bassham" cycle (named after the three principal researchers, Andrew Benson, Melvin Calvin, and James Bassham, among the many other collaborators and students), and also called the C_3 cycle or the photosynthetic carbon reduction cycle (PCRC) (Osmond *et al.* 1982, Ogren 1984). The PCRC is the basic and the universally occurring photosynthetic CO_2 reduction pathway in all green plants.

The elucidation of the photosynthetic carbon metabolic pathway was a *milestone* in the fields of biochemistry and biology that received the Nobel Prize in Chemistry (awarded to Melvin Calvin in 1961). Equally important details of the light reaction involved in capturing the photosynthetically active solar radiation and converting it, *via* multi-steps of electron flow systems, into usable chemical energy required for CO_2 assimilation and sugar formation were also worked out by many researchers in Europe and the United States of America (Arnon 1984, Duysens 1989).

Parallel to the above mentioned biochemical advances that greatly enhanced interest in photosynthetic research, plant physiologists and agronomists made efforts to study leaf photosynthetic rates of various plant species that were aided by the modern infrared CO_2 analysers (Williamson 1951) associated with leaf chamber techniques (Bosian 1955, Gaastra 1959, Egle 1960, Lister *et al.* 1961, Hesketh 1963). The open-circuit system in which a stream of air passes into transparent chambers enclosing attached or detached leaves under illumination was employed to investigate the interrelationships between photoperiodism and CO_2 assimilation in *Kalanchoe* (Gregory *et al.* 1954, Spear and Thimann 1954); the effect of ecological factors on plant photosynthesis (Parker 1953, Bohning and Burnside 1956, Burnside and Bohning 1957); effects of petroleum oils on respiration (Helson and Minshall,1956); and effects of ozone on respiration and photosynthesis (Todd 1958). In most of this early research, plants were grown in pots or containers and either left outdoors or in greenhouses and controlled cabinets, growing conditions that often resulted in the underestimation of the potential photosynthetic rates. It was a common conclusion then that rates of all studied plant species were light saturated at less than 50 % of full sun light (Bohning and Burnside 1956, Burnside and Bohning 1957). Furthermore, another common conclusion at the time was that within a large group of herbaceous mesophytes of the temperate zone, leaf photosynthetic rates were much the same and less than 15 μmol CO_2 m^{-2} s^{-1} when measured in full sun light, normal air, and optimum temperatures (Verduin

1953, Verduin *et al.* 1959). Plant physiology text books, proceedings, and treatises written in the1950's and early 1960's reflected this era in photosynthesis research (Rabinowitch 1945, 1951, 1955, Ruhland 1960).

The anatomical, biochemical and physiological aspects of the C_4 syndrome that was discovered in many plant species in the early 1960's by several research groups (Karpilov 1960, El-Sharkawy and Hesketh 1965, Kortschak *et al.* 1965, Forrester *et al.* 1966b, Hatch and Slack 1966, 1970, Jackson and Volk 1969, 1970, Volk and Jackson 1972, Laetsch 1974) [where CO_2 is first fixed into C_4 dicarboxylic acids by the C_4-phospho*enol*-pyruvate carboxylase (PEPC) in cytosols of mesophyll tissue] have been the subject of numerous reviews and books published in the past four decades (*e.g.* Hatch and Slack 1970, Black 1971,1973, Black *et al.* 1976, Burris and Black 1976, Hatch 1976, Ray and Black 1979, Edwards and Walker 1983, Hatch 1992a,b, 1999, von Caemmerer and Furbank 1997, Kanai and Edwards1999, Sage and Monson 1999, Sage 2004, Boote and Sinclair 2006). Unfortunately, in most of these references, pioneering agronomic, anatomical, and physiological research on C_4 photosynthesis (El-Sharkawy 1965, El-Sharkawy and Hesketh 1965, El-Sharkawy *et al.* 1967, 1968), which laid the foundations for the elucidation of further anatomical and biochemical details, was largely overlooked. Moreover, in many cases original findings were used either without the proper citations of the original authors or were incorrectly attributed to researchers who had nothing to do with them (*e.g.* Black 1973, Black *et al.* 1976, Ray and Black 1979, Edwards and Walker 1983, Hatch 1992a,b, von Caemmerer and Furbank 1997, Sage and Monson 1999, Boote and Sinclair 2006).

Interestingly, most, if not all, of these pioneering research achievements were published in the then new *Crop Science* journal that was dominated by crop-related genetic and breeding publications. The results of this research were also presented during various scientific meetings of professional societies, including those held by the American Society of Agronomy and the American Society of Plant Physiology. Several of these publications were highly cited in the literature (*e.g.* El-Sharkawy and Hesketh 1964b,c, El-Sharkawy *et al.* 1965, 1967, 1968, Muramoto *et al.* 1965), according to assessments by the Institute of Scientific Information (ISI). It is noteworthy that these research findings were at the time controversial and contradicted most of the previously known photosynthetic information and plant physiology literature (*e.g.* Verduin 1953, Verduin *et al.* 1959, Rabinowitch 1945, 1951, 1955, Ruhland 1960). The Ph.D. thesis (El-Sharkawy 1965), that was presented to the faculty and accepted in the final doctoral defence exam, was held up days before my scheduled graduation, all because of political interdepartmental fightings at the University of Arizona. This episode that could have delayed my graduation and the start of my post-doc appointment at the University of California, Davis, called for the immediate corrective intervention by the highest university managerial level. Yet, one article highlighting the many discoveries in plant photosynthesis (El-Sharkawy and Hesketh 1965) was easily accepted for publication and later cited as a Citation Classic by the ISI in1986 and was among the 20 most cited publications in *Crop Science* until 1990 (El-Sharkawy and Hesketh 1986, Garfield 1992, at the time Garfield pointed out to the author that his other related Crop Science papers from Tucson, AZ, were cited almost as many times). These discoveries were made before the biochemical characteristics of the C_4 syndrome (Kortschak *et al.* 1965, Hatch and Slack 1966, 1970) were known and published [although it is claimed that the work on sugarcane (*Saccharum officinarum*) in Hawaii by Kortschak and co-workers was initiated during the mid and late 1950's but was never allowed to be published in peer reviewed journals until 1965 for reasons unknown to us]. It is also noteworthy to point out that Russian work (Karpilov 1960) on the primary C_4 products, *via* $^{14}CO_2$ fixation, by illuminated maize (*Zea mays*) leaves and the much earlier leaf anatomy on sugarcane leaves by German botanists in the late 19th century (Haberlandt 1904) were not known until the 1970's in English speaking countries, after the 1960's research in the USA.

It is warranted, therefore, to correct this negligence and inaccuracy in the scientific literature concerning the history of the discovery of C_4 syndrome. Moreover, young scientists and the coming generations of science students need to assess critically relevant old literature and find out who made the discoveries and where they were made. On one hand, for example, in the second edition of the standard textbook *Plant Physiology* by Salisbury and Ross (1978) a table appears on page 165 containing original comparative C_3 and C_4 data apparently taken from El-Sharkawy and Hesketh (1965). That table does not cite their work and refers the readers to *Annual Review of Plant Physiology* (Black 1973). Black (1973) does not cite the pioneering work of El-Sharkawy and Hesketh (1965) where specific criteria of physiological, anatomical, environmental and growth characteristics separating the C_3 and C_4 species were given. On the other hand, Devlin in the third edition of his textbook *Plant Physiology* (1975) and Zelitch in his book *Photosynthesis, Photorespiration, and Productivity* (1971) properly cited original information on C_3 and C_4 systems reported by El-Sharkawy and Hesketh (1965). Leopold (1988) commenting in the *Citation Classic* awarded to his classical textbook *Plant Growth and Development* (McGrow-Hill, New York, 1st edn 1964, and 2nd edn 1975) stated that "for many years I have held a conviction that reviews of a given science area would be more meaningfull if generalisations were presented along with hard experimental data to support them. Without supportive experimental results, the reader is deprived of the kind of factual basis upon which science is built." These remarks by Leopold substantiate the purpose of the current review. Textbooks should be revised, and authors of future review papers on history of photosynthesis should be more diligent in their reporting.

In this review, ground-breaking pioneering agronomical, physiological, and anatomical research conducted in the late 1950's and early 1960 is highlighted for the sake of scientific records and readers. This research established much higher photosynthetic rates of various crop/weed plant species than values reported before, as well as large differences among species, on properly grown plants, particularly under field conditions. The link between a particular kind of leaf anatomy and the physiological characteristics of photosynthesis in several species (*i.e.*, the so-called Kranz anatomy in C_4 leaves where the vascular bundles are enclosed by sheaths of large chlorenchymatous cells tightly surrounded with mesophyll cells, as compared to the typical internal leaf anatomy of C_3 plants with palisade/mesophyll cells) was first determined at the University of Arizona, Tucson, between 1962 and 1965 (El-Sharkawy *et al.* 1964, El-Sharkawy 1965, El-Sharkawy and Hesketh 1965). Moreover, plant factors that limit rates of photosynthesis and the underlying differences among species were elucidated, which led to the discovery of the so-called C_4 syndrome in photosynthesis. The implications of these discoveries for crop water relations and productivity are also discussed.

Pioneering Research On C_4 And C_3 Photosynthetic Systems

Early measurements of photosynthesis in the field

Tropical grasses (C_4 species)

In the late 1950's and 1960's at Cornell University, in Ithaca, NY, the agronomist Robert Musgrave and his students studied maize photosynthesis in the field (Musgrave and Moss 1961, Moss *et al.* 1961, Hesketh and Musgrave 1962, Baker and Musgrave 1964, Moss and Musgrave 1971).These were followed by studies at the Connecticut Agricultural Experiment Station (Moss 1963, Hesketh 1963, Hesketh and Moss 1963, Waggoner *et al.* 1963). Photosynthetic rates measured under field conditions were two to three times higher than those quoted by Verduin (1953) and Verduin *et al.* (1959). Maize leaves showed unsaturated photosynthetic responses up to the highest light levels tested, and rates in normal air exceeded 38 μmol(CO_2) m^{-2} s^{-1} (Hesketh 1963, Hesketh and Moss 1963). Large differences among species in leaf photosynthetic rates in response to illumination were also reported. A Russian researcher (Strogonova 1964) also reported high photosynthetic rates in field-grown maize. At the University of Arizona, El-Sharkawy and co-workers reported similar rates and responses in several tropical grasses such as sugarcane, maize, grain sorghum (*Sorghum bicolor*), elephantgrass or napiergrass (*Pennisetum purpureum*), Johnsongrass (*Sorghum halepense*), and bermudagrass (*Cynodon dactylon*) (El-Sharkawy and Hesketh 1964a,b,c). In all these tropical grasses, leaf photosynthetic rates were not light-saturated at the highest-used irradiance levels (El-Sharkawy 1965), confirming the research at Cornell University. These species also showed a broad range of optimum temperatures, from 30 to 40 ^{o}C, for leaf photosynthesis (El-Sharkawy and Hesketh 1964b). Šesták *et al.* (1971) provided an extensive and critical review of early measurements of photosynthesis in various plant species and the methods used.

Nevertheless, while the above discussed pioneering research on C_4 photosynthetic system was done mainly by agronomists, it is puzzling to *see* the indifferent and careless attitude by some among the mainstream plant physiologists and biochemists who missed these discoveries. For example, in a paper dedicated to a famous biochemist-scientist, Martin Gibbs, upon his recent death, Black (2008) quotes the scientist saying that "those farmers know nothing about photosynthesis", meaning Cornell's agronomist Musgrave and his students. The early research on C_4 photosynthetic characteristics in crop and weed plants was done by agronomists of the Musgrave school (myself included), as well as by Canadian scientists (Tregunna *et al.* 1964, Forrester *et al.* 1966a,b); the research on the biochemistry involved was done by employees of the private Hawaiian and Australian Sugar Planters Association; tax payers and top level research managers of public-funded science should be aware of this lesson and remedy their system. Notable in particular among these agronomy-related scientists was the research of the New Zealand prominent biochemist Roger Slack in Australia. Clanton Black certainly knew that this research was going on and been reported upon when he was a post-doc at Cornell with Martin Gibbs. Later, Black got involved with plant photosynthesis research and since then he and his colleagues and former students at the University of Georgia, Athens, GA, USA, have repeatedly discussed results from the early agronomic research (Ithaca, New Haven, Tucson, and Davis, USA) without properly citing sources, thereby in effect assuming credit for what was done earlier; this does not reflect well on the history of American plant physiological-biochemical scientists. Other biochemical plant physiologists working on C_4 photosynthesis often cited Black and co-workers as the historical source for what was discovered. At the same time, Israel Zelitch built up a reputation with reviews and a book (*e.g.* Zelitch 1971, 1982) on what the agronomists did some being done at his location at Connecticut Agricultural Experiment Station, carefully citing most of the proper sources; the early agronomic work was also cited enough to win two citation classics. The American mainstream plant physiological-biochemical photosynthetic scientists also missed the Berkeley, CA, ground-breaking work on photosynthetic C_3 biochemistry (for the history of the elucidation of the C_3 cycle *see* Calvin 1989), as well as work on internode-dwarfing and flowering genes and their role in the photosynthesis of crop canopies and subsequent yield that impacted positively agricultural productivity and food security worldwide in the past five decades (the so-called “Green Revolution”, mainly funded by non-profit private agencies) (Borlaug 1983, El-Sharkawy 2005, 2006a, Begonia and Begonia 2007, Andre 2006). American mainstream plant physiological-biochemical photosynthetic scientists seem to have drifted somewhat during all this. When I once confronted that famous biochemist-scientist about all this during his talk in a 1989 photosynthesis meeting at New Haven, Connecticut, on the research involved, he was not ‘diplomatic’, as Black suggested he was, with third world scientists. Such attitude must be avoided; a lesson in humility has yet to be learned.

Rice (C_3 species)

Japanese scientists also had been studying in the field for sometime photosynthesis of *japonica* rice (*Oryza sativa*) as affected by environmental conditions and plant nutritional status (Murata 1961, 1969, Murata and Iyama 1963a,b). They measured leaf contents of protein, potassium, phosphorus, chlorophyll, and leaf age on attached rice leaves. Maximum photosynthetic rates exceeding 24 μmol(CO_2) m^{-2} s^{-1} were reported in normal air, 30 °C leaf temperature, and at high illumination for recently expanded leaves. As leaves aged, photosynthetic rates decreased with protein, chlorophyll, and potassium. Varietal differences in photosynthesis were recorded. Osada (1964) also found varietal differences in leaf photosynthesis among *indica* rices. This research on rice photosynthesis laid a foundation for further investigation conducted at the International Rice Research Institute (IRRI) in the Philippines (Tanaka *et al.* 1966, Yoshida 1972, Yoshida *et al.* 1972, Cock and Yoshida 1972, 1973). At high leaf area index (LAI), the improved short-stemmed variety *IR8* had higher leaf photosynthetic rate than the tall traditional variety *Peta* (Cock and Yoshida 1973). Also, genotypic variation in leaf photosynthesis of tall-early-and-late maturing *indica* rice was reported (Janardhan *et al.,* 1983). Differences in photosynthetic rates were positively associated with chlorophyll content, leaf nitrogen concentration, shorter interveinal distances, and specific leaf weight. These differences were heritable but with high phenotypic variances. Some traditional tall- and late-duration types like *Peta*, *Mashuri*, and GEB 24, with high heritability and genetic advances, were identified with higher photosynthetic rates. The easily determined interveinal distance was recommended as a selectable trait for higher photosynthetic efficiency. Path coefficient analysis of all plant traits tested for both tall and short types showed a residual effect that ranged from 19 to 24 % .This may imply effects of the photosynthetic enzymes.

Cotton (C_3 species)

El-Sharkawy *et al.* (1965) investigated the effects of greenhouse versus field culture on leaf photosynthetic rates of a wide range of cotton (*Gossypium* sp.) species and cultivars. Photosynthetic rates of cotton plants in the field or grown outdoors in large pots were almost twice as great as photosynthetic rates for similar plants grown in the greenhouse in winter. The plants grown outdoors or in the field showed much higher levels of saturating irradiance and higher and broader optimum leaf temperatures (30–40 °C), as compared to cotton grown in greenhouses (El-Sharkawy and Hesketh 1964b, El-Sharkawy *et al.* 1965). There were also two-fold differences among 26 cotton species, with the cultivated upland cotton *G. hirsutum* L. having the highest photosynthetic rates that exceeded 28 μmol(CO_2) m^{-2} s^{-1}. Some of the wild cotton species were also found to have high leaf photosynthetic rates under field conditions. Muramoto *et al.* (1965) measured in the field photosynthetic rates of varieties of cotton in two species, *G. hirsutum* L. and *G. barbadense* L., and they found no significant varietal differences. Crop growth rates were related more to seed size and early rapid rate of leaf area development than to photosynthetic rate. Later research in the USA with different leaf-types of upland cotton showed that Okra leaf-types (*i.e.*, smaller narrow leaves with large leaf lobes) had higher leaf photosynthetic rates per unit leaf area than values in the Normal broad-leaf varieties (Pettigrew *et al.* 1993, Pettigrew and Gerik 2007). Across a group of near-isogenic lines representing the different leaf types (*i.e.* Normal, Sub-Okra, Okra, Super Okra leaves and the F_1 of a cross between Normal and Okra types), lint yield was significantly correlated with the integrated canopy photosynthetic rate ($r^2 = 0.53$) (Wells *et al.* 1986), indicating the importance of canopy leaf area and the intercepted irradiances in carbon fixation.

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 PEPC and PEP kinase (this enzyme catalyses the regeneration of the CO_2 acceptor phospho*enol*pyruvate in the C_4 pathway) were greatly enhanced, whereas activity of Rubisco decreased. Nasyrov (1981) believed that the possibility exists to improve genetically photosynthesis by selecting for higher carboxylation efficiency of both Rubisco and PEPC. Moreover, Bhatt and Rao (1981) reported that the photosynthetic rates of cotton F_1 hybrids (both intraspecific *G. hirsutum* hybrids and interspecific *G. hirsutum* x *G. barbadense* hybrids) were comparable with those of maize and sorghum and much higher than the average rates in the parents. These hybrids also showed a high level of heterosis over the parents in terms of seed cotton yield as well as in number of produced fruiting branches and bolls.

Sunflower (C_3 species)

El-Sharkawy *et al.* (1965) reported that leaf photosynthetic rates were slightly depressed in indoor-grown sunflower (*Helianthus annuus*) plants, but plants grown in a shaded greenhouse had photosynthetic rates one half those of field-grown plants that were around 30 μmol(CO_2) m^{-2} s^{-1}. The high leaf photosynthetic rate at saturating irradiances and high optimum temperatures

(30–35 °C), the rapid early leaf area development, and the relatively high crop growth and net assimilation rates, were the main plant traits explaining the high productivity of sunflower cultivated in a warm climate and with high solar radiation (Muramoto *et al.*1965).

Weedy amaranth (C_4 species)

In the summer of 1964 at the University of Arizona Agricultural Experiment Station, the weed *Amaranthus palmeri* (known as palmer amaranth) was the first discovered dicotyledonous species with a rapid growth rate in sunny, hot climates (for more information on the discovery of this amaranth *see* El-Sharkawy and Hesketh comments in Current Contents on their Citation Classic, 1986). It had high field photosynthetic rates, under high irradiances and in normal air, equivalent to rates of the tropical C_4 monocotyledonous species such as maize, sorghum, and bermudagrass. These species possess the leaf Kranz anatomy features (Fig. 1; El-Sharkawy 1965) and did not release photorespiratory CO_2 into a rapid stream of CO_2-free air (El-Sharkawy *et al.* 1964, El-Sharkawy and Hesketh 1965, 1986). Elmore and Paul (1983) published a list of C_4 weeds, including 11 species of the genus *Amaranthus*, indicating their competitive and adaptive abilities.

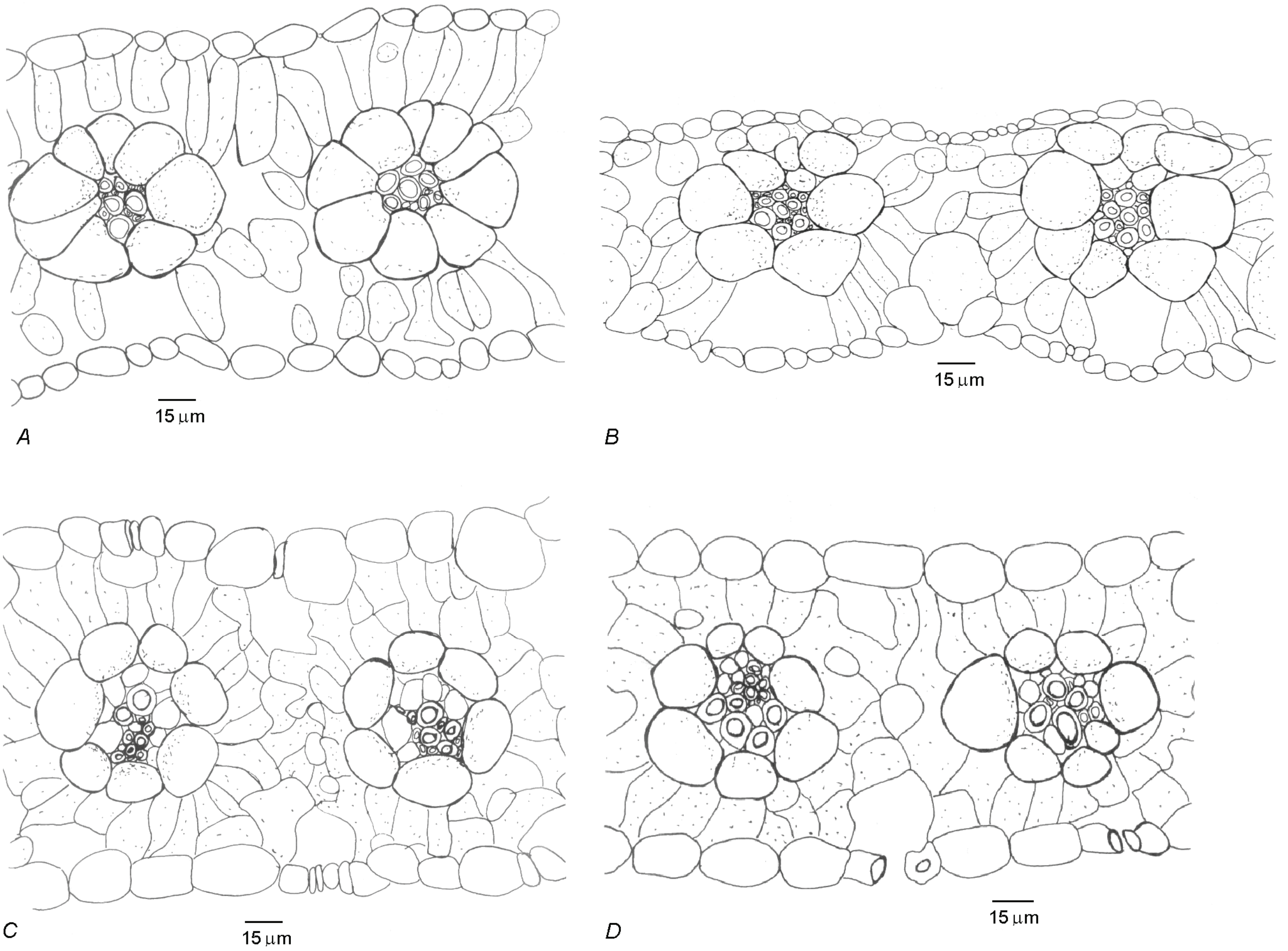

Fig. 1. Camera lucida drawings of transverse sections of leaves. *A*: Palmer weed (*Amaranthus palmeri*); *B*: Bermuda grass (*Cynodon dactylon*); *C*: Grain sorghum (*Sorghum bicolor*); *D*: Maize (*Zea mays*). Notice the arrangement of vascular bundles and the large compact and thick-walled cells of 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), also *see* El-Sharkawy and Hesketh (1965, 1986).

Cultivated grain amaranth (C_4 species)

The above-cited field research findings on leaf photosynthetic rates were crucial in separating different plant species according to their ability to assimilate carbon under normal conditions, on the basis of their leaf anatomy, and their environmental adaptation. Further research conducted in 1965 and 1966 at the University of California, Davis (El-Sharkawy *et al.* 1967, 1968) led to the discoveries of two more species within the genus *Amaranthus*, *i.e.*, the weedy amaranth (*A. retroflexus* L., known as redroot pigweed) and the cultivated grain amaranth (*A. edulis* Speg.) (syn. *A. caudatus* ‘edulis’) that possessed leaf Kranz anatomy and had photosynthetic rates similar to values observed in tropical C_4 grasses under high irradiances and 35–40 °C leaf temperature [≈ 40 μmol(CO_2) m^{-2} s^{-1}]. On one hand, no apparent photorespiration was detected in recently fully expanded leaves that developed under high irradiances, and as measured by CO_2 release into rapid streams of CO_2-free air. On the other hand, older leaves from plants grown under high irradiances and fully expanded leaves from plants grown under low irradiances leaked measurable amounts of CO_2 under illumination and in CO_2-free air, indicating the existence of photorespiration in amaranths. Thus, in old leaves and in shade-grown young leaves, photorespiratory CO_2 was partially reassimilated as compared to a complete reassimilation in young leaves developed under high illumination (El-Sharkawy *et al.* 1967, 1968). This conclusion was further confirmed by later research with *A. edulis* that had a rapid post-illumination CO_2 burst (indicative of photorespiration) (Björkman 1968, El-Sharkawy *et al.* 1967,1968); on *A. hybridus* where the sub-cellular organelles and enzymes required for the operation of the two-carbon photorespiratory cycle were present (Tolbert *et al.* 1969, Tolbert 1971); on *A. lividus* that released substantial amounts of photorespiratory $^{14}CO_2$ into CO_2-free air in light (Laing and Forde 1971); by the observed high CO_2 compensation point in greenhouse-grown *A. edulis* (Lester and Goldsworthy 1973); by the oxygen inhibition of photosynthesis of *A. graecizans* L. (Ku and Edwards 1980); and by the relatively high carbon isotope discrimination ratios (Δ = 14.9 ‰) and the high intercellular CO_2 concentration (C_i) (208 μmol mol^{-1}) in illuminated leaves of *A. tricolor* (Lin and Ehleringer 1983), and by the relatively lower quantum yields (as compared with C_4 monocot and dicot species) in *A. palmeri, A. retroflexus,* and *A. tricolor* (Ehleringer and Pearcy 1983), and by the rapid (within 2–3 minutes) post-illumination CO_2 burst in *A. retroflexus* (El-Sharkawy and Cock 1987). Moreover, by using *in situ* immunofluorescent localization of Rubisco, Castrillo *et al.* (1997) found that the enzyme was detected in the mesophyll cells of *A. caudatus* (syn. *A. edulis*) and *A. dubius*, indicating incomplete compartmentalisation of Rubisco in the bundle sheath cells. Furthermore, by using the immunogold-labeling technique in the leaf tissues of *A. tricolor*, Hong *et al.* (2005) reported that both Rubisco and Rubisco activase were localised in mesophyll cells as well as in outer vascular bundle sheath cells. These amaranth species had also broad optimum leaf temperatures for photosynthesis (30–40 °C), unsaturated responses up to the maximum irradiance levels tested, and increasing responses to CO_2 levels in leaf chambers up to 500 μmol mol^{-1} (El-Sharkawy *et al.*1964, 1967, 1968). Pearcy and Ehleringer (1984) reviewed the comparative ecophysiology of C_3 and C_4 systems and emphasised the phenomena of CO_2 leakage out of the bundle sheath cells, which is greater in NAD-dependent malic enzyme (NAD-ME) and PEP-carboxykinase (PCK) C_4-subpathway types than in NADP-dependent malic enzyme (NADP-ME) subpathway type, and greater in dicots than in monocots. The leakage can occur *via* apoplastic regions of the unsuberized bundle sheath cells. A manifestation of such CO_2 leakage is the relatively high carbon isotope discrimination ratio noted above in *A. tricolor* [also *see* photosynthetic responses to CO_2 in C_4 species with leaky bundle sheath cells (LeCain and Morgan 1998, Ziska *et al.* 1999)]. El-Sharkawy and Hesketh (1965) indicated the importance of CO_2 leakage from the whole leaf tissue in CO_2-free air in light as a factor underlying differences in photosynthetic rates among C_3 and C_4 species. Farquhar (1983) indicated the importance of CO_2 leakage from bundle sheath cells of some C_4 species in estimating the level of CO_2 within these cells, attributed to the CO_2 concentrating mechanism, as well as on carbon isotope discrimination. Moreover, the leakiness of the bundle sheath cells can complicate modeling the photosynthetic process at the biochemical level (Boote and Loomis 1991, von Caemmerer and Furbank 1997).

This research helped in elucidating factors underlying photosynthetic characteristics of different plant species and in enhancing photosynthesis research in general. For example, Hatch, Slack and co-workers (Hatch and Slack 1970) repeated their work done on sugarcane CO_2 fixation by using *A. edulis* [they obtained seeds of the same cultivar researched by El-Sharkawy *et al.* (1967, 1968) for photosynthetic characteristics at the University of California, Davis, USA]. Also, *A. edulis* is extensively used today as a model plant system in studying, *via* mutations deficient in genes controlling some C_4 enzymes, the genetic base of the C_4 system (Dever *et al.* 1997, Maroco *et al.* 1998, Sheen 1999, Bailey *et al.* 2000).

Plant traits associated with leaf photosynthetic characteristics among C_3 and C_4 species

Leaf anatomical characteristics and their relations to gas exchange

For the first time in the modern history of photosynthesis research, leaf anatomical parameters were investigated in a wide range of plant species (15 monocotyledon and dicotyledon species, representing important field crops) in relation to photosynthetic characteristics (El-Sharkawy 1965, El-Sharkawy and Hesketh 1965). Thin-leaf transverse and tangential sections of the same plants used for gas exchange measurements were made and examined with an ordinary microscope. Camera lucida drawings were made and the method and equations proposed by Turrell (1936) were used to calculate the internal cell surface areas. The Turrell methodology and equations were modified in the case of monocot leaves, because the original equations were satisfactory only for dicot leaves. The work was tedious in nature and required painstaking effort in sectioning, staining, and in carefully drawing the internal cellular structure and tissue configurations. But the effort was rewarding, because it led to the discovery of the many anatomical features separating plant species with different photosynthetic characteristics.

Discovery of leaf Kranz anatomy and its implications for photosynthesis and photorespiration in C_4 versus C_3 plants

Fig. 1 illustrates camera lucida drawings of transverse sections of the monocot leaves of maize, sorghum, and bermudagrass, and the dicot palmer weed, *Amaranthus palmeri* (*see* also El-Sharkawy 1965, El-Sharkawy and Hesketh 1965, 1986). In these species, having the greatest leaf photosynthetic rates as compared to the many other species studied, all had vascular bundles on both sides of the main vein that were tightly enclosed with a chlorenchymatous sheath consisting of large thick-walled and compact cells without air spaces. The chlorenchymatous mesophyll cells were longer in shape and smaller in diameter and were in close contact with- and radiating from- the vascular bundle sheath, and hence, this formed a circle with many airspaces between cells. This leaf structure was in contrast with that observed in other dicot and monocot species having lower photosynthetic rates, such as sunflower, cotton, soybean (*Glycine max*), sugarbeet (*Beta vulgaris)*, and oats (*Avena sativa*). The vascular bundle sheath cells in the less efficient species, in terms of CO_2 uptake, were either void of chloroplasts or had a few, small chloroplasts (El-Sharkawy 1965). Moreover, the cells of the vascular bundle sheath in the less efficient species were smaller, irregularly arranged, and were not distinguishable from the rest of the chlorenchymatous mesophyll cells. In sorghum, maize, bermudagrass, and amaranthus, most of the chloroplasts in the bundle sheath cells were located centripetally (*i.e.*, located at the inner end of the cells).

This unique leaf structure that was first observed at the University of Arizona, Tucson (El-Sharkawy 1965, El-Sharkawy and Hesketh 1965, 1986), and later at the University of California, Davis, in efficient tropical grasses and in amaranthus (*A. palmeri*, *A. retroflexus, A. edulis*) is now called 'C_4 leaf Kranz anatomy' after the term coined by the German botanist Haberlandt (1904), who conducted original anatomic work in the late 19th century and found similar structures in sugarcane leaves. Haberlandt's book was written in German and was apparently not known in English speaking countries, including the USA, until the work conducted at the University of Arizona was published (El-Sharkawy *et al.* 1964, El-Sharkawy 1965, El-Sharkawy and Hesketh 1965). Haberlandt (1904) speculated on the possible function of the chloroplasts observed in sugarcane vascular bundle sheath cells, stating "whether there is a division of labor between the chloroplasts in the vascular bundle sheath cells and those in the surrounding mesophyll cells." Such speculative and perhaps intuitive remarks awaited another century to be proved to be true. It was found that, after the unique anatomical features of the photosynthetically efficient tropical grasses and amaranthus species had been revealed (El-Sharkawy *et al.* 1964, El-Sharkawy 1965, El-Sharkawy and Hesketh 1965), the leaf Kranz anatomy is essential for separating the key C_4 enzyme PEPC, which is confined in the cytosol of mesophyll cells, from the key C_3 Rubisco, which is confined in the stroma of the chloroplasts of the bundle sheath cells. Such compartmentalisation is necessary for the proper functioning of both the primary fixation of CO_2 into C_4 dicarboxylic acids catalysed by PEPC and the decarboxylation of these acids within the bundle sheath cells, which is required for the operation of Rubisco (although deviations from this generalisation now exist). This coordination of function and structure conferred an adaptive advantage for the C_4 plants. In this system, CO_2 concentration resulting from the decarboxylation of C_4 acids, could be elevated around Rubisco, and, hence, restrains the photorespiratory cycle by reducing the oxygenase reaction *via* Rubisco, and at the same time, enhances the carboxylation reaction (Hatch and Slack 1970, Jackson and Volk 1970,Raven, 1972, Black 1971,1973, Laetsch 1974). Laetsch (1974) stated that "perhaps the first investigation linking Kranz anatomy with physiological aspects of photosynthesis was conducted by El-Sharkawy and Hesketh in 1965. They found that species with high photosynthetic rates, which did not leak CO_2 to the environment in the light, had Kranz anatomy. These taxa are now known to be C_4 plants." Other researchers (Downton and Tregunna, 1968, Hatch and Slack, 1970, Black, 1971, 1973, Black *et al.*, 1976, Ray and Black, 1979, Hatch, 1992a,b), however, overlooked the pioneering research done by El-Sharkawy and Hesketh (1965) and wrongly attributed the discoveries of Kranz leaf anatomy in relation to C_4 plants to researchers who had nothing to do with them. This obvious negligence on the part of some mainstream photosynthetic plant physiologists/biochemists and the recent outrageous comments by a prominent biochemist-scientist on the pioneering agronomists in the field of photosynthetic research (Black, 2008) are not only counterproductive but unacceptable by any means. Scientists must adhere to scientific methods and ethics.

The location of PEPC in mesophyll cells also helps in refixing any photorespiratory CO_2 that may leak from the bundle sheath cells, a mechanism explaining the absence of CO_2 release into a rapid stream of CO_2-free air from illuminated leaves of maize and *Amaranthus* spp. under a wide range of irradiances and temperatures (El-Sharkawy *et al.* 1964, El-Sharkawy 1965,El-Sharkawy and Hesketh 1965, El-Sharkawy *et al.* 1967, 1968, Rathnam1977, El-Sharkawy and Cock 1987). El-Sharkawy and Hesketh (1965) reported that C_3 plants, such as cotton and sunflower, had leaf photosynthetic rates (at high irradiances, high humidity in leaf chambers, and at optimum temperatures, conditions that kept stomata wide open during measurements) similar to those of C_4 maize at elevated external CO_2 concentrations [$\approx$ 1600 $\mu mol(CO_2)\ mol^{-1}$]. For example, sunflower rates increased from about 30 to 60 $\mu mol(CO_2)\ m^{-2}\ s^{-1}$ when CO_2 was increased from 310 to 1600 $\mu mol\ mol^{-1}$, whereas rates of maize leaves increased from about 40 to 64 $\mu mol(CO_2)\ m^{-2}\ s^{-1}$, when exposed to the lower and higher CO_2 concentrations, respectively. In another experiment with grain amaranth grown at high irradiances in cabinet-controlled environments, El-Sharkawy *et al.* (1968) reported that photosynthetic rates in recently fully expanded leaves increased from about 40 to 60 $\mu mol(CO_2)\ m^{-2}\ s^{-1}$ at 310 and 500 $\mu mol\ mol^{-1}$ of external CO_2 concentrations, respectively. Also responses to CO_2 concentration were dependent on leaf temperature and irradiance levels. These early findings indicated that leaves of both C_3 and C_4 plants responded to short-term increases of CO_2, with greater relative responses in the former, probably because the oxygenase reactions of Rubisco were restrained as well as its carboxylase reactions were enhanced. Ziska *et al.* (1999) reported that leaf photosynthetic rates in three C_4 species (*Flaveria trinervia, Panicum miliaceum,* and *Panicum maximum*), grown at ambient and double-ambient CO_2 levels, increased with increasing CO_2, were light and temperature dependent, and, hence, confirmed earlier observations by El-Sharkawy and Hesketh (1965) and El-Sharkawy *et al.* (1968). These species are known to show different rates of CO_2 leakage out of their bundle sheath cells.

In later work, however, by several plant physiologists and biochemists, it was suggested that the higher rates in C_4 leaves in normal air and optimal temperatures and irradiances, as compared to rates in C_3 leaves, were due mainly to a much higher CO_2 concentration around Rubisco in the bundle sheath cells that saturated the carboxylation capacity (Hatch and Slack 1970, Black 1973, Laetsch 1974). On one hand, in some of this research, the C_4 plants showed little or no response to elevated CO_2 above the ambient concentration and their photosynthetic rates were not sensitive to variations in oxygen levels below that in normal air as compared to C_3 plants, thus implying that photorespiration is absent in C_4 plants (*see*, for example, Forrester *et al.* 1966a,b, Chollet and Ogren 1975, Ehleringer and Björkman 1977, Ku and Edwards 1978, Edwards and Walker 1983, Ogren 1984, Edwards *et al.* 1985, Hatch 1987). [In these early studies rates were compared normally at 21 % versus 1–2 % O_2, but later it was found that photosynthetic rates of C_4 plants were enhanced when measured at 5-10 % O_2, relative to rates in normal air or in 1–2 % O_2, which indicates the existence of photorespiration. The explanations given for these responses were that photosynthesis is inhibited in normal air by photorespiration and inhibited at 1–2 % O_2 probably by limitations of the light reactions Dai *et al.*1993, Maroco *et al.* 1998)]. On the other hand, research with oxygen isotopes (^{18}O) revealed that illuminated maize leaves absorbed substantial amounts of oxygen, an indication that photorespiration exists in C_4 plants but at lower rates compared to C_3 plants (Jackson and Volk 1969, 1970, Volk and Jackson 1972, de Veau and Burris 1989). Irvine (1970) reported evidence for the existence of photorespiration in sugarcane, maize, and sorghum leaves using the $^{14}CO_2$ labeling method in a closed system to trace released photorespiratory CO_2 under high irradiances. In addition, research with various species of C_4 *Amaranthus*, as discussed above, clearly illustrated the existence of photorespiration. Thus, it can be concluded that the apparent lack of CO_2 release into a rapid stream of CO_2-free air and the very low CO_2 compensation point often observed in C_4 species, as compared to the much higher values in C_3 species (Meidner 1962, Moss 1962, Tregunna and Downton 1967, Krenzer *et al.* 1975) are manifestations of the ability of these plants to refix/recycle their photorespiratory CO_2 before it can leak out of their leaves, as suggested in earlier investigations (El-Sharkawy and Hesketh 1965, El-Sharkawy *et al.* 1964, 1967,1968, Mansfield 1968, Stoy 1969, Ogren 1984).

Furthermore, earlier estimation by modeling of the extent of the CO_2 concentration around Rubisco in bundle sheath cells of C_4 plants, *via* the so-called 'CO_2-concentrating mechanism,' ranged from about 2000 to more than 18 000 $\mu mole\ mol^{-1}$ (Furbank and Hatch 1987, Jenkins *et al.* 1989). Such very high CO_2 concentrations seemed unrealistic, and leakage of CO_2 from bundle sheath into mesophyll tissues, even with partially suberized and thick-walled cells (Hattersley and Browning 1981), is likely to occur (Ehleringer and Pearcy 1983, Pearcy and Ehleringer 1984, LeCain and Morgan 1998, Ziska *et al.* 1999). Later research, however, indicated more realistic estimates of CO_2 levels in bundle sheath cells of maize leaves that were less than 1000 $\mu mol\ mol^{-1}$ (Dai *et al.* 1993), and, hence, confirmed the earlier findings by El-Sharkawy and co-workers where they reported that at an external CO_2 ranging from 500 to 1600 $\mu mol\ mol^{-1}$ differences in photosynthetic rates among C_3 and C_4 species practically disappeared. Recent research, using the sophisticated Free-Air-CO_2 Enrichment (FACE) method, concerning the response of field-grown crops to elevated CO_2 ($\approx 550\ \mu mol\ mol^{-1}$), as compared to rates at ambient air, demonstrated photosynthetic enhancement of 10–20 % in some C_3 crops above rates in normal air, whereas in C_4 crops the enhancement was less than 10 % (Long *et al.* 2006). This range of percent enhancement in the field is in contrast to higher enhancement rates previously reported for indoor-grown potted-plants as well as in field-grown plants enclosed normally in open-top chambers (Kimball *et al.* 2002). Assuming uniformity in the CO_2 profile within the FACE-grown crops with elevated CO_2, discrepancies in these cases could possibly be attributed, among other plant and environmental factors, to lower air humidity in the case of FACE-grown crops as compared to indoor and enclosed plants, and, hence, to partial stomatal closure that would have led to lower C_i. Thus, in these complex and expensive FACE trials that require large interdisciplinary/interinstitution team of researchers, it would be of importance to extensively study effects of edapho-climatic conditions, particularly soil-water-nutrient factors and air humidity within crop canopy, and to construct complementary crop-soil-atmosphere sub-models. Such information is essential in order to elucidate mechanisms underlying responses to elevated CO_2 in the field, to maximise benefit/cost ratio of doing research as well as to reasonably predict the effect of global climate change on agricultural productivity (Rosenzweig and Parry 1994, El-Sharkawy 2005, 2006a, IPCC 2006). In any case, however, these data indirectly illustrate the invalidity of the high estimates of CO_2 around Rubisco in bundle sheath cells of C_4 plants *via* the so-called 'CO_2-concentrating mechanism' that were based on unrealistic theoretical models. Yet, while the later research by the mainstream plant physiologists and biochemists generally confirmed what was done at Tucson, AZ, and at Davis, CA, comparing C_4 versus C_3 species, they almost unanimously (but with few exceptions) did not cite that work. Perhaps, some researchers have not known about the original discoveries and when and where they were made, particularly in times when there is an overproduction of papers (the publish or perish concept!) and young scientists do not take the trouble to look for original literature. However, searching and assessing critically scientific literature is a *fundamental* requirement for doing research and in fulfilling a basic element in the scientific method that should be adhered to. Another unacceptable excuse which I often encounter some researchers saying is that "the original discoveries are too old and are so distant in time that citing them is not of importance to their work".

Garfield (1991) wrote on the problem of literature citation violations and omissions by researchers coining it "*bibliographical negligence and citation amnesia*" and suggested that authors should sign a pledge or oath that they have done a minimal search of the literature and that to the best of their knowledge there is no other relevant work not being cited (*see* Gallagher, 2009). Nevertheless, I believe that not only the researchers are responsible for such *citation amnesia* but also the editors of scientific journals and peer reviewers who are supposedly vigilant of the publishing process without prejudice. Thus, it might be advisable in this regard to eliminate the journals restrictions often imposed on the number of allowed literature citations provided that they are relevant to the work under consideration. The effort made by Šesták and his colleagues, before the advent of the internet, over the years to compile long lists of literature related to photosynthesis research and regularly published in Photosynthetica was a model for facilitating the tracking of relevant works. The electronic searching tools, now common via internet, must further enhance tracking relevant literature that are digitally available.

Another adaptive advantage of the leaf Kranz anatomy is that the shorter distances between the veins of C_4 leaves (Fig. 1) make it much easier for transport of photosynthetic products *via* the large bundle sheath cells and it facilitates the loading of them efficiently into the enclosed phloem tissues (Crookston and Moss 1974, Giaquinta, 1983 Wardlaw 1990). This interveinal trait was also negatively associated with photosynthetic rates among *indica* rice genotypes (Janardhan *et al.* 1983). Fast translocation of photoassimilates would probably enhance photosynthesis in source leaves *via* mitigating possible feed-back inhibition effects by carbohydrate accumulation (Neales and Incoll 1968, Ho 1988, Wardlaw 1990). The ontogeny and genetic and environmental regulations of Kranz anatomy were reviewed by Nelson and Langdale (1989, 1992) and Langdale and Nelson (1991). Compared to advances in the physiological and biochemical components of the C_4 syndrome in terrestrial plants, the understanding of the structural components and its regulatory mechanisms are still awaiting much research, which perhaps can be aided by advances in molecular biology tools.

This pioneering research in unravelling the C_4 Kranz anatomy was crucial and laid the foundations for consequent physiological, anatomical, and biochemical research (Begonia and Begonia 2007). The equally important pioneering biochemical work on the nature of the primary products involved in CO_2 fixation in sugarcane leaves done in Hawaii was published in the journal *Plant Physiology* (Kortschak *et al.* 1965) at about the same time the research done in Arizona was published in *Crop Science*. The research was presented at several annual meetings of professional societies, including those held by the American Society of Agronomy (El-Sharkawy and Hesketh 1964b, 1965, El-Sharkawy *et al.* 1964, El-Sharkawy 1965). The earlier Russian work on the nature of the primary products of CO_2 fixation in maize leaves was published in Russian (Karpilov 1960). This work went unnoticed in English speaking countries until the biochemical work of Hatch and Slack (1966), which was done in Australia on sugarcane biochemical CO_2 fixation and was based on the Hawaiian discoveries, had been published (*see*, for example, Hatch 1992a,b, for more information on the story of the Karpilov discoveries and how they were misinterpreted).

Relations between gas exchange and the internal anatomy of leaves

Table 1 contains information about several leaf characteristics of some of the studied species (El-Sharkawy and Hesketh 1965). There were large interspecific differences in leaf thickness, diameter of palisade cells in dicot leaves, diameter of mesophyll cells in monocot leaves, percent volume of air, internal exposed surface per unit external leaf surface (S) (calculation based on both sides) and internal surface per cell volume [S/V or the cell ratio = S ÷ (% cell volume x leaf thickness in μm)]. Across 15 species, significant negative correlations were found between leaf photosynthetic rate (as measured in normal air, high irradiance, and optimum temperature) and diameter of palisade cells or mesophyll cells ($r = -0.77$, $p< 0.01$) and with leaf thickness ($r = -0.51$, $p< 0.05$), but positive correlations with S/V or the cell ratio ($r = 0.84$, $p< 0.01$). Also, there was strong negative correlation between diameter of palisade or mesophyll cells and S/V cell ratio ($r = -0.84$, $p< 0.01$). Leaf photosynthesis was not significantly correlated with S. From these results it seemed that a physical relationship exists between the size of photosynthetic cells, the internal exposed surface per volume of cell, and leaf photosynthetic rate. The smaller the diameter of the palisade or mesophyll cells the larger S/V or the cell ratio. The positive correlation coefficient between S/V or the cell ratio and the photosynthetic rate indicates that the more internal surface exposed to the air the higher the photosynthetic rate will be. Thus, CO_2 will diffuse faster in leaves with higher S/V or the cell ratio. The C_4 species had smaller internal resistances to CO_2 diffusion [*i.e.*, mesophyll resistances (r_m) and intracellular resistances (r_k)] than the C_3 species (Table 1), and, hence, a higher carboxylation capacity and leaf water use efficiency (*i.e.*, photosynthesis/transpiration) (El-Sharkawy *et al.* 1964, 1967, El-Sharkawy 1965, El-Sharkawy and Hesketh 1965, 1967, Ludlow 1976).

The higher photosynthetic rates in C_4 plants are partially attributed to both a higher carboxylation capacity *via* restraining the oxygenase reaction by Rubisco at elevated CO_2 and to the ability of PEPC in mesophyll cells to reassimilate photorespiratory CO_2 before it can leak outside the leaves. The combined favorable effects of the leaf structure and biochemical traits in C_4 species underlie the high productivity of these plants as well as their higher crop water use efficiency, compared to values obtained in C_3 plants, particularly in hot dry environments (Briggs and Shantz 1914, Shantz and Piemeisel 1927, Stanhill 1986, Boyer 1996). However, exceptions to this generalisation occurred in natural ecosystems as reported by Pearcy and Ehleringer (1984). Such exceptions are well illustrated by the winter C_3 ephemeral *Camissonia claviformis* that showed high photosynthetic rates (probably because of high stomatal conductances) comparable to rates in C_4 plants, when measured under its favorable environments, and by the discovery of the C_4 syndrome in the subtropical shade-adapted tree *Euphorbia forbesii*, which had a very low maximum photosynthetic rate at much reduced saturation irradiances. These authors concluded, "When similar ecological forms are compared under similar environmental conditions, the value of the C_4 pathway is not necessarily manifested in a higher photosynthetic rate or productivity rate."

In the C_3 perennial ryegrass (*Lolium perenne)*, Wilson and Cooper (1969a,b, 1970) reported a negative correlation between leaf photosynthetic rate and mesophyll cell size and found that selected lines with smaller mesophyll cells possessed greater CO_2 uptake rates, which were highly heritable among lines. Moreover, the selected lines had heavier seeds that resulted in faster seedling growth rates and in greater plant mass. Dunstone and Evans (1974) found that leaf photosynthetic rates vary widely among species of *Triticum*. The rates were higher in the diploid wild wheat than rates found in the cultivated hexaploid genotypes. The photosynthetic rates were negatively associated with the size of mesophyll cells. The size of mesophyll cells was smaller in the diploid than cell size in hexaploid wheats. Similar findings were reported in Germany with six wheat genotypes representing different levels of ploidy (Lieckfeldt 1989). Also, LeCain *et al.* (1989) compared two near-isolines of winter wheat (*T. aestivum)* and found that the higher leaf photosynthetic rate in the semidwarf line, as compared to rates in the tall line, was associated with smaller and numerous mesophyll cells with greater exposed internal mesophyll surface area. Such findings corroborate the negative trends observed between leaf photosynthesis and the diameter of palisade or mesophyll cells encountered across C_3 and C_4 species as reported earlier by El-Sharkawy (1965) and El-Sharkawy and Hesketh (1965).

Nobel and co-workers (1975) also emphasised the importance of leaf anatomy in relation to leaf gas exchanges and developed a model to estimate the internal exposed surface areas of the mesophyll by expressing it based only on one leaf surface. The model also separates components of gas exchange diffusion resistances in the gas phase within the mesophyll from the liquid components and the carboxylation capacity, which are confounded in the term (r_m). In this model the intracellular resistance to CO_2 diffusion (R_{CO2}^{cell}) is given as: $R_{CO2}^{cell} = (R_{CO2}^{mes}) (A^{mes}/A)$; where R_{CO2}^{mes} is the mesophyll resistance as estimated from leaf gas exchange using Gaastra's resistance model (Gaastra 1959), and A^{mes}/A is the ratio of exposed mesophyll surface area to the external leaf area of one leaf side. Applications of the model have yielded useful information about gas exchanges as affected by plant and environmental factors (Nobel 1980, Longstreth *et al.* 1980, El-Sharkawy *et al.* 1984, Patton and Jones 1989). Moreover, Patton and Jones (1989) studied the relationships between leaf anatomy and photosynthesis in unshaded field-grown cultivars of willow trees (*Salix viminalis, S. burjatica,* and *S. dasyclados*). They found that irradiance-saturated leaf photosynthetic rates in normal air were positively correlated ($r^2 = 0.81$, $p < 0.05$) with A^{mes}/A. El-Sharkawy *et al.* (1984) found that differences in photosynthetic rates among 10 cassava cultivars from different habitats could be accounted for by differences in both A^{mes}/A and R_{CO2}^{cell}. There was also a significant positive correlation between leaf photosynthetic rate across a group of C_3 and C_4 plants and A^{mes}/A ($r^2 = 0.84$, $p < 0.01$). By applying the model to data of Table 1, a highly significant negative correlation existed between leaf photosynthetic rate and R_{CO2}^{cell} ($r^2 = 0.88$, $p < 0.01$). Moreover, R_{CO2}^{cell} values were similar to values of r_k (Table 1) for both C_4 and C_3 species, as estimated by the El-Sharkawy *et al.* (1967) model, suggesting the validity of both models in estimating intracellular resistances to CO_2 diffusion. These findings highlight the importance of the internal anatomical features as well as the biochemical components of leaves in controlling differences in photosynthetic rates across species and cultivars. Moreover, taking into consideration the effects of anatomy of non-uniform leaf photosynthesis or non-uniform stomatal opening and distribution on leaf surfaces (Parkhurst 1978, Terashima 1992) on estimated values of stomatal, mesophyll, and intracellular CO_2 diffusion resistances should further

Table 1. Photosynthetic rate (P_N), mesophyll CO_2 diffusion resistance (r_m), intracellular CO_2 diffusion resistance (r_k), and leaf anatomical characteristics among C_4 and C_3 species. Data from El-Sharkawy 1965, El-Sharkawy and Hesketh (1965), and El-Sharkawy *et al.* (1967). Leaf photosynthetic rate across 15 monoct and dicot species was significantly correlated with: internal exposed mesophyll surface area per volume of cell (S/V) ($r = 0.84$, $p < 0.01$); diameter of mesophyll or palisade cells ($r = -0.77$, $p < 0.01$); leaf thickness ($r = -0.51$, $p < 0.05$). S/V was correlated with diameter of mesophyll or palisade cells ($r = -0.84$, $p < 0.01$). $^*r_m$ was calculated from leaf CO_2 uptake rates in normal air, optimum temperatures, and saturating irradiance, and the rates of H_2O losses using Gaastra's (1959) model of gas diffusion resistances. The estimated r_m combines the gaseous phase within mesophyll tissue, the wall/intracellular liquid phase, and the biochemical carboxylation reactions. $^{**}r_k$, was calculated using El-Sharkawy *et al.* (1967) model. The estimated r_k combines intracellular resistances to CO_2 diffusion in liquid phase and charachteristics related to carboxylation reactions. Equations, assumptions, and measured parameters used for calculations of r_m and r_k are given in the cited references. S – internal exposed mesophyll surface area per external leaf area (two sides); LT –leaf thickness; CD – mesophyll or palisade cell diameter; VA – relative volume of intercellular spaces to total mesophyll.

Species	Pathway	P_N [μmol(CO_2) m^{-2} s^{-1}]	LT [μm]	CD [μm]	VA [%]	S	S/V	r_m^* [s cm^{-1}]	r_k^{**} [s cm^{-1}]
Maize *Zea mays*	C_4	40	106	7	34	10	1.9	1.0	14.8
Grain sorghum *Sorghum bicolor*	C_4	40	127	7	35	10	1.8	-	15.6
Bermudagrass *Cynodon dactylon*	C_4	40	82	7	33	7	2.1	-	-
Palmer weed *Amaranthus palmeri*	C_4	40	140	8	56	9	2.0	-	-
Upland cotton *Gossypium hirsutum*	C_3	28	155	9	25	10	1.0	2.9	29.0
Sunflower *Helianthus annuus*	C_3	32	245	8	46	16	1.2	1.5	34.0
Soybean *Glycine max*	C_3	18	133	12	47	7	1.0	5.5	-
Oats *Avena sativa*	C_3	23	180	13	44	8	0.8	4.1	-
C_4/C_3		1.6	0.64	0.69	1.0	0.88	2.0	0.29	0.49

improve modeling the path of CO_2 within leaf mesophyll. Parkhurst (1977) suggested a complex three-dimensional model (as compared to the simpler Gaastra's model 1959) for estimating gas diffusion resistances. Application of the three-dimensional model may improve estimates of gas diffusion resistances, and, hence, estimates of leaf mesophyll CO_2 concentrations.

Kubinová (1993) and, recently, Albrechtová *et al.* (2007) described a novel method for measuring mesophyll anatomical characteristics using fresh leaf sections in combination with stereological and confocal microscopy assessment. It has been tested with pine needles as well as with bifacial-leaves (dicot leaves with typical palisade and spongy tissues) and grass leaves for measuring mesophyll surface area, volume density, and other cell-related parameters. This new technique apparently has some advantages over the old Turrell method (1936), that is, the new method avoids tissue fixation, paraffine embedding, sectioning, and staining that might cause deformations in leaf tissues. Emphasis should be given to leaf anatomical studies that are conducted along with photosynthetic physiological and biochemical investigations. Anatomical research again played a crucial role in unraveling recently the existence of functional C_4 photosynthesis in single cells of *Bieneria cyclopetra* and *Borszczowia aralocaspica* (Chenopodiaceae), which lack typical C_4 leaf Kranz anatomy (Edwards *et al.* 2004, Voznesenskaya *et al.* 2004). In these species, compartmentalisation of the key C_4 and C_3 enzymes occurs in cytosols as well as in dimorphic chloroplasts located at opposite ends within the same cell. This important finding represents a novel structural variant in the evolution of C_4 photosynthesis and may lead to more discoveries in the near future. Another variant of C_4 leaf structure without bundle sheath cells found in typical Kranz anatomy was reported in the succulent leaves of the dicot *Suaeda monoica* (Shomer-Ilan *et al.* 1975). In this species, leaf anatomy is characterised by two types of chlorenchymatous cell layers underneath the epidermis: an outer layer with relatively small chloroplasts and an inner layer with larger and centripetally located choloroplasts, which surrounds the water tissue. It was suggested that the coordinated functions of the C_4 and C_3 cycles occur between these two chlorenchymatous cell layers without the need of the bundle sheath cells.

Comparative productivity and plant-water relations in C_4 and C_3 systems

The discovery of the C_4 syndrome and the much higher photosynthetic rates coupled with lower stomatal conductances to gas diffusion in plants possessing this system, as compared to the C_3 system, has stimulated research on comparative crop productivity, agroclimate, and plant-water relations [*see*, for example, the recent review by Steiner and Hatfield (2008) on historical advances in agroclimatology and soil-plant-water-atmosphere relations]. Attempts were made to investigate if there are relationships between photosynthetic rates and yield in various crops. In most of this early research no unequivocal evidence could be found between instantaneous measurements of maximum leaf photosynthetic rate (measured in normal air with saturation irradiances and near optimum temperatures) and crop yield (Elmore,1980, Gifford and Evans 1981). Zelitch (1982) reviewed these early attempts and concluded that "Crop yield is closely related to net photosynthetic assimilation of CO_2 throughout an entire season, but instantaneous measurements of photosynthesis may be misleading." Also, Zelitch stressed the importance of improving other crop traits that may affect both yield and photosynthesis such as sink capacity for utilizing and storing photoassimilates. El-Sharkawy (2004, 2005) emphasised the importance of extended field measurements of photosynthesis in relation to productivity and cautioned against using data collected on inappropriately grown plants, particularly when used for crop modeling. Moreover, El-Sharkawy (2006a) reviewed recent research on photosynthesis of various crops and presented many examples on positive relationships between yield and field-measured photosynthetic rates, whether of single leaves or in whole canopy. Gifford (1974) in his early report compared the C_3 and C_4 systems with regard to maximum leaf photosynthesis in relation to yield and stated, "The large potential advantage of the C_4 mechanism at the biochemical level is progressively attenuated in moving from the microscopic to the macroscopic parameters until, at the level of crop growth rate, there is no apparent difference between the best examples of the two groups when grown in their preferred natural environments." Loomis and Gerakis (1975) and Monteith (1978) in their reassessments of maximum short-term crop growth rates (CGR) for C_3 and C_4 crops criticised Gifford's report. These authors discarded the unrealistically high C_3 CGR that were reported in the literature and were included in Gifford's analysis [in the case of sunflower CGR as high as 79–104 g m^{-2} d^{-1} , and carrot (*Daucus carota*) CGR ≈ 146 g m^{-2} d^{-1}] because of inadequate control of edaphoclimatic conditions during experiments, plot border effects, and deficiency in sampling these two crops. When these outlaw values were eliminated from calculations, large differences in maximum short-term CGR became apparent [*e.g.*, C_4 species including sudangrass (*Sorghum sudanense*), maize, and elephantgrass had maximum rates of 51–54 g m^{-2} d^{-1} versus 27–37 for C_3 species as soybean (*Glycine max*), potato (*Solanum tuberosum*), sugarbeet (*Beta vulgaris*), and rice]. Also, differences between the two systems in CGR and total productivity persist in seasonal long-term estimates. For example, under favorable environments for the two systems, estimates from the literature of long-term CRG values for C_3 crops normally range from ≈ 8 to 15 (mean = 12±3) g m^{-2} d^{-1} and from ≈ 12 to 25 (mean = 21±5) for C_4 crops. There is, however, a crossover between the two systems when crops are grown under unfavorable environments [for more comparisons in productivity between the two photosynthetic systems in natural ecosystems *see* Pearcy and Ehleringer (1984), Bazzaz (1990)]. At higher latitudes, for example, with lower temperatures and irradiances, C_3 species outperform the C_4 ones [for more information on comparative productivity of C_3 and C_4 cultivated species, *see* Loomis *et al.* (1971), Ludlow (1985), Loomis and Connor (1992)].

Muramoto *et al.* (1965) studied the relationships among rate of leaf area development, photosynthetic rate, and rate of dry matter (DM) production among several genotypes of *Gossypium* sp., tropical grasses, and sunflower. Net assimilation rate (NAR) in cotton ranged from 9.7 to 14.7 g(DM) m^{-2}(leaf area) d^{-1} as compared to rates as high as 21.4 in sunflower and from 13.7 to 14.3 for grain sorghum and maize. Thus, it appears that sunflower has superior net assimilation rate than cotton and the C_4 crops. This apparent superiority in NAR is because sunflower plants possess larger horizontal leaves that intercept higher amounts of irradiances as compared to the smaller values normally intercepted by mostly vertical leaves in tropical grasses. The greater maximum CGR observed in tropical C_4 grasses than values observed in sunflower must then be attributed to both an inherent superior photosynthetic rate and larger leaf area index in the former species. The higher NAR in sunflower than in cotton was due mainly to greater leaf photosynthesis of sunflower (El-Sharkawy, 1965, El-Sharkawy and Hesketh, 1965).

Yet, Evans (1993) believes that the inherent advantages of the higher leaf photosynthetic rates in C_4 plants could become less important at the crop canopy level, because of the complexity of the many interactive plant and environmental factors that may erode the biochemical advantage of the C_4 system as compared to the C_3 system. He stated, "It is not the record CGR that should be compared but typical rates for crops growing under characteristic conditions."

Almost five decades before the discoveries of the physiological and biochemical characteristics of the C_4 system were made, research on plant-water relations conducted by using large containers in the field in the USA (Briggs and Shantz, 1914, Shantz and Piemeisel, 1927) revealed the large differences between plant species possessing the two photosynthetic systems. Crops with the C_4 system such as maize, sorghums (*Sorghum* spp), sudangrass, and *Panicum miliaceum* had a mean transpiration ratio of ≈ 300 kg(H_2O) kg^{-1}(DM), as compared to ratios ≈ 500–900 in the C_3 crops. These findings were *milestones* in plant-soil-water-atmosphere relations research. Moreover, recently under field rainfed conditions at Prosper, North Dakota, USA, crop water use patterns, productivity, and water use efficiency were investigated in four growing seasons (1989–1992) with four cultivars representing tall, intermediate, and short-stemmed C_4 grain amaranths (Johnson and Henderson, 2002). The water use efficiency for total biomass production under the rainfed conditions recorded in these trials is equivalent to a transpiration ratio of 314 kg(H_2O) kg^{-1}(DM), which is within the range of values reported for C_4 tropical grasses and cereal crops. El-Sharkawy and Hesketh (1965, 1967) and El-Sharkawy *et al.* (1967) reported large differences in the instantaneous leaf water use efficiency (photosynthesis/transpiration) among several crop species, with the C_4 species having the highest efficiency. The differences among species were related to both stomatal and mesophyll characteristics controlling gas diffusion and net leaf photosynthetic rates. Compared to C_3 plants, the C_4 species have greater net leaf photosynthesis and tend to have higher stomatal resistances to water vapor diffusion, hence, lower transpiration, and lower mesophyll resistances to CO_2 diffusion (*i.e.*, higher carboxylation efficiency). Similar findings were also reported for several tropical forage legume and grass species by Ludlow and Wilson (1972).

Another advantage of crops with the C_4 system, as nutrient resources allocations are concerned, is the lower percent investment of protein in their Rubisco (fraction-I protein). For example, the C_4 saltbush *Atriplex* spp. have ≈ 20 % of the total leaf soluble protein allocated into Rubisco whereas in the C_3 *Atriplex* spp. Rubisco constitutes ≈ 50 % of the leaf soluble protein (Björkman *et al.*, 1976). Rubisco uses both CO_2 and O_2 as substrates in reaction with the five-carbon 'ribulose-1,5-bisphosphate' and is known for its lower affinity to CO_2 under normal air. In C_3 system, the oxygenation reaction under normal air conditions is high resulting in substantial photorespiratory carbon losses and this leads to lower net CO_2 fixation rate compared to C_4 system. Because of the primary fixation of CO_2 by the more efficient PEPC and consequently the elevated CO_2 concentrations around Rubisco in the bundle sheath cells of the C_4 species during photosynthesis, the carboxylation efficiency of Rubisco is higher and the oxygenation is lesser and this leads to a higher CO_2 fixation per unit of protein invested in Rubisco. These patterns of the leaf protein allocation and the enzyme carboxylation efficiency result in a much higher photosynthetic nitrogen use efficiency (PNUE) (*i.e.*, amount of carbon fixed per unit leaf nitrogen) in the C_4 species than that in the C_3 species (Brown 1978, Sage and Pearcy 1987, Oaks 1994). The high PNUE may confer an adaptive advantage and a high level of competitivness for the C_4 species in low-fertility soils. However, in C_3–C_4 intermediate cassava, yield was positively correlated with PNUE across a large group of genotypes grown under rainfed field conditions (El-Sharkawy 2004, 2007, El-Sharkawy *et al.* 2008). This finding may explain why cassava has a high level of adaptability to low-nitrogen soils (CIAT 1986–1996, Howeler 2002, Howeler and Cadavid 1990, Pellet and El-Sharkawy 1993a,b, 1997). After nine consecutive years of cassava cropping in low-fertility acidic Inceptisols at Santander de Quilichao, Cauca Dept., Colombia, cassava dry root yields without fertilization were greater than 5 t ha^{-1} (CIAT 1992). In sandy soils very low in organic carbon and nutrients at northern Colombia, dry root yields were 2. 2 t ha^{-1} after eight consecutive years of cassava cropping without fertilization (Cadavid *et al.* 1998). Thus, high PNUE in cassava apparently confers an adaptive advantage in low-nitrogen soils and points to the importance of selection for higher photosynthetic rates in breeding programes in order to maximize nutrient, as well as water, use efficiency. Cassava PEPC activity was in leaf extracts found to be 10–30 % of levels observed in C_4 species such as maize and sorghum with significant differences among genotypes under field conditions in different environments (El-Sharkawy and Cock 1990, El-Sharkawy 2004, 2006b, 2007, El-Sharkawy *et. al.* 2008); and the activity was positively correlated with leaf photosynthesis and crop storage root yield. Therefore, selection for higher content and activity of PEPC in cassava is warranted. Under prolonged drought of more than 3–4 months, cassava can remain photosynthetically active and produces reasonable yields as compared to C_4 cereal crops such as millets and grain sorghum (El-Sharkawy 1993, De Tafur *et al.* 1997a,b, El-Sharkawy and Cadavid 2002, El-Sharkawy 2006b, 2007). Cassava, as a major food and feed crop in the tropics and subtropics, is anticipated to further play an important role in developing world regions that will suffer from prolonged drought conditions due to global climate changes such as sub-Saharan Africa (Rosenzweig and Parry 1994, Kamukondiwa 1996, El-Sharkawy 2005).

Moreover, the role of PEPC in leaf photosynthesis of species other than C_4 crops should be investigated in relation to productivity. Jenkins (1989) in studies using the PEPC inhibitor 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)propenoate (DCDP) found that P_N was inhibited by about 79 to 98% in a range of C_4 species, including maize, sorghum and grain amaranth, as compared to about 12 to 46% inhibition in several C_3 crop species such as wheat, barley, *Pisum sativum* and *Brassica napus*, when treated with DCDP.

Concluding remarks

This review has been done not only to review C_4 photosynthetic research (summarized in the next paragraph), but also to correct the history of the pioneering research done on the topic and to draw attention to the negligence in some previously published reports, which distorted that history for many years. For the sake of the younger generations of scientists and students, it is the responsibility of scientific societies and journals involved to rectify the situation.

More than four decades ago important discoveries were made in the C_3 and C_4 photosynthetic systems of terrestrial plants by agronomists, physiologists, and biochemists across continents. Against the commonly-held belief in the 1950's that all terrestrial plants have similar photosynthetic rates, agronomists were able to differentiate plant species, including many important cultivated crops, on the basis of their photosynthetic capacities. El-Sharkawy and Hesketh (1965) found that the highly productive monocot tropical grasses such as maize and sorghum, as well as several other grasses, and the dicot *Amaranthus* spp. had the highest leaf photosynthetic rates, followed by warm-climate crops such as sunflower and cotton. Cool-climate crops such as oats, sugarbeet, and soybeans were less efficient, as compared to tropical grasses and warm-climate species. Shrubs were the least efficient among the many species studied (but *see* Nelson 1984 for woody species with high leaf photosynthetic rates). For the first time in the modern history of photosynthesis, El-Sharkawy and Hesketh (1965) and El-Sharkawy *et al.* (1967, 1968) discovered the many physiological and anatomical plant traits that underpin differences in photosynthetic efficiency among species. Most notable among traits of the C_4 species that differentiated them from the C_3 ones are: (I) high optimum temperature and high irradiance saturation for maximum leaf photosynthetic rates; (II) apparent lack of CO_2 release into a rapid stream of CO_2-free air in illuminated leaves in varying temperatures and high irradiances; (III) a very low CO_2 compensation point; (IV) lower mesophyll resistances to CO_2 diffusion coupled with higher stomatal resistances, and, hence, higher instantaneous leaf water use efficiency; (V) the existence of the so-called ''Kranz leaf anatomy'' and the higher internal exposed mesophyll surface area per cell volume; and (VI) the ability to recycle respiratory CO_2 by illuminated leaves. These findings laid the foundation for a series of subsequent physiological, anatomical, and biochemical researches that helped in the elucidation of the C_4 photosynthetic pathway. Nevertheless, the majority of the mainstream physiologists and biochemists overlooked these pioneering discoveries and in their publications neglected citing the original papers. Worse, in many cases they attributed the discovery of the C_4 leaf Kranz anatomy to scientists who had nothing to do with it. Yet, all these pioneering findings were confirmed by the consequent physiological, anatomical, and biochemical research. Moreover, El-Sharkawy and Hesketh's 1965 paper was identified a " Citation Classic" by the Institute of Scientific Information (ISI), USA, in 1986 (El-Sharkawy and Hesketh 1986), and their other companion papers were cited almost as much, all published in *Crop Science*. Recent research on cassava at CIAT demonstrated the importance of PEPC in leaf photosynthesis and yield. Both leaf photosynthesis and PEPC activity were positively correlated with yield. Therefore, it is warranted to investigate PEPC in wild *Manihot* species in order to further enhance leaf photosynthesis and productivity in improved cassava cultivars targeted to dry areas where food and feed shortages are common such as in sub-Saharan Africa (Kamukondiwa 1996).

Finally, I wish to conclude with the following statements directed to young scientists and future generations of science students: the photosynthetic process is the *machinery of nature* to which humanity, as the ultimate in the chain of organisms on earth, owes its existence. Our need for renewable sources of energy, food, feed, clothes, and shelter depends entirely on plant photosynthesis in the far past (e.g., fossil energy), in the present and in the future. Surely, you are better equipped and trained than our generation was and this should enable you to achieve what most of us failed to do. Apply your acquired knowledge and research to reality in the *field* where problems constraining agricultural productivy, along with environmental degradation, are awaiting solutions. Human population is ever increasing at an accelerating pace, particularly in developing countries, more than the rate of supplying their highly wanted essential materials (Gilland 2002). Apparent global climate change is a real threat to our mere existence (IPCC 2006). The role of photosynthetic research in mitigating this mostly man-made threat can not be overlooked. The up to date technological advances such as the electronically easily accessed information *via* the internet must help you to get acquainted with the scientific literature worldwide, old and new, a miracle that we the old folks just starting to appreciate. In writing this review, so many references that were not available in paper prints were traced in the web using key words. Drafts of the manuscript were easily shuffled and exchanged with colleagues across continents for comments in a matter of days.

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