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Photosynthetic Carbon Metabolism: Plasticity and Evolution

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1. Introduction

Carbon metabolism is the important part of the photosynthetic process in that plant green cells convert physical and chemical sources of energy into carbohydrates. Over the last 50 years, knowledge and understanding of carbon metabolism has improved considerably. Photosynthetic carbon metabolism can no longer be explained by a single, invariable cycle. It is no longer restricted to just the chloroplast or even to a single cell. In addition to carbon reduction, nitrogen assimilation, sulphate reduction and other aspects of intermediary metabolism are tightly connected with this process.

Like other physiological processes, photosynthesis differs greatly among various plant species and under different environmental conditions. Over the evolutionary history of land plants, selection pressures led to evolution of variants of photosynthetic carbon metabolism namely C_4 and crassulacean acid metabolism (CAM) pathways. In this chapter we will focus on the effect of environmental factors on photosynthetic carbon metabolism in order to show the considerable plasticity of this process. In addition, the processes in the evolution of main types of photosynthetic carbon metabolisms will be discussed regarding anatomical, physiological and molecular evidences.

2. Photosynthetic carbon metabolism: A general description

The pathway by which all photosynthetic eukaryotic organisms incorporate CO_2 into carbohydrates is known Calvin cycle or photosynthetic carbon reduction (PCR) cycle. The PCR cycle can be divided into three primary stages: (1) carboxylation which fixes the CO_2 in the presence of the five-carbon acceptor molecule, ribulose bisphosphate (RuBP), and converts it into two molecules of a three-carbon acid. The carboxylation reaction is catalyzed by the enzyme ribulose-1,5-bisphospahate carboxylase-oxygenase (Rubisco). (2) reduction, which consumes the ATP and NADPH produced by photosynthetic electron transport to convert the three-carbon acid to trios phosphate, and (3) regeneration, which consumes additional ATP to convert some of the triose phosphate back into RuBP to ensure the capacity for the continuous fixation of CO_2 (Fig. 1).

The first stable intermediate of Calvin cycle is a three-carbon acid, 3-phosphoglycerate. Therefore, the PCR cycle is commonly referred to as the C_3 cycle.

2.1 Photorespiration, principles and significance

An important property of Rubisco is its ability to catalyze both the carboxylation and the oxygenation of RuBP. Oxygenation is the primary reaction in a process known as photorespiration. Photosynthesis and photorespiration work in opposite directions, photorespiration results in loss of CO_2 from cells that are simultaneously fixing CO_2 by the Calvin cycle. The C_2 glycolate cycle, also known as the photosynthetic carbon oxidation cycle, begins with the oxidation of RuBP to 3P-glycerate and P-glycolate (Fig. 2).

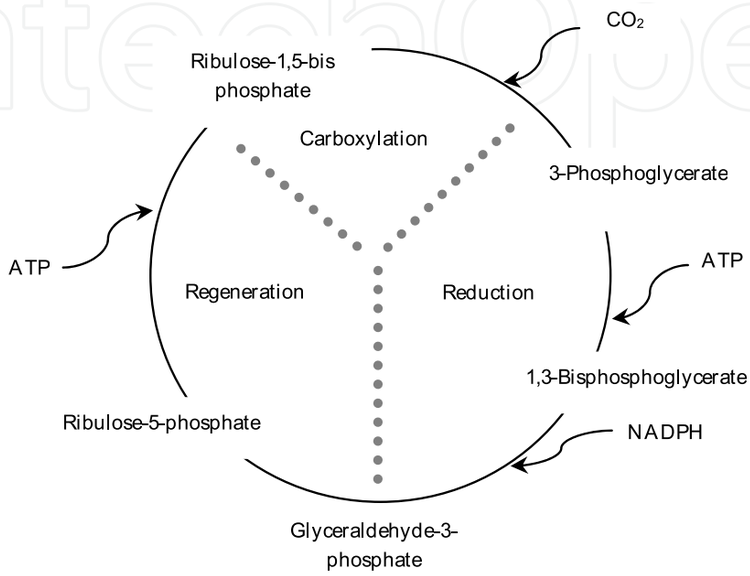


Fig. 1. The three stages of the photosynthetic carbon reduction (PCR) cycle or Calvin cycle.

In normal air (21% O_2), the rate of photorespiration in sunflower leaves is about 17% of gross photosynthesis. Every photorespired CO_2, however, requires an input of two molecules of O_2 and the true rate of oxygenation is about 34% and the ratio of carboxylation to oxygenation is about 3 to 1. The ratio of carboxylation to oxygenation depends, however, on the relative levels of O_2 and CO_2 since both gases compete for binding at the active site on Rubisco. Increase in the relative level of O_2 (or decrease in CO_2) shifts the balance in favor of oxygenation. An increase in temperature will also favor oxygenation. Increase in temperature declines the solubility of gases in water, but O_2 solubility is less affected than CO_2. Thus O_2 will inhibit photosynthesis, measured by net CO_2 reduction. There is also an energy cost associated with photorespiration and the glycolate pathway. Not only is the amount of ATP and NAD(P)H expended in the glycolate pathway following oxygenation (5ATP+3NADPH) greater than that expended for the reduction of one CO_2 in the PCR cycle (3ATP+2NADPH), but there is also a net loss of carbon. Photorespiration appears to be a costly energy and carbon acquisition. It is logical to ask why should the plants indulge in such an apparently wasteful process? Several ideas have been proposed (Hopkins and Hüner, 2004; Foyer et al., 2009; Bauwe, 2011).

2.1.1 Oxygenation is an unavoidable consequence of evolution

It has been proposed that the oxygenase function of Rubisco is an inescapable process. Rubisco evolved at a time when the atmosphere contained large amounts of CO_2 but little oxygen. Under these conditions, an inability to discriminate between the two gases would

have had little significance to the survival of the organism. It is believed that oxygen began to accumulate in the atmosphere primarily due to photosynthetic activity and the atmospheric content of O_2 had increased to significant proportions during the following stages of evolution of land plants. By this view, then, the oxygenase function is an evolutionary "hangover" that has no useful role.

However, this is an oversimplified view of photorespiration since photorespiratory mutants of *Arabidopsis* proved to be lethal under certain growth conditions, indicating the essential nature of the photorespiratory pathway in C_3 plants. There is no evidence that selection pressures have caused evolution of a form of Rubsico with lower affinity to O_2.

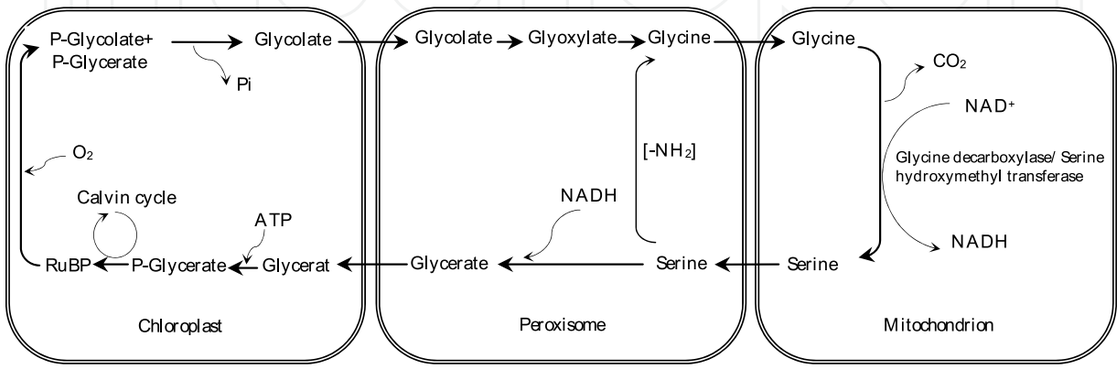


Fig. 2. The photorespiratory glycolate pathway.

2.1.2 Plants have turned this apparent evolutionary deficiency into a useful metabolic sequence

The glycolate pathway, undoubtedly serves as a scavenger function. For each two turns of the cycle, two molecules of phosphoglycolate are formed by oxygenation. Of these four carbon atoms, one is lost as CO_2 and three are returned to the chloroplast. The glycolate pathway thus recovers 75% of the carbon that would otherwise be lost as glycolate. There is also the possibility that some of the intermediates, serine and glycine, for example, are of use in other biosynthetic pathways, although this possibility is still subject of some debate.

2.1.3 Photorespiration functions as a safety valve for dissipation of excess excitation energy

A significant decline in the photosynthetic capacity of leaves irradiated in the absence of CO_2 and O_2 has been reported. Injury is prevented, however, if sufficient O_2 is present to permit photorespiration to occur. Apparently, the O_2 consumed by photorespiration is sufficient to protect the plant from photo-oxidative damage by permitting continued operation of the electron transport system. This could be of considerable ecological value under conditions of high light and limited CO_2 supply, for example, when the stomata are closed due to water stress. Photorespiratory mutants of *Arabidopsis* are more sensitive to photoinhibition than their wild type counterparts.

In order to increase crop productivity efforts have been made on the inhibition or genetically eliminating photorespiration. Effort has been expended in the search for chemicals that inhibit the glycolate pathway or selective breeding for low-photorespiratory strains through finding a Rubisco with lower affinity for oxygen. All of these efforts have

been unsuccessful, presumably because the basic premise that photorespiration is detrimental to the plant and counterproductive is incorrect.
Clearly, success in increasing photosynthesis and improving productivity lies in other directions. A mechanism for concentrating CO_2 in the photosynthetic cells could be one way to suppress photorespiratory loss and improve the overall efficiency of carbon assimilation. That is exactly what has been achieved by C_4 and CAM plants. A limited extent of photorespiration in C_4 and CAM plants is a consequence of mechanisms that concentrate CO_2 in the Rubisco environment and thereby suppress the oxygenation reaction (Hopkins and Hüner, 2004; Foyer et al., 2009; Bauwe, 2011)

3. C_4 mode of carbon assimilation

C_4 plants are distinguished by the fact that the first product is a four-carbon acid oxaloacetate (OAA). The key to the C_4 cycle is the enzyme phosphoenol pyruvate carboxylase (PEPC), which catalyzes the carboxylation of PEP using the bicarbonate ion (HCO_3^-) as the substrate (rather than CO_2). C_4 plants also exhibit a number of specific anatomical, physiological and biochemical characteristics that constitute the "C_4 syndrome". One particular anatomical feature characteristic of most C_4 leaves is the presence of two distinct photosynthetic tissues. In C_4 leaves the vascular bundles are quite close together and each bundle is surrounded by a tightly fitted layer of cells called the bundle sheath. Between the vascular bundles and adjacent to the air spaces of the leaf are the more loosely arranged mesophyll cells (Fig. 3). This distinction between mesophyll and bundle sheath photosynthetic cells called Kranz anatomy plays a major role in the C_4 syndrome (Bhagwat, 2005; Edwards and Voznesenskaya, 2011).

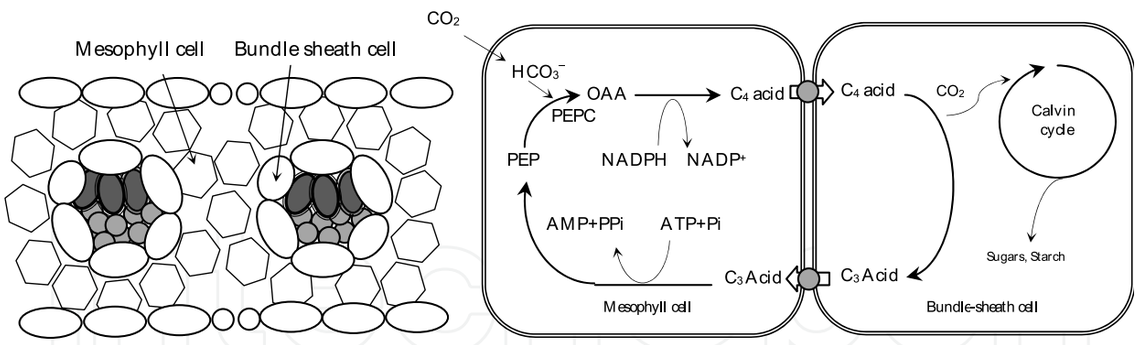


Fig. 3. Leaf anatomy of a C_4 plant (left). Note the tightly fitted bundle sheath cells (ovals) surrounded by a concentric layer of mesophyll cells (hexagons). Schematic of the C_4 photosynthesis carbon assimilation cycle (right).

There are certain similarities between the PCR cycle and C_4 metabolism. Like Rubisco, the PEPC carboxylation reaction is virtually irreversible and, consequently, energetically very favorable. Reducing potential is required at some point to remove the product and ATP is required to regenerate the acceptor molecule, PEP (Fig. 3). A very significant difference between the PCR cycle and C_4 metabolism, however, is that once in the bundle sheath cell, the C_4 acid is decarboxylated, giving up the CO_2 originally assimilated in the mesophyll cell. This decarboxylation means that, unlike the C_3-cycle, the C_4 cycle does not of itself result in

any net carbon reduction. The plant relies ultimately on the operation of the PCR cycle in the bundle sheath chloroplast for the synthesis of triose phosphates.

Within the general pattern of the C_4 cycle described above there are three variations include NADP-malic enzyme type, NAD-malic enzyme and PEP carboxykinase types. Regardless of these variations, the principal effect of the C_4 cycle remains to concentrate CO_2 in the bundle-sheath cells where the enzymes of the PCR cycle are located. By shuttling the CO_2 in the form of organic acids it is possible to build much higher CO_2 concentrations in the bundle-sheath cells than would be possible relying on the diffusion of CO_2 alone. The concentration of CO_2 in bundle-sheath cells may reach 60 mM about tenfold higher than that in C_3 plants. Higher CO_2 concentrations would suppress photorespiration and support higher rates of photosynthesis. Under optimal conditions, C_4 crop species can assimilate CO_2 at rates two to three times that of C_3 species. All this productivity, however, has an energy cost to building the CO_2 concentration in the bundle-sheath cells. For every CO_2 assimilated, two ATP must be expended in the regeneration of PEP. This is in addition to the ATP and NADPH required in the PCR cycle. Thus the net energy requirement for assimilation of CO_2 by the C_4 cycle is five ATP and two NADPH (Bhagwat, 2005; Edwards and Voznesenskaya, 2011).

4. CAM mode of carbon assimilation

Another CO_2 concentrating mechanism is CAM. This specialized pattern of photosynthesis was originally studied in the family Crassulaceae. One of the most striking features of CAM plants is an inverted stomatal cycle i.e. the stomata open mainly during the nighttime hours and are usually closed during the day. This means that CO_2 uptake also occurs mainly at night. In addition, CAM plants are characterized by an accumulation of malate at night and its subsequent depletion during hours and storage carbohydrate levels. Nocturnal stomatal opening supports a carboxylation reaction producing C_4 acids that are stored in the large vacuoles. Accumulation of the organic acids leads to a marked acidification of these cells at night. The acids are subsequently decarboxylated during daylight hours and the resulting CO_2 is fixed by the PCR cycle. As in C_4 plants, the enzyme PEPC is central to CAM operation (Fig. 4). CAM species may be distinguished by the enzymes which catalyse organic acid decarboxylation, NAD-malic enzyme, NADP-malic enzyme and PEP-carboxykinase types (Bhagwat, 2005; Dodd et al., 2002; Holtum et al., 2005).

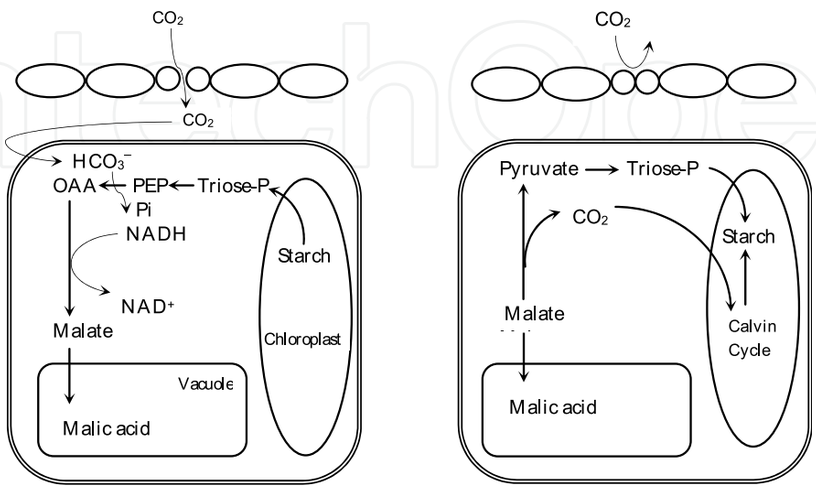


Fig. 4. Schematic of crassulacean acid metabolism (CAM).

5. Carbon isotope discrimination in carbon assimilation pathways

There are two naturally occurring stable isotopes of carbon, ^{12}C and ^{13}C. Most of the carbon is ^{12}C (98.9%), with 1.1% being ^{13}C. The overall abundance of ^{13}C relative to ^{12}C in plant tissue is commonly less than in the carbon of atmospheric carbon dioxide, indicating that carbon isotope discrimination occurs in the incorporation of CO_2 into plant biomass. Variation in the ^{13}C /^{12}C ratio is the consequence of so called "isotope effects," which are expressed during the formation and destruction of bonds involving a carbon atom, or because of other processes that are affected by mass, such as gaseous diffusion (Farquhar et al., 1989). Isotope effect, denoted by α, is defined as the ratio of carbon isotope ratios in reactant and product:

$$\alpha = R_r / R_p \quad (1)$$

R_r is the ^{13}C /^{12}C molar ratio of reactant and R_p is that of the product. For plants R_a (R_r) is isotopic abundance in the air and R_p is defined isotopic abundance in the plant. For numerical convenience, instead of using the isotope effect ($\alpha = R_a/R_p$), it has been proposed to use the Δ, the deviation of α from unity, as the measure of the carbon isotope discrimination by the plant:

$$\Delta = \alpha - 1 = R_a/R_p - 1 \quad (2)$$

Isotopic composition is another parameter is specified as $\delta^{13}C$ values, R_s is the $^{13}C/^{12}C$ molar ratio of the standard:

$$\delta^{13} = R_p/R_s - 1 \quad (3)$$

Isotopic composition of plants (δ^{13}) is negative, whereas the process of CO_2 diffusion and carboxylation by Rubisco have positive discrimination (i.e. against $^{13}CO_2$). Therefore, Δ values are usually positive while those of δ^{13} are usually negative. Typically, Δ and δ^{13} values are ~$10\text{-}35\times10^{-3}$, which is normally presented as 10-35‰ ("per mil").

Because Rubisco preferentially fixes the light ^{12}C isotope over the heavier ^{13}C, plant tissues are enriched in ^{12}C relative to the bulk atmosphere. However, C_4 plants exhibit much lower rates of Δ (and higher rates of δ^{13}) than C_3 plants. Plants exhibiting CAM have intermediate values which appear to be related to the relative proportions of C_3 and C_4 fixation by these species (Farquhar et al., 1989). The evolutionary modifications that lead to the enhancement of fixation by the C_4-cycle in C_3-C_4 intermediates are also associated with reduction in Δ values and increase in δ^{13} values from C_3 to near C_4 values (Hobbie and Werner, 2004)(See below).

6. Effect of environmental factors on photosynthetic carbon metabolism

6.1 Effect of water availability

Plant and cell water balance is determined by water lost in transpiration and water absorption from the soil. When transpiration exceeds absorption, cell turgor and relative water content (RWC) decrease, while the concentration of cellular contents increases. Under these conditions, osmotic potential and water potential fall.

RWC normalizes water content by expressing it relative to the fully turgid state and is an easily measured indicator of water status:

$$\text{RWC\%}=(\text{fresh mass} - \text{dry mass})/(\text{water saturated mass} - \text{dry mass})\times 100 \quad (4)$$

Low cell turgor and RWC slow growth and decrease the stomatal conductance for H_2O (g_s). Water deficiency covers the range from fully hydrated cells (100% RWC), as the control state with metabolism functioning at the potential rate to very dehydrated cells (50% RWC or less) at which the cell will not recover when rehydrated.

Progressive decrease in RWC decreases A (net CO_2 assimilation rate) of leaves. A depends on the activity of Rubisco per unit leaf, the rate of RuBP synthesis (hence on capture of photosynthetically active radiation, PAR) and on the CO_2 supply, determined by g_s and the ambient CO_2 concentration (C_a) (Lawlor, 2001). CO_2 supply to the PCR cycle in the chloroplast is determined by C_a and conductance of the pathway for diffusion between air and enzyme active sites, principally g_s in the gas phase and g_m in the liquid phase, which includes all physicochemical and biochemical factors (von Caemmerer 2000). The CO_2 concentration within the leaf, C_i, depends on A, g_s and C_a:

$$A = g_s (C_a - C_i) \quad (5)$$

The CO_2 concentration at the active sites of Rubisco in the chloroplasts (C_c) is given by:

$$A = g_m (C_i - C_c). \quad (6)$$

By measuring A as a function of C_i (A/C_i response curve) under standard PAR flux, the limitations to A could be assessed. The maximum rate of A under saturating CO_2 (C_a, C_i and C_c) and light in fully hydrated leaves is defined as A_{pot}. To achieve the same A_{pot} at small RWC as at large RWC, C_c must saturate Rubisco and so Ca must be sufficient to overcome the limitation of g_s. If A_{pot} at small RWC does not attain the value of A_{pot} at large RWC, despite CO_2 saturation, then metabolism is inhibited (Lawlor and Cornic, 2002).

Experimental studies on CO_2 assimilation of C_3 plants under decreasing RWC show that there are fundamental differences between species in the relative roles of g_s limitation of CO_2 supply and metabolic limitation of A_{pot}. Basically data obtained from various species fall into two groups, which have been called Type 1 and Type 2 responses (Fig. 5).

Type 1: With RWC=90% –75%, increasing C_a to 5% restores A fully to the A_{pot} of control leaves (Cornic and Massacci 1996). At RWC<75% restoration of A_{pot} to the value at 100% RWC is not achieved and the response to CO_2 becomes progressively smaller. Therefore, in Type 1 response, there are two main, relatively distinct phases with a transition between them. The stomatal limitation phase occurs at RWC between 100 and 75%, without effect on A_{pot}, so that A may be restored to A_{pot} by large concentration of CO_2. The metabolic phase of limitation is at lower RWC <75% where A_{pot} is limited by metabolism (Lawlor and Cornic, 2002).

Type 2: Elevated CO_2 increases A to A_{pot} in unstressed leaves (RWC 100 to 75%), but A is progressively less stimulated as RWC decreases, i.e. A is not restored to the unstressed A_{pot}, so the potential rate of CO_2 assimilation is decreased (Tezara *et al*. 1999). Inhibition of A with 10% or greater C_a, rather than restoration, shows that metabolism is affected by elevated CO_2, but as A is not restored to A_{pot}, photosynthetic metabolism must be impaired. The evidence is therefore of partial metabolic inhibition of A by moderate stress and substantial inhibition at more severe stress. In Type 2, the phases are not distinct but progressive, lacking the two clearly distinguished phases of Type 1. Stomatal regulation, i.e. decreased g_s, dominates at relatively large RWC, leading to a lower C_i and C_c. g_s becomes progressively less important and metabolic limitations more important as RWC falls (Lawlor and Cornic, 2002).

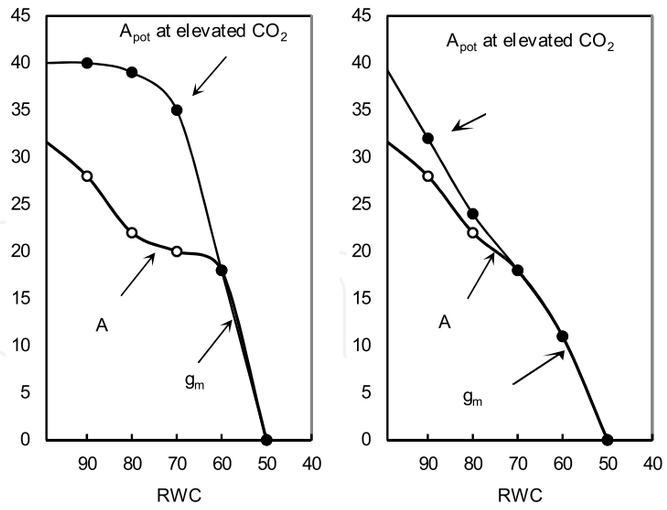


Fig. 5. There are two types of response of A_{pot} to decreasing RWC: Type 1 (left) with no decrease as RWC falls from 100 to~75% and Type 2 with progressive decrease over this range (right). Redrawn according to Lawlor and Cornic (2002) with permission.

Although effect of different experimental approaches could not be excluded in different response curve of species as described by type 1 and 2, this difference is most likely related to differences in the particular characteristics of metabolism in different species. They may reflect differences in the cell water balance and differences in cell elastic modulus. Alternatively, they could reflect differences in sensitivity of a basic process to changing cellular conditions in different species or under different conditions, e.g. if ionic concentration in chloroplasts differed and thus resulted in different rates of ATP synthesis (Lawlor and Cornic, 2002).

The potential capacity for light harvesting, energy transduction, electron transfer in reaction centers of the photosystems (PSII and PSI) and electron transport in thylakoids are unaffected by a wide range of RWC, only severe loss of RWC decreases photosystem activity and alters the structure of PSII (Giardi *et al*. 1996). The rate of electron transport at saturating PAR is determined by sink capacity for electrons, principally *A* at large RWC. Decreased sink capacity for electrons results in increased non-photochemical energy dissipation in Type 1 and 2 responses (Müller et al., 2001). However, maintenance of A_{pot} in the Type 1 response will enable greater CO_2 recycling and energy use within the tissue than if A_{pot} decreases as in Type 2. At low RWC, where A_{pot} decreases in both responses, electron transport is decreased because of biochemical, as opposed to biophysical, limitations (Lawlor and Cornic, 2002).

As *A* falls with decreasing RWC, the amount of assimilate available for export as triose-phosphate from chloroplast to cytosol and sucrose synthesis diminishes. Sucrose content in leaves falls in rapidly stressed leaves at RWC<80%, caused by low *A* and continued respiration. Thus, it is very unlikely that accumulation of assimilates would result in feedback inhibition of *A* or that the capacity of the triose-phosphate-P_i transporter in the chloroplast envelope is affected by low RWC. The rate of sucrose synthesis also depends on the activity of sucrose phosphate synthase, which is greatly decreased by even small loss of RWC. Sucrose phosphate synthase is subjected to complex control, including allosteric

modulation by glucose 6-phosphate and phosphorylation by a protein kinase using ATP (S.C. Huber and J.L. Huber, 1996). The latter activates SPS under osmotic stress, suggesting that inactivation may be related to decreased ATP content (Lawlor and Cornic, 2002).
Water deficiency changes the proportion of different carbohydrates. Starch, glucose and fructose concentrations increase with mild drought but sucrose change little. Such changes may be adaptive i.e. osmoregulation, and is associated with increased soluble (vacuolar) acid invertase activity in C_3 species (Pelleschi *et al*. 1997).

6.2 Effect of temperature

6.2.1 Carbon metabolism under low temperature and during cold acclimation

Low temperature is one of the most important factors affecting plant performance and distribution (Stitt and Hurry, 2002). Photosynthetic carbon metabolism is greatly influenced by low temperature, directly via the modulation of enzymes activity and indirectly via changes in sink demand of plants experiencing low temperature stress.
Ecological and physiological studies have uncovered a strong correlation between sugar concentrations and frost resistance (Guy et al., 1992). Sugars either act as osmotica or protect specific macromolecules during dehydration. Changes in the subcellular concentration and distribution of sugars might also provide a mechanism to protect specific compartments. For example, although sucrose is largely restricted to the cytosol of leaves at high temperatures, there are reports that it accumulates in the chloroplast in cold-acclimated cabbage (Fowler et al., 2001).
In recent years, molecular studies unravel mechanisms underlying responses of photosynthetic metabolism under low temperatures. The constitutive increases in the frost tolerance was observed in an *Arabidopsis* mutant over-expressing one dehydration-responsive element gene that is correlated with increased sugar contents (Gilmour et al., 2000). A freezing sensitive mutant with impaired cold acclimation has sugar levels that are lower than those of wild type *Arabidopsis* plants (McKown et al., 1996).
Decreased temperatures lead to an acute Pi-limitation of photosynthesis. Indeed, some of the changes in photosynthetic metabolism that occur during cold acclimation are reminiscent of the response to low Pi (Nielsen et al., 1998). Evidence that changes in Pi concentration or availability to metabolism contribute to cold acclimation has been provided by studies of *pho1* and *pho2* mutants. *pho1* mutant with decreased shoot Pi concentration shows accentuation of the low-temperature-induced increase of sucrose phosphate synthase activity and of cytosolic fructose-1,6-bisphosphatase and sucrose phosphate synthase gene expression, abolishment of the decrease in the transcript levels of genes encoding for Rubsico and light harvesting chlorophyll a/b binding protein after chilling, accentuation of the cold-induced shift in carbon allocation from starch to sucrose and increase in the proline accumulation after chilling (Hurry et al., 2000). The opposite of these metabolic characteristics was observed in *pho2* mutant with higher shoot Pi concentration compared with control (Hurry et al., 2000). These results reveal that signals relating to altered Pi concentration or availability to metabolism lead to the activation and increased expression of enzymes in the sucrose synthesis pathway. They also lead to changes in the relative activities of enzymes in the Calvin cycle (Hurry et al., 2000).
Functional importance of sugar metabolism has also been demonstrated during cold acclimation. Optimal rates of photosynthesis require an appropriate balance between the rates of carbon fixation and sucrose synthesis (Stitt, 1996). Excessive sucrose synthesis

depletes the phosphorylated Calvin cycle intermediates and inhibits the regeneration of ribulose-1,5-bisphosphate. Conversely, inadequate sucrose synthesis leads to accumulation of phosphorylated intermediates and depletion of Pi, resulting in inhibition of ATP synthesis, accumulation of 3-phosphoglycerate and inactivation of Rubisco. A sequence of events reverses the inhibition of sucrose synthesis and photosynthesis as the plants acclimate to low temperatures (Hurry et al., 2000). Short- and mid-term adjustments act primarily on sucrose synthesis but also stimulate photosynthesis by relieving the acute Pi-limitation. Longer-term adjustments affect photosynthesis directly. The recovery has two important functions: increased sucrose production (Strand et al., 1999) and protection against photoinhibition by allowing increased turnover of the photosynthetic electron chain (Hurry et al., 2000). The transfer of warm-grown *Arabidopsis* plants to 4°C leads to the post-translational activation of sucrose phosphate synthase within 30 min. Over the next few days, sucrose synthesis is stimulated by two further adjustments. One is a selective increase in the expression of cytosolic fructose-1,6-bisphosphatase and sucrose phosphate synthase genes, the two key regulated enzymes in the pathway of sucrose synthesis (Strand et al., 1999). The second is a shift in the subcellular distribution of Pi. In the leaves, most of the Pi is in the vacuole. Indirect evidence indicates that the Pi distribution shifts towards the cytoplasm at low temperatures allowing phosphorylated metabolites to increase without depleting the free Pi (Hurry et al., 2000).

Full acclimation occurs in leaves that develop at low temperature. They retain the selective increase in the expression of sucrose synthesis enzymes, and also have higher activities of all of the Calvin cycle enzymes on a fresh weight or leaf area basis. Two factors contribute to this increase. First, whereas transcripts for light harvesting chlorophyll a/b binding protein and Rubisco genes decrease after transfer to low temperature, they recover in leaves that develop at low temperature. This recovery occurs even though leaf sugars rise, indicating that sugar-repression of these genes is overridden at low temperature in acclimated leaves. Second, leaves that mature at low temperature have reduced water and increased protein contents due to an increase in the volume of the cytoplasm relative to that of the vacuole (Strand et al., 1997; 1999) .

6.2.2 Carbon metabolism under higher temperatures

Photosynthesis is very responsive to high temperatures (Knight and Ackerly, 2003). In semi-arid regions, temperature and precipitation are often negatively correlated, with lower rainfall in warmer environments. Therefore, studies on photosynthetic thermal tolerance of plants are complicated by the fact that a variety of environmental factors can affect photosynthesis, including plant water status, soil salinity and light levels. Photosynthetic acclimation can occur on the scale of minutes to hours in response to moderately elevated temperatures. During long-term effects of various co-existing factors, however, morphological processes may also contribute to the plastic acclimation responses of photosynthesis (Knight and Ackerly, 2003). Reduction of specific leaf area and increased expression levels of small heat shock proteins are form adaptations of plants carbon metabolism to high temperatures. Small heat shock proteins dominate protein synthesis during and after high temperature stress. Variation between species for expression levels of the chloroplast small heat shock proteins following heat stress is positively correlated with the maintenance of PSII electron transport (Preczewskiet at al., 2000).

6.3 Effect of CO_2 concentration

Human activities have caused the concentration of atmospheric CO_2 to increase continuously from about 280 parts per million (ppm) at the beginning of the 19th century to 369 ppm at the beginning of the 21st century (Prentice et al., 2001). Future projections of atmospheric CO_2 concentration range between about 450 and 600 ppm by the year 2050 and are strongly dependent on future scenarios of anthropogenic emissions.

Long-term studies on the effects of CO_2 enrichment on plants have provided a rich suite of data and understanding about a wide variety of plant responses (McLeod and Long, 1999). Initial short-term experiments demonstrated that elevated CO_2 concentrations partially alleviated the limitation of C_3 (but not C_4) photosynthesis by CO_2 supply and acted as a negative feedback on transpiration in both C_3 and C_4 species (Long, 1991). Subsequent and often longer-term experiments have shown that photosynthesis could acclimate downwards in response to CO_2 enrichment, and there is now some evidence to suggest that photosynthesis is stimulated in C_4 species in response to CO_2 enrichment (Ghannoum et al 2000). In species with the C_3 photosynthetic pathway, high irradiance can lead to photoinhibition. Field studies have now demonstrated that CO_2 enrichment can reduce the severity of photoinhibition, although this effect is dependent on rubisco activity (Hymus et al., 2000).

6.3.1 Effect on stomatal conductance and water use efficiency

Leaf thickness generally increases whereas specific leaf area decreases as a result of CO_2 enrichment. A detailed analysis of leaf development in Scots pine (*Pinus sylvestris*) after four years of exposure to CO_2 enrichment confirmed that leaf thickness was increased but also indicated reductions in stomatal density (Lin et al., 2001). This stomatal-density response confirms observations of reduced stomatal conductance in response to CO_2 enrichment.

Recently, a gene *HIC* (*HI*gh *C*arbon dioxide) has been identified whose disruption leads to large increases in the number of stomata initiated in response to CO_2 enrichment (Gray et al., 2000). The *HIC* gene encodes an enzyme involved in the synthesis of those long-chain fatty acids that are typically found in the cuticle of leaves. Changes in these fatty acids may influence the cell-to-cell signaling of stomatal development. The short-distance cell-to-cell signaling of stomatal development is complimented by longer distance systemic signaling of stomatal development. The systemic signal allows the development of stomata in immature leaves to be controlled after CO_2 concentration is detected by mature leaves (Lake et al., 2001).

Early experiments demonstrated significant reductions in stomatal conductance under CO_2 enrichment. Analysis of 13 long-term (i.e. duration of more than one year) field-based studies on tree species demonstrated an overall reduction of 21% in stomatal conductance (Medlyn et al., 2001). The observation of reduced stomatal conductance was much more consistent in the longer-term than in the shorter-term studies. In combination with the partial down regulation of photosynthetic rate as the plants acclimatized to elevated CO_2, the reduction in stomatal conductance led to a 40% increase in instantaneous water use efficiency (Woodward, 2002).

6.3.2 Assimilates allocation

Carbon allocation to reproduction is strongly stimulated after a long-term of CO_2 enrichment. In a long-term study, trees growing in the enriched CO_2 were twice as likely to

be reproductively mature, and produced three times more seeds than control plants growing in ambient CO_2 concentrations (LaDeau and Clark 2001). This result indicates that CO_2 enrichment hastens significantly the onset of seed production, a feature that may prove to be effective in tracking climatic change. In contrast, flowering and seed set in grasslands, where species may have deterministic life cycles, were unaffected, reduced or stimulated under CO_2 enrichment. The species-specific nature of these responses indicates a strong potential for CO_2 enrichment to change the composition of plant communities (Woodward, 2002).

6.3.3 Acclimation of plants to CO_2 enrichment

Long-term field experiments indicate that leaf photosynthesis is stimulated by CO_2 enrichment in C_3 species from 7% for legume herbs to 98% for *Pinus radiata* (Long et al., 2004). However, in the longer term when photosynthesis exceeds the capacity for carbohydrate export and utilization, a down regulation process is expected. This response is exacerbated by genetic limitations, such as determinate growth patterns, and environmental limitations, such as N deficiency or low temperature (Ainsworth et al., 2004).

The causes of photosynthetic downregulation have been variously ascribed to a reduction in carbohydrate sink strength, a limited capacity to sequester carbon in a storage form, changes in nitrogen allocation and a reduction of rubisco concentration (Woodward, 2002). These responses indicate not only a decreased expression of photosynthetic genes but also a co-ordination of the carbon to nitrogen balance (Paul and Foyer 2001). For example, nitrate and ammonium uptake, and nitrate reductase activity are sensitive to CO_2. These co-ordinating activities match photosynthetic capacity with the capacities for growth and carbon storage (Walch-Liu et al., 2001).

Respiration rates have been observed to decline, or remain unchanged with CO_2 enrichment, depending on the species. Rates of dark respiration are directly correlated with leaf nitrogen content (Hamilton et al., 2001). Therefore, when CO_2 enrichment leads to a reduction in leaf nitrogen concentration, respiration also declines. Surprisingly, CO_2 enrichment increases the average number of mitochondria in each cell, even though leaf respiration rate decreases in response to elevated CO_2 across a diverse selection of plant species (Griffin et al., 2001).

At elevated CO_2 concentrations, Rubisco content was decreased by about 20%, but in contrast there was little change in capacity for Ribulose-1,5-bisphosphate regeneration and little or no effect on photosynthetic rate. In long-term CO_2 enrichments, the loss of Rubisco cannot be explained as the result of an overall decline in leaf N, but instead appears specific and accounts for most of the decrease in N per unit of leaf area. These results suggest that loss of Rubisco is more appropriately described as an acclimatory change benefiting N use efficiency rather than as down-regulation (Long et al., 2004). Both genetic and experimental modifications of source-sink balance provide results consistent with current models of carbohydrate feedback on Rubisco expression. There is no evidence of acclimation in C_4 species under long term CO_2 enrichment, and increases in photosynthesis and production are consistent with the hypothesis that this results from improved water use efficiency. The findings have important implications both for predicting the future terrestrial biosphere and understanding how crops may need to be adapted to the changed and changing atmosphere.

7. Plasticity in CAM

Photosynthetic gas exchange pattern of CAM consists of four phases (Osmond 1978). Phase I consisted of nocturnal uptake of CO_2 via open stomata, fixation by PEPC and vacuolar storage of CO_2 in the form of organic acids, mainly malic acid. Daytime remobilization of vacuolar organic acids, decarboxylation and refixation plus assimilation of CO_2 behind closed stomata in the Calvin-cycle were named phase III. Between these two phases there are transitions when stomata remain open for CO_2 uptake for a short time during the very early light period (phase II) and reopen again during the late light period for CO_2 uptake with direct assimilation to carbohydrate when vacuolar organic acid is exhausted (phase IV) (Fig. 6).

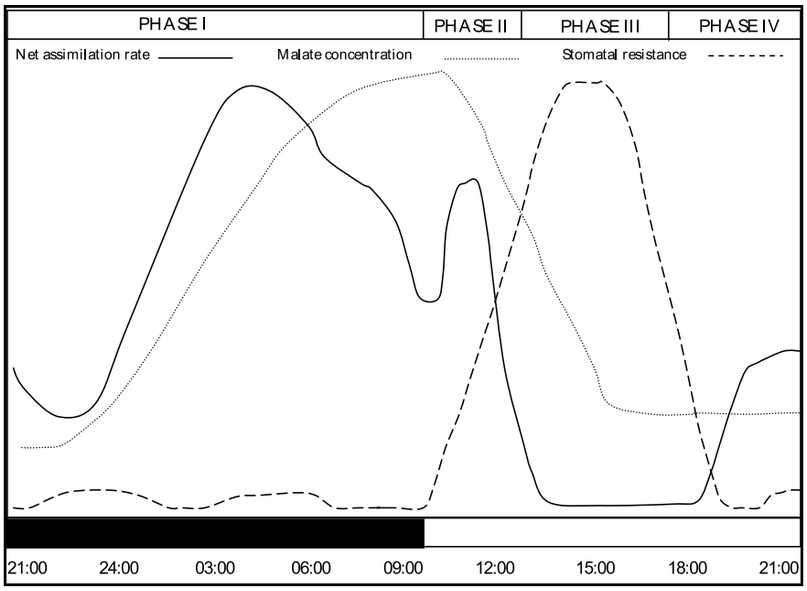


Fig. 6. Diurnal course of net CO_2 assimilation rate, malate accumulation and stomatal resistance in a CAM plant.

High internal CO_2-concentrations in phase III resulting from malate decarboxylation repress photorespiration, due to a generally low O_2/CO_2-ratio in CAM plants. The main advantage of CAM, in comparison to C_3 photosynthesis, is that it offers much higher water use efficiency, due to stomatal closure during the day. Such an adaptation is beneficial to plants living in dry and saline environments.

Plasticity in the expression of various CAM phases described above is a ubiquitous feature of the majority of CAM plants. CAM is intimately linked with the environment and can be perturbed by temperature, light level and water status. Plasticity in expression of CAM has been shown in the members of Crassulaceae. Thinner-leaved species of Crassulaceae are highly plastic in photosynthetic expression and behave like C_3 species, both in terms of the duration of diurnal atmospheric CO_2 uptake and light use efficiency. In contrast, thicker-leaved, more succulent species suffer from extreme CO_2 -diffusion limitation and are more strongly bound to nocturnal CO_2 fixation for the daytime supply of carbon (Winter, 1985). Carbon isotope discrimination studies showed also that CAM photosynthesis is inducible (facultative) or constitutive (obligate) (Kluge et al., 1995). In addition, the stage of plant

development affects CAM expression in plants. Several C_3/CAM intermediate species change their mode of metabolism in response to stress conditions (Dodd et al. 2002).
Facultative or inducible CAM species use the C_3 pathway to maximize growth at times of sufficient water supply but switch to CAM as a means of reducing water loss while maintaining photosynthetic integrity during periods of limited water supply. This C_3/CAM intermediate pathway is predominantly found among the Aizoaceae, Crassulaceae, Portulacaceae and Vitaceae (Smith and Winter 1996). CAM expression in facultative species is primarily determined by genotype and the severity of water limitation, but may also be induced by ontogenetic and other environmental factors. Although no unique enzymes are required to facilitate the C_3–CAM transition, the imposition of water stress has a profound influence on the abundance and regulation of enzymes involved in organic acid and carbohydrate formation, turnover and intracellular transport functions (Cushman and Borland, 2002). Salt-induced C_3–CAM transition is linked with an increased activity of antioxidative enzymes. It has been suggested that, the redox status in the proximity of PSII in the C_3/CAM intermediate plants controls the expression of key genes encoding scavengers of reactive oxygen species such as superoxide dismutase, ascorbate peroxidase and activity of NADP-malic enzyme (Ślesak et al., 2002).

7.1 Effect of environmental factors on CAM

Generally, water is considered to be the most important factor and CAM to be an adaptation to water-shortage stress. However, CAM is also observed in submerged freshwater plants (Keeley, 1996). CO_2 has been considered as the central factor and most important driving force for CAM. It has been assumed that early CAM evolution occurred during geological times when atmospheric CO_2 concentration was low (Raven and Spicer, 1996). CAM is a CO_2-concentrating mechanism due to the much higher substrate affinity of PEPC for HCO_3^- than of the C_3-photosynthesis/Calvin cycle carboxylase Rubisco for CO_2. Thus, during the dark period a concentrated CO_2 pool is built up in the form of vacuolar malic acid accumulation, and during phase III its remobilization in the light leads to internal CO_2 concentrations that may be 2-60 times more than atmospheric CO_2 concentration. For aquatic plants, this CO_2-concentrating mechanism provides a benefit for CO_2 acquisition. For terrestrial plants, the benefit of CO_2-concentrating by CAM is considered to be related to water use as will be discussed below (Lüttge et al., 2004).

7.1.1 Water availability

For terrestrial plants, the greatest benefit of CAM is considered to be increased water use efficiency because stomatal opening during the dark period causes much less transpirational loss of water than opening during the light period. With this high water use efficiency, CAM plants not only inhabit arid habitats e.g. cacti, agaves and euphorbs, but also inhabit tropical rainforests. These CAM species are mainly epiphytic and subjected also to the particular problems of water supply in this habitat (Zotz and Hietz, 2001). In addition to CAM phase-dependent stomatal responses affecting WUE, CAM plants have other structural and functional ways for water storage. The large vacuolar concentrations of nocturnally accumulated organic acids are osmotically active. The increased osmotic pressure drives water uptake into the cells, which is associated with increased turgor pressure. This allows CAM plants extra acquisition of water, particularly towards the end of the dark period when vacuolar organic acid levels become rather high. It may be a particular advantage in

moist, tropical forests with dew formation occurring mainly during the late dark period. During acid remobilization in phase III, osmotic and turgor pressures decline again but the water gained is available to the plants (Lüttge, 2004). CAM also occurs in some resurrection plants such as *Haberla rhodopensis* and *Ramonda serbica* (Gesneriaceae) that are desiccation-tolerant and can shift between biosis and anabiosis as they dry out and are rewatered, respectively (Markovska et al., 1997).

7.1.2 Light

Light quality and intensity affects CAM in different ways. Intensity of photosynthetically active radiation during the day (phase III) determines the rate of organic acid mobilization from the vacuole. A signaling function of light is also obvious i.e. long-day dependent induction of CAM. Phytochrome, the red-light receptor involved in photoperiodism, elicits CAM expression (Brulfert et al., 1985). In C_3/CAM intermediate species, light responses of stomata change dramatically when CAM is induced. In *Portulacaria afra*, blue-light and red-light responses of stomata in the C_3-state are lost in the CAM-state. In *M. crystallinum* after the C_3-CAM transition, the opening response of guard cells to blue and white light is lost in parallel with light-dependent xanthophyll formation. The xanthophyll zeaxanthin is involved in the signal transduction chain from light to stomatal opening (Tallman et al., 1997).

7.1.3 Salinity

One of the major effects of salinity is osmotic stress, and hence there are intimate relationships to drought stress. Therefore, considering CAM as a major photosynthetic accommodation to water stress, CAM might be expected to be a prominent trait among halophytes. Moreover, halophytes are often succulent as they sequester NaCl in large central vacuoles, which is called salt succulence (Ellenberg, 1981). However, observations do not support this expectation as, in general, halophytes are not CAM plants and CAM plants are not halophytes. Generally CAM plants, including desert succulents, are highly salt sensitive (Lüttge, 2004). CAM plants inhabiting highly saline ecosystems are either effectively functional salt excluders at the root level, such as some cacti or complete escape from the saline substrate by retreat to epiphytic niches (Lüttge, 2004). The single exception is the annual facultative halophyte and facultative CAM species *Mesembryanthemum crystallinum* (Cushman and Bohnert, 2002). This plant can grow well in the absence of NaCl but has its growth optimum at several hundred mM NaCl in the medium and can complete its life cycle at 500 mM NaCl (Lüttge, 2002).

7.2 CAM physiotypes

There are some photosynthetic physiotypes for the metabolic cycle of CAM include full CAM, CAM idling, CAM cycling, C_3/CAM and C_4/CAM (Table 1). In CAM idling stomata remain closed day and night and the day/night organic acid cycle is fed by internal recycling of nocturnally re-fixed respiratory CO_2. In CAM cycling, stomata remain closed during the dark period but some nocturnal synthesis of organic acid fed by respiratory CO_2 occurs, and stomata are open during the light period with uptake of atmospheric CO_2 and direct Calvin-cycle CO_2 reduction (C_3-photosynthesis) in addition to assimilation of CO_2 remobilized from nocturnally stored organic acid. CAM idling is considered as a form of very strong CAM, while CAM cycling is weak CAM (Sipes and Ting, 1985). In the epiphytic

Codonanthe crassifolia (Gesneriaceae), CAM cycling was observed in well-watered plants and CAM idling in drought-stressed plants. CAM cycling that scavenges respiratory CO_2 appears to be a starting point for CAM evolution (Guralnick et al., 2002). The various forms of weak and strong CAM may be restricted to different individual species or may also be expressed temporarily in one given species. For example, *Sedum telephium* has the potential to exhibit pure C_3 characteristics when well-watered and a transition to CAM when droughted, including a continuum of different stages of CAM expression which are repeatedly reversible under changing drought and watering regimes (Lee and Griffiths, 1987).

CAM physiotypes	Phase of CO_2 fixation	Phase of stomatal closure	Diel Fluctuation of malate concentration	Diel pH Fluctuation
Full CAM	I	II, III, IV	>15	High
CAM idling	---	I, II, III, IV	>15	High
CAM cycling	II, III, IV	I	>5	Low
C_3/CAM				Intermediate
C_4/CAM				Intermediate

Table 1. Various CAM physiotypes with different degrees of CAM expression.

There are true intermediate species (C_3/CAM) that can switch between full C_3 photosynthesis and full CAM. The large genus *Clusia*, comprises three photosynthetic physiotypes, i.e. C_3, C_3/CAM and CAM. There are also some C_4/CAM intermediate species, e.g. *Peperomia camptotricha*, *Portulaca oleracea* and *Portulaca grandiflora* (Guralnick et al., 2002). Only succulent C_4 dicotyledons are capable of diurnal fluctuations of organic acids, where dark-respiratory CO_2 is trapped in bundle sheaths by PEPC and the water storage tissue in the succulent leaves may also participate in the fixation of internally released CO_2. In *Portulaca*, this may be a form of CAM cycling in leaves with C_4 photosynthesis, while stems perform CAM idling (Guralnick et al., 2002). However, although C_4 photosynthesis and weak CAM occur in the same leaves, they are separated in space and do not occur in the same cells.

Compatibility of CAM and C_4 photosynthesis has been questioned (Sage, 2002a). Incompatibility of C_4 photosynthesis and CAM may be due to anatomical, biochemical and evolutionary incompatibilities. The separation of malate synthesis and decarboxylation in space in C_4 photosynthesis and in time in CAM, respectively, and the primary evolution of C_4 photosynthesis for scavenging photorespiratory CO_2 and of CAM for scavenging respiratory CO_2 (CAM cycling) may be the most important backgrounds of these incompatibilities. Although single cells may perform C_4 photosynthesis, there is intracellular compartmentation of carboxylation and decarboxylation, and these cells never perform CAM. Unlike C_3-CAM coupling, there is never C_4-CAM coupling and both pathways only occur side by side in C_4/CAM intermediate species (Sage, 2002a).

7.3 CAM evolution

CAM occurs in approximately 6% of plants, comprising monocots and dicots, encompassing 33 families and 328 genera including terrestrial and aquatic angiosperms, gymnosperms and *Welwitschia mirabilis* (Sayed, 2001). Its polyphyletic evolution was facilitated because there

are no unique enzymes and metabolic reactions specifically required for CAM. CAM in the terrestrial angiosperms is thought to have diversified polyphyletically from C_3 ancestors sometime during the Miocene, possibly as a consequence of reduced atmospheric CO_2 concentration (Raven and Spicer, 1996). There is strong evidence that the evolutionary direction has been from C_3/CAM intermediates to full CAM, paralleled by specialization to and colonization of new, increasingly arid habitats (Kluge et al., 2001). A rearrangement and appropriately regulated complement of enzyme reactions present for basic functions in any green plant tissue are sufficient for performing CAM (Lüttge 2004). However, CAM-specific isoforms of key enzymes have evolved. Analysis of PEPC gene families from facultative and obligate CAM species led to the conclusion that during the induction of CAM, in addition to the existing housekeeping isoform, a CAM-specific PEPC isoform is expressed, which is responsible for primary CO_2 fixation of this photosynthetic pathway (Cushman and Bohnert 1999). A single family member of a small gene family (e.g. four to six isogenes) is recruited to fulfill the increased carbon flux demand of CAM. The recruited family member typically shows enhanced expression in CAM-performing leaves. Remaining isoforms, which presumably fulfill anapleurotic 'housekeeping' or tissue-specific functional roles, generally have lower transcript abundance and show little change in expression following water deficit. This 'gene recruitment' paradigm is likely to apply to other gene families as well (Cushman and Borland, 2002). In addition to enzymes involved in malate synthesis and mobilization, CAM induction involves large increases in carbohydrate-forming and -degrading enzymes (Häusler *et al*. 2000). Such activity changes are matched by corresponding changes in gene expression of at least one gene family member of glyceraldehyde-3-phosphate dehydrogenase, enolase and phosphoglyceromutase (Cushman and Borland, 2002). CAM induction causes a dramatic increase in transcripts encoding PEP-Pi and glucose-6-phosphate-Pi translocators, with expression peaking in the light period, whereas transcripts for a chloroplast glucose transporter and a triose-phosphate transporter remain largely unchanged (Häusler *et al*. 2000).

Duplication events appear to be the source of CAM-specific genes recruited from multigene families during CAM evolution (Cushman and Bohnert 1999). Enzyme isoforms with different subcellular locations are also thought to have evolved through gene duplication of pre-existing. Following gene duplication, modification of multipartite cis-regulatory elements within non-coding 5′ and 3′ flanking regions is likely to have occurred, conferring water-deficit-inducible or enhanced expression patterns for CAM-specific isogenes (Cushman and Borland, 2002).

Transcriptional activation appears to be the primary mechanism responsible for increased or enhanced expression of CAM-specific genes following water-deficit stress. Most changes in transcript abundance correlate with changes in protein amounts arising from *de novo* protein synthesis. Alterations in the translational efficiency of specific mRNA populations may also contribute significantly to the expression of key CAM enzymes (Cushman and Borland, 2002).

8. C_3-C_4 intermediate species

Evolution of C_4 species undoubtedly involved steps in which anatomical characteristics were between those of C_3 and C_4 species.

Evidences suggest that C_4 plants have evolved from ancestors possessing the C_3 pathway of photosynthesis and this has occurred independently over 45 times in taxonomically diverse

groups (Sage, 2004). Naturally occurring species with photosynthetic characteristics intermediate between C_3 and C_4 plants have been identified in the genera *Eleucharis* (Cyperaceae), *Panicum* (Poaceae), *Neurachne* (Poaceae), *Mollugo* (Aizoaceae), *Moricandia* (Brassicaceae), *Flaveria*, (Asteraceae) *Partheniurn* (Asteraceae), Salsola (Chenopodiaceae), Heliotropium (Boraginaceae) and *Alternanthera* (Amaranthaceae) (Brown and Hattersley 1989; Rawsthorne, 1992; Voznesenskaya et al., 2001; Muhaidat, 2007). All of these genera include C_3 species and most also include C_4 species.

The intermediate nature of these species is reflected in the isotopic composition (δ^{13}), CO_2 compensation point (Γ) as well as in the differential distribution of organelles in the bundle sheath cells (Table 2).

Photosynthetic type	δ^{13}Value (‰)	Γ (µmol mol^{-1})	Organelles in bundle sheath cells (%)	
			Chloroplasts	Mitochondria +Peroxisomes
C_3	~ −30	48−62	9-11	8-19
C_3−C_4	~ −28	9−17	13-25	25-52
C_4	~ −15	3−5	28-53	30-74

Table 2. Main characteristics of C_3-C_4 species from various genera showing the intermediate nature of these species.

Intermediate species are also recognized in their CO_2 net assimilation rate as a function of intercellular CO_2 concentration and in the CO_2 compensation point as a function of O_2 concentration in the medium (Fig. 7).

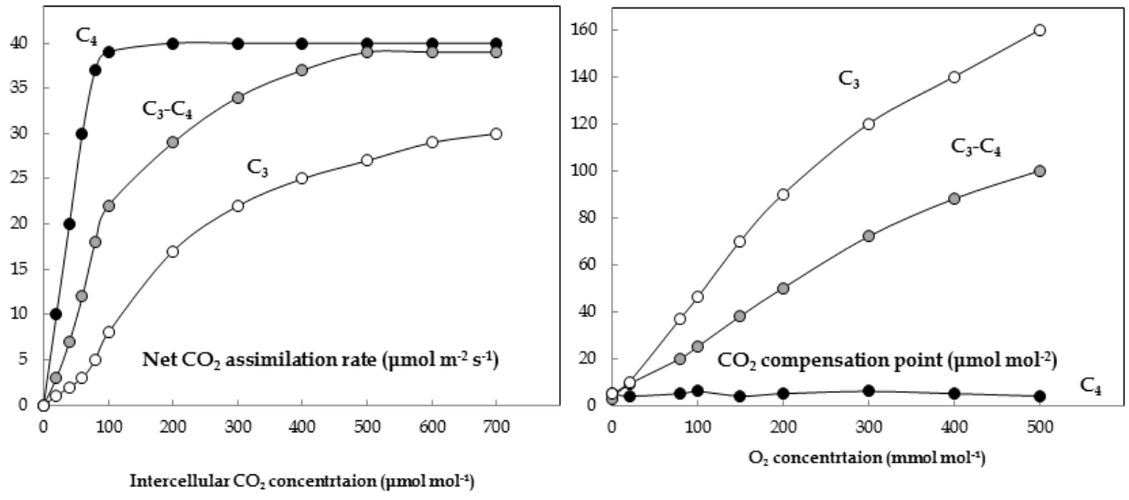


Fig. 7. Generalized curves for net assimilation rate (left) and compensation point (right) of CO_2 in C_3, C_4 and C_3-C_4 intermediate species.

8.1 Leaf anatomy

C_3-C_4 species have anatomical characteristics between those of C_3 and C_4. The vascular bundles are surrounded by chlorenchymatous bundle sheath cells reminiscent of the Kranz anatomy of leaves of C_4 plants (Fig. 8). However, the mesophyll cells are not in a concentric

ring around the bundle sheath cells as in a C_4 leaf, but are arranged as in leaves of C_3 species where interveinal distances are also much greater. In all intermediate species, the bundle sheath cells contain large numbers of organelles. Numerous mitochondria, the peroxisomes and many of the chloroplasts are located centripetally in the bundle sheath cells. The mitochondria are found along the cell wall adjacent to the vascular tissue and are overlain by the chloroplasts. Quantitative studies have shown that the mitochondria and peroxisomes are four times more abundant per unit cell area than in adjacent mesophyll cells and that these mitochondria have twice the profile area of those in the mesophyll (Brown and Hattersley, 1989; McKown and Dengler, 2007, 2009).

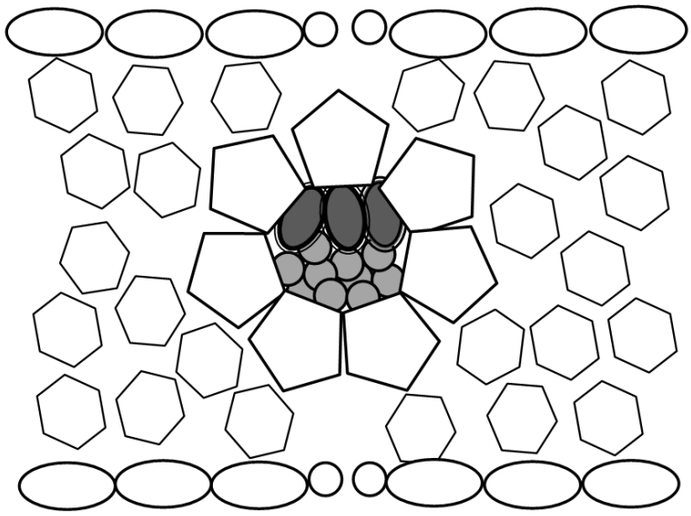


Fig. 8. Leaf anatomy in a C_3-C_4 intermediate species. Note the concentric layer of not well-developed bundle sheath cells (large hexagons) surrounded by not concentrically-arranged mesophyll cells (small hexagons).

Although some of the C_3-C_4 species, notably in *Flaveria* and *Moricandia*, do not have very well developed Kranz anatomy, they all exhibit a tendency to partition more cells to the bundle sheath and to concentrate organelles in bundle sheath cells. The tendency to partition organelles to the bundle sheath was not accomplished in a parallel way in the various C_3-C_4 species. The small bundle sheath cells in *Neurachne minor*, for example, resulted in only 5% of the total cell profile area being in the bundle sheath. But the high concentration of organelles in bundle sheath cells compensated for their small size. In other C_3-C_4 species, increased partitioning of organelles in bundle sheath cells compared to C_3 species resulted from both higher organelle concentrations and increased bundle sheath cells size and/or number relative to mesophyll cells (Brown and Hattersley, 1989; McKown and Dengler, 2007, 2009). In addition, C_3-C_4 intermediate species plasmodesmatal densities at the bundle sheath/mesophyll interface approach those of C_4 species and are much greater than those of the C_3 species studied (Brown et al, 1983).

8.2 Leaf gas exchange in C_3-C_4 intermediate species

Photosynthetic rates of C_3 and C_3-C_4 intermediate species are comparable in a range of light and atmospheric gas compositions, but the responses of gas exchange parameters which

provide a measure of photorespiratory activity differ widely between these two photosynthetic groups. In contrast to C_3 plants where Γ is essentially unaffected by light intensity, Γ is strongly light-dependent in C_3-C_4 intermediate species. There is no evidence that the oxygenation reaction of Rubisco was itself being suppressed to any major extent by a C_4-like mechanism. Whereas about 50% of the photorespiratory CO_2 of a C_3 leaf is recaptured before it escapes from the leaf, it was estimated that up to 73% is recaptured in a C_3-C_4 leaf. Clearly, the improved recapture of CO_2 could account for a low Γ in C_3-C_4 species but a mechanism was required to explain how this improvement occurred (Hunt et al., 1987; Sudderth et al., 2007).

8.3 Biochemical mechanisms in C_3-C_4 intermediate species

Because of the intermediate nature of Γ and the somewhat C_4-like leaf anatomy of the C_3-C_4 species, many researchers attempted to show that these species had a partially functional C_4 cycle which accounted for their low rates of photorespiration and hence Γ. However, there is now good evidence that C_3-C_4 intermediates in the genera *Alternanthera, Moricandia, Panicurn* and *Parthenium* do not have a C_4 cycle which could account for their low rates of photorespiration. Activities of PEPC and the C_4 cycle decarboxylases are far lower than in C_4 leaves, and Rubisco and PEPC are both present in mesophyll and bundle sheath cells. Label from $^{14}CO_2$ is not transferred from C_4 compounds to Calvin cycle intermediates during photosynthesis. There was clearly another explanation for low apparent photorespiration in these species. Since gas exchange measurements indicated that CO_2 was being extensively recaptured via photosynthesis, and the unusual leaf anatomy was at least in part consistent with this mechanism, the location of the photorespiratory pathway in leaves of the C_3-C_4 species has been examined (Rawsthorne, 1992).

It was shown that, the differential distribution of glycine decarboxylase is a major key to the unusual photorespiratory metabolism and Γ of C_3-C_4 intermediate species. This enzyme is abundant in the mitochondria of leaves of higher plants but is only detected at very low levels in mitochondria from other tissues. Glycine decarboxylase has four heterologous subunits (P, H, T, and L) which catalyse, in association with serine hydroxymethyltransferase, the metabolism of glycine to serine, CO_2 and ammonia. The P, H, T, and L subunits are all required for activity of gdc but the P subunit catalyses the decarboxylation of glycine. Immunocytological and in-situ hybridization studies have shown that the P subunit, is absent from the mesophyll mitochondria and the expression of the P subunit gene in the mesophyll is specifically prevented in the leaves of C_3-C_4 intermediate species. It seems likely, therefore, that the differential distribution of glycine decarboxylase must contribute to the observed reduction in apparent photorespiration in the C_3-C_4 species (Rawsthorne, 1992; Yoshimura et al., 2004).

9. Evolution of C_4 photosynthesis

C_4 photosynthesis is a series of biochemical and anatomical modifications that concentrate CO_2 around the carboxylating enzyme Rubisco. Many variations of C_4 photosynthesis exist, reflecting at least 45 independent origins in 19 families of higher plants. C_4 photosynthesis is present in about 7500 species of flowering plants, or some 3% of the estimated 250 000 land plant species. Most C_4 plants are grasses (4500 species), followed by sedges (1500 species) and dicots (1200 species). C_4 photosynthesis is an excellent model for complex trait

evolution in response to environmental change (Furbank et al., 2000; Sage, 2001; Keeley and Rundel 2003; Sage, 2004; Sage et al., 2011).

Molecular phylogenies indicate that grasses were the first C_4 plants, arising about 24–34 million yr ago. Chenopods were probably the first C_4 dicots, appearing 15 –20 million yr ago. By 12–14 million yr ago, C_4 grasses were abundant enough to leave detectable fossil and isotopic signatures. By the end of the Miocene, C_4-dominated grasslands expanded across many of the low latitude regions of the globe, and temperate C_4 grasslands were present by 5 million yr ago (Cerling et al., 1999).

Rubisco and the C_3 mode of photosynthesis evolved early in the history of life and apparently were so successful that competing forms of net photosynthetic carbon fixation have gone extinct. In high CO_2 atmospheres, Rubisco operates relatively efficiently. However, the active site chemistry that carboxylates RuBP can also oxygenate i.e. photorespiration. In the current atmosphere, photorespiration can inhibit photosynthesis by over 30% at warmer temperatures (> 30°C). Evolving a Rubisco that is free of oxygenase activity also appears unlikely because the active site biochemistry is constrained by similarities in the oxygenase and carboxylase reactions. In the absence of further improvements to Rubisco, the other solution to the photorespiratory problem is to enhance the stromal concentration of CO_2 or to reduce O_2. Reducing O_2 is unlikely due to unfavorable energetics. Increasing CO_2 around Rubisco by 1000 ppm would nearly eliminate oxygenase activity, and under circumstances of high photorespiration could justify the additional energy costs required to operate a CO_2 pump (von Caemmerer and Furbank, 2003).

PEPC is the other major carboxylase in C_3 plants. In its current configuration, however, PEP carboxylation does not allow for net CO_2 fixation into carbohydrate, because the carbon added to PEP is lost as CO_2 in the Krebs cycle. For PEPC to evolve into a net carboxylating enzyme, fundamental rearrangements in carbon flow would also be required, while the existing role of PEPC would have to be protected or replaced in some manner (Sage, 2004).

Instead of evolving novel enzymes, CO_2 concentration requires changes in the kinetics, regulatory set points, and tissue specificity of existing enzymes. This pattern of exploiting existing biochemistry rather than inventing new enzymes is the general rule in complex trait evolution. Given these considerations, it is no surprise that the primary means of compensating for photorespiration in land plants has been the layering of C_4 metabolism over existing C_3 metabolism. All C_4 plants operate a complete C_3 cycle, so in this sense the C_4 pathway supplements, rather than replaces, C_3 photosynthesis. Because it uses existing biochemistry, the evolutionary trough that must be crossed to produce a C_4 plant is relatively shallow, and could be bridged by a modest series of incremental steps (Furbank et al., 2000; Sage, 2001; Keeley and Rundel 2003; Sage, 2004; Sage et al., 2011).

9.1 Effect of environmental factors on C_4

C_4 photosynthesis has been described as an adaptation to hot and dry environments or to CO_2 deficiency. These views, however, have been challenged in recent publications. C_4 plants do not appear to be any more drought-adapted than C_3 species from arid zones and a diverse flora of C_4 grasses occurs in the tropical wetland habitats. In addition, there is a disparity between the timing of C_4 expansion across the earth and the appearance of low atmospheric CO_2. C_4-dominated ecosystems expanded 5 and 10 million yr ago, but no obvious shift in CO_2 has been documented for this period (Cerling, 1999). Indeed, C_4

photosynthesis is not a specific drought, salinity or low-CO_2 adaptation, but it as an adaptation that compensates for high rates of photorespiration and carbon deficiency. In this context, all environmental factors that enhance photorespiration and reduce carbon balance are responsible for evolution of C_4 photosynthesis. Heat, drought, salinity and low CO_2 are the most important factors, but others, such as flooding, could also stimulate photorespiration under certain conditions (Sage, 2004).

9.1.1 Heat. Salinity and drought

High temperature is a major environmental requirement for C_4 evolution because it directly stimulates photorespiration and dark respiration in C_3 plants. The availability of CO_2 as a substrate also declines at elevated temperature due to reduced solubility of CO_2 relative to O_2. Aridity and salinity are important because they promote stomatal closure and thus reduce intercellular CO_2 level, again stimulating photorespiration and aggravating a CO_2 substrate deficiency. Relative humidity is particularly low in hot, arid regions, which will further reduce stomatal conductance, particularly if the plant is drought stressed. The combination of drought, salinity, low humidity and high temperature produces the greatest potential for photorespiration and CO_2 deficiency (Ehleringer and Monson, 1993), so it is not surprising that these environments are where C_4 photosynthesis would most frequently arise. Many C_3-C_4 intermediates are from arid or saline zones, for example intermediate species of *Heliotropium, Salsola*, *Neurachne*, *Alternanthera* and a number of the *Flaveria* intermediates (Sage, 2004).

C_4 photosynthesis may have evolved in moist environments as well, which can be consistent with the carbon-balance hypothesis if environmental conditions are hot enough to promote photorespiration. The sedge lineages largely occur in low-latitude wetlands, indicating they may have evolved on flooded soils and the aquatic C_4 species certainly evolved in wet environments (Bowes et al., 2002). In the case of the aquatic, single-celled C_4 species, warm shallow ponds typically become depleted in CO_2 during the day when photosynthetic activity from algae and macrophytes is high. Many of the C_3-C_4 intermediates such as *Flaveria linearis, Mollugo verticillata* also occur in moist, disturbed habitats such as riverbanks, roadsides and abandoned fields indicate that disturbance is also an important factor in C_4 evolution, particularly for lineages that may have arisen in wetter locations (Monson 1989).

9.1.2 Low CO_2 concentration

In recent geological time, low CO_2 prevailed in the earth's atmosphere. For about a fifth of the period of past 400 000 yr, CO_2 was below 200 ppm. Because low CO_2 prevailed in recent geological time, discussions of C_4 evolution must consider selection pressures in atmospheres with less CO_2 than today. In low CO_2, C_3 photosynthesis is impaired by the lack of CO_2 as a substrate in addition to enhanced photorespiration (Ehleringer, 2005). As a result, water and nitrogen-use efficiencies and growth rates are low, competitive ability and fecundity is reduced and recovery from disturbance is slow (Ward, 2005). There is a strong additive effect between heat, drought and salinity and CO_2 depletion, so that, the inhibitory effects of heat, drought and salinity increase considerably in low CO_2.

Manipulation of the biosphere by human and increases in atmospheric CO_2 could halt the rise of new C_4 life forms and may lead to the reduction of existing ones (Edwards *et al.*, 2001). However, certain C_4 species are favored by other global change variables such as climate warming and deforestation. Hence, while many C_4 species may be at risk, C4

photosynthesis as a functional type should not be threatened by CO_2 rise in the near term (Sage, 2004).

9.2 Evolutionary pathways to C_4 photosynthesis

Evolution was not directed towards C_4 photosynthesis, and each step had to be stable, either by improving fitness or at a minimum by having little negative effect on survival of the genotype. The predominant mechanisms in the evolution of C_4 genes are proposed to be gene duplication followed by nonfunctionalization and neofunctionalization (Monson, 1999, 2003), and alteration of *cis*-regulatory elements in single copy genes to change expression patterns (Rosche and Westhoff, 1995). Major targets for non- and neofunctionalization are the promoter and enhancer region of genes to allow for altered expression and compartmentalization, and the coding region to alter regulatory and catalytic properties. Both non- and neofunctionalization can come about through mutations, crossover events, and insertions of mobile elements (Kloeckener-Gruissem and Freeling, 1995; Lynch & Conery, 2000). A model for C_4 evolution has been presented that recognizes seven significant phases (Sage, 2004) (Table 3).

10. Single cell C_4 photosynthesis

The term Kranz anatomy is commonly used to describe the dual-cell system associated with C_4 photosynthesis, consisting of mesophyll cells containing PEPC and initial reactions of C_4 biochemistry, and bundle sheath cells containing enzymes for generating CO_2 from C_4 acids and the C_3 carbon reduction pathway, including Rubisco. Kranz anatomy is an elegant evolutionary solution to separating the processes, and for more than three decades it was considered a requirement for the function of C_4 photosynthesis in terrestrial plants (Edwards et al., 2001).

This paradigm was broken when two species, *Borszczowia aralocaspica* and *Bienertia cycloptera*, both representing monotypic genera of the family Chenopodiaceae, were shown to have C_4 photosynthesis within a single cell without the presence of Kranz anatomy (Voznesenskaya et al., 2001; Sage, 2002b; Edwards and Voznesenskaya, 2011). *Borszczowia* grows in central Asia from northeast of the Caspian lowland east to Mongolia and western China, whereas *Bienertia* grows from east Anatolia eastward to Turkmenistan and Pakistani Baluchestan (Akhani et al., 2003).

Single-cell C_4 plants can capture CO_2 effectively from Rubisco without Kranz anatomy and the bundle sheath cell wall barrier. Photosynthesis in the single-cell systems is not inhibited by O_2, even under low atmospheric levels of CO_2, and their carbon isotope values are the same as in Kranz-type C_4 plants, whereas the values would be more negative if there were leakage of CO_2 and overcycling through the C_4 pathway (Voznesenskaya et al., 2001; Edwards and Voznesenskaya, 2011).

Borszczowia has a single layer of elongate, cylindrical chlorenchyma cells below the epidermal and hypodermal layers, which surround the veins and internal water storage tissue. The cells are tightly packed together with intercellular space restricted to the end of the cells closest to the epidermis. The anatomy of *Bienertia* leaves with respect to photosynthetic tissue is very different in that there are two to three layers of shorter chlorenchyma cells that surround the centrally located water-storage and vascular tissue in the leaf. The cells are loosely arranged, with considerable intercellular space around them (Edwards et al., 2004).

Stage	Events
General Preconditioning	**Modification of the gene copies without losing the original function:** multiplication of genes by duplication → selection and screen for adaptive functions in the short-lived annuals and perennials → reproductive barriers → genetically isolated populations.
Anatomical Preconditioning	**Decline of distance between mesophyll (MC) and bundle sheath cells (BSC) for rapid diffusion of metabolites:** reduction of interveinal distance and enhancement of BSC layer size → adaptive traits without relationship with photosynthesis: improvement of structural integrity in windy locations and enhancement of water status of the leaf in hot environments → selection. Easier reduction of MC and BSC distance in species with parallel venation (grasses) than in species with reticulate venation (dicots) → C_4 photosynthesis first arose in grasses and is prolific in this family.
Creating Metabolic Sink for Glycine Metabolism and C_4 Acids	**Increase in bundle sheath organelles:** the number of chloroplasts and mitochondria in the bundle sheath increases in order to maintain photosynthetic capacity in leaves with enlarged BSC→ increased capacity of BSC to process glycine from the mesophyll → subsequent development of a photorespiratory CO_2 pump → further increase in organelle number → greater growth and fecundity in high photorespiratory environments → maintaining incremental rise in BSC organelle content → significant reduction in CO_2 compensation points.
Glycine Shuttles and Photorespiratory CO_2 Pumps	**Changes in the glycine decarboxylase (GDC) genes:** duplication of GDC genes, production of distinct operations with separate promoters in the MC and BSC → loss of function mutation in the MC GDC → movement of glycine from MC to the BSC to prevent lethal accumulation of photorespiratory products → subsequent selection for efficient glycine shuttle.
Efficient Scavenging of CO_2 Escaping from the BSC	**Enhancement of PEPC activity in the MC**: reorganization of expression pattern of enzymes: specific expression of C_4 cycle enzymes in the MC and localization of Rubisco in BSC, increase in the activity of carboxylating enzymes: NADP-ME, NAD-ME through increasing transcriptional intensity, increased PPDK activity in the later stages.
Integration of C_3 and C_4 Cycles	**Avoidance of competition between PEPC and Rubisco in the MC for CO_2 and ATP increase in the phases of C_4 cycle:** further reorganization of the expression pattern of enzymes: reduction in the carbonic anhydrase activity in chloroplasts of BSC for preventing its conversion to bicarbonate and its diffusion out of the cell without being fixed by Rubisco, increase in the cytosol of MC to support high PEPC activity → large gradient of CO_2 between BSC and MC, reduction of MC Rubisco activity in the later stages.
Optimization and Whole-Plant Coordination	**Selection for traits that allow plants to exploit the productive potential of the C_4 pathway to the maximum:** adjustment and optimization of photosynthetic efficiency, kinetic properties and regulatory set-points of enzymes to compensate for changes in the metabolic environment: (1) Optimization of NADP-ME regulation in the earlier phases of C_4 evolution: increase in the specific activity of NADP-ME and reduction of Km for malate. (2) Optimization of PEPC in the final stages of C_4 evolution: reduction of sensitivity of PEPC to malate, increased sensitivity to the activator glucose-6-phosphate, increased affinity for bicarbonate and reduced for PEP. (3) Optimization of Rubisco: evolving into a higher catalytic capacity but lower specificity with no negative consequences. (4) Improvement of water-use efficiency: increased stomatal sensitivity to CO_2 and light→ enhancing the ability of stomata to respond to environmental variation at relatively low conductances, reduction of leaf specific hydraulic conductivity by increasing leaf area per unit of conducting tissue.

Table 3. The main evolutionary pathways towards C_4 photosynthesis (Adapted from Sage, 2004).

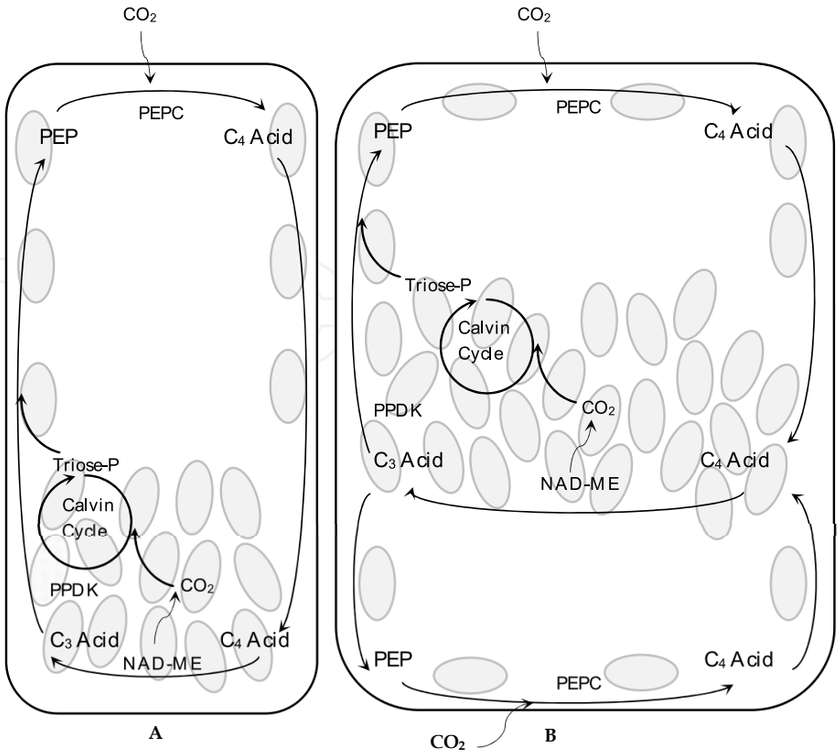


Fig. 9. Model of proposed function of C_4 photosynthesis in the two types of single cell systems in *Borszczowia* (A) and *Bienertia* (B). Note that chloroplasts are in two distinct cytoplasmic compartments.

A model has been proposed for the operation of C_4 photosynthesis in a single chlorenchyma cell in *Borszczowia* and *Bienertia* (Edwards et al., 2004; Edwards and Voznesenskaya, 2011). In *Borszczowia*, atmospheric CO_2 enters the chlorenchyma cell at the distal end, which is surrounded by intercellular air space. Here, the carboxylation phase of the C_4 pathway assimilates atmospheric CO_2 into C_4 acids. Two key enzymes in the process are pyruvate-Pi dikinase (PPDK), located in chloroplasts at the proximal part and PEPC, located in the cytosol. The C_4 acids diffuse to the proximal part of the cell through a thin, cytoplasmic space at the periphery of the middle of the cell, which is devoid of organelles. In the proximal end, the C_4 acids are decarboxylated by NAD-malic enzyme (NAD-ME) in mitochondria that appear to be localized exclusively in this part of the cell. The CO_2 is captured by Rubisco that is localized exclusively in chloroplasts surrounding the mitochondria in the proximal part of the cell (Fig. 9A).

In *Bienertia* there is a similar concept of organelle partitioning in a single cell to operate the C_4 process. However, it has a very different compartmentation scheme (Fig. 9B). Atmospheric CO_2 enters the cell around the periphery, which is exposed to considerable intercellular air space, and here the carboxylation phase of the C_4 pathway functions to convert pyruvate and CO_2 into OAA through the combined action of PPDK in the chloroplast and PEPC in the cytosol. C_4 acids diffuse to the central cytoplasmic compartment through cytoplasmic channels and are decarboxylated by NAD-ME in mitochondria, which are specifically and abundantly located there. Chloroplasts in the central cytoplasmic compartment surround the mitochondria and fix the CO_2 by Rubisco,

which is only present in the chloroplasts of this compartment, through the C_3 cycle (Edwards et al., 2004; Edwards and Voznesenskaya, 2011).
Single-cell C_4 photosynthesis could simply be an alternative mechanism to Kranz type C_4 photosynthesis. Although it may be equally complex in its control of compartmentation of functions, is less complex in that it does not require the cooperative function of two cell types, nor does it require development of Kranz anatomy. Single-cell C_4 allows more flexibility in mode of photosynthesis than Kranz-type C_4 plants by, for example, shifting from C_3 to C_4 depending on environmental conditions (Edwards et al., 2004; Edwards and Voznesenskaya, 2011).

11. Conclusion

Life on earth largely depends on the photosynthetic carbon fixation using light energy. Energy-rich sugar molecules are the basis of many growth and developmental processes in plants. Reduced carbon products in the leaves, however, are used not only for synthesis of carbohydrates but also in a number of primary and secondary metabolic pathways in plants including nitrogen assimilation, fatty acid synthesis and phenolic metabolism.
Photosynthetic carbon assimilation is an investment of resources and the extent of this investment responds to the economy of the whole plant. Maintenance of energy homeostasis requires sophisticated and flexible regulatory mechanisms to account for the physiological and developmental plasticity observed in plants. It this regard, sugars not only are the prime carbon and energy sources for plants, but also play a pivotal role as a signaling molecule that control metabolism, stress response, growth, and development of plants.
Environmental factors determine the distribution and abundance of plants and evolutionary adaptation is an inevitable response to environmental change. Throughout the course of geological time, the environments in which plants grew have been changing, often radically and irreversibly. Physiological adaptation to environmental variables cannot improve without associated changes in morphology and anatomy. Evolution of C_4 plants is an excellent example of parallel evolution of leaf physiology and anatomy. Finally, any physiological evolution must be associated with changes at biochemical and molecular level. This chapter provides an introduction to theses area with a focus on plasticity in the carbon metabolism and evolution of variants of the carbon assimilation pathways.

12. References

Ainsworth, E.A.; Rogers, A.; Nelson, R. & Long, S.P. (2004). Testing the source-sink hypothesis of down-regulation of photosynthesis in elevated [CO_2] with single gene substitutions in *Glycine max*. *Agricultural and forest meteorology,* Vol. 122, pp. 85-94, ISSN 0168-1923

Akhani, H.; Ghobadnejhad, M. & Hashemi, S.M.H. (2003). Ecology, biogeography, and pollen morphology of *Bienertia cycloptera* Bunge ex Boiss. (Chenopodiaceae), an enigmatic C_4 plant without Kranz anatomy. *Plant Biology,* Vol. 5, pp. 167-78, ISSN 1435-8603

Bauwe, H. (2011). Photorespiration: The Bridge to C_4 Photosynthesis. In: *C_4 Photosynthesis and Related Concentrating Mechanisms*, *Advances in Photosynthesis and Respiration*, *Vol.*

32, Raghavendra, A. S. & Sage, R. F. (Eds.), pp. 81-108, ISBN 978-90-481-8530-6, Springer, Dordrecht, The Netherlands.

Bhagwat, A.S. (2005). Photosynthetic carbon assimilation of C_3, C_4 and CAM pathways. In: *Hand Book of Photosynthesis*, 2nd Edition, Pessarakli, M. (Ed.). pp. 376-389, ISBN 0-8247-5839-0, CRC Press, Taylor & Francis Group, Boca Raton, FL, USA.

Bowes, G.; Rao, S.K.; Estavillo, G.M. & Reiskind, J.B. (2002). C_4 mechanisms in aquatic angiosperms: comparisons with terrestrial C_4 systems. *Functional Plant Biology,* Vol. 29, pp. 379–392, ISSN 1445-4408

Brown, R.H.; Bouton, J.H.; Rigsby, L. & Rigler, M. (1983). Photosynthesis of grass species differing in carbon dioxide fixation pathways. VIII. Ultrastructural characteristics of *Panicum* species in the Laxa group. *Plant Physiology,* Vol. 71, pp. 425-431, ISSN 0032-0889

Brown, R.H. & Hattersley, P. W. (1989) Leaf anatomy of C_3-C_4 species as related to evolution of C_4 photosynthesis. *Plant Physiology,* Vol. 91, pp. 1543-1550, ISSN 0032-0889

Brulfert, J.; Vidal, J.; Keryer, E., Thomas, M.; Gadal, P. & Queiroz, O. (1985). Phytochrome control of phosphoenolpyruvate carboxylase synthesis and specific RNA level during photoperiodic induction in a CAM plant and during greening in a C_4 plant. *Physiologie Vegetale,* Vol. 23, pp. 921-928, ISSN 0570-1643

Cerling, T.E. (1999). Paleorecords of C_4 plants and ecosystems. In: *C_4 Plant Biology*, Sage, R.F. & Monson, R.K. (Eds.), pp. 445–469, ISBN 0126144400, Academic Press, San Diego, CA, USA.

Cornic, G. & Massacci, A. (1996). Leaf photosynthesis under drought stress. In: *Photosynthesis and the Environment,* Baker, N. R. (Ed.), pp. 347–366, ISBN 9780792343165, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Cushman, J.C. & Bohnert, H.J. (1999). Crassulacean acid metabolism: molecular genetics. *Annual Review of Plant Physiology and Plant Molecular Biology,* Vol. 50, pp. 305-332, ISSN 1040-2519

Cushman, JC. & Bohnert, H.J. (2002). Induction of Crassulacean acid metabolism by salinity—molecular aspects. In: *Salinity: Environment – Plants – Molecules*, Läuchli, A. & Lüttge, U. (Eds.), pp 361–393, ISBN 978-90-481-5965-9, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Cushman, J.C. & Borland, A.M. (2002). Induction of crassulacean acid metabolism by water limitation. *Plant, Cell and Environment,* Vol. 25, pp. 297-312, ISSN 0140-7791

Dodd, A.N.; Borland, A.M.; Haslam, R.P.; Griffith, H. & Maxwell, K. (2002). Crassulacean acid metabolism: plastic fantastic. *Journal of Experimental Botany,* Vol. 53, pp. 569-580, ISSN 0022-0957

Edwards, G.E. & Voznesenskaya, E.V. (2011). C_4 Photosynthesis: Kranz forms and single-cell C_4 in terrestrial plants. In: *C_4 Photosynthesis and Related Concentrating Mechanisms*, *Advances in Photosynthesis and Respiration*, *Vol. 32,* Raghavendra, A. S. & Sage, R. F. (Eds.), pp. 29-61, ISBN 978-90-481-8530-6, Springer, Dordrecht, The Netherlands.

Edwards, G.E.; Franceschi, V.R.; Ku, M.S.B.; Voznesenskaya, E.V.; Pyankov, V.I. & Andreo, C.S. (2001). Compartmentation of photosynthesis in cells and tissues of C_4 plants. . *Journal of Experimental Botany,* Vol. 52, pp. 577–90, ISSN 0022-0957

Edwards, G.E.; Franceschi, V.R. & Voznesenskaya, E.V. (2004). Single-cell C_4 photosynthesis versus the dual-cell (Kranz) paradigm. *Annual Review of Plant Biology,* Vol. 55, pp. 173–96, ISSN 1543-5008

Ehleringer, J.R. & Monson, R.K. (1993). Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics,* Vol. 24, pp. 411–439, ISSN 0066-4162

Ehleringer, J.R. (2005). The influence of atmospheric CO_2, temperature, and water on the abundance of C_3/C_4 taxa. In: *A History of Atmospheric CO_2 and its Effects on Plants, Animals and Ecosystems*, Ehleringer, J.R., Cerling, T.E. & Dearling, D. (Eds.), pp. 214-231, ISBN 978-0-387-22069-7, Springer, Berlin, Germany.

Ellenberg, H. (1981). Ursachen des Vorkommens und Fehlens von Sukkulenten in den Trockengebieten der Erde. *Flora,* Vol. 171, pp. 114-169, ISSN 0367-2530

Farquhar, G.D.; Ehleringer, R. & Hubick, K.T. (1989). Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology,* Vol. 40, pp. 503–37, ISSN 1040-2519

Fowler, D.B.; Breton, G.; Limin, A.E.; Mahfoozi, S. & Sarhan, F. (2001). Photoperiod and temperature interactions regulate low-temperature-induced gene expression in barley. *Plant Physiology,* Vol. 127, pp. 1676-1681, ISSN 0032-0889

Foyer, C.H.; Bloom, A.J.; Queval, G. & Noctor, G. (2009). Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. *Annual Review of Plant Biology,* Vol. 60, pp. 455–484, ISSN 1543-5008

Furbank, R.T.; Hatch, M.D. & Jenkins, C.L.D. (2000). C_4 photosynthesis: mechanism and regulation. In: *Photosynthesis: Physiology and Metabolism*, Leegood, R.C., Sharkey, T.D. & von Caemmerer, S. (Eds.), pp. 435-457, ISBN 978-0-7923-6143-5, Kluwer Academic Publishers, The Netherlands.

Ghannoum, O.; Von Caemmerer, S.; Ziska, L.H. & Conroy, J.P. (2000). The growth response of C_4 plants to rising atmospheric CO_2 partial pressure: a reassessment. *Plant, Cell and Environment,* Vol. 23, pp. 931-942, ISSN 0140-7791

Giardi, M.T.; Cona, A.; Geiken, B.; Kučera, T.; Maojídek, J. & Mattoo, A.K. (1996). Long-term drought stress induces structural and functional reorganization of photosystem II. *Planta,* Vol. 199, pp. 118–125, ISSN 0032-0935

Gilmour, S.J.; Sebolt, A.M.; Salazar, M.P.; Everard, J.D. & Thomashow, M.F. (2000). Overexpression of the *Arabidopsis* CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. *Plant Physiology*, Vol. 124, pp. 1854-1865, ISSN 0032-0889

Gray, J.E.; Holroyd, G.H.; Van der Lee, F.; Bahrami, A.R.; Sijmons, P.C.; Woodward, F.I.; Schuch, W. & Hetherington, A.M. (2000). The *HIC* signaling pathway links CO_2 perception to stomatal development. *Nature*, Vol. 408, pp. 713-716, ISSN 0028-0836

Griffin, K.L.; Anderson, O.R.; Gastrich, M.D.; Lewis, J.D.; Lin, G., Schuster, W.; Seemann, J.R.; Tissue, D.T.; Turnbull, M.H. & Whitehead, D. (2001). Plant growth in elevated CO_2 alters mitochondrial number and chloroplast fine structure. *Proceedings of the National Academy of Sciences USA*, Vol. 98, pp. 2473-2478, ISSN 0027-8424

Guralnick, L.J.; Edwards, G.; Ku, M.S.B.; Hockema, B. & Franceschi, V.R. (2002). Photosynthetic and anatomical characteristics in the C_4-crassulacean acid

metabolism-cycling plant, *Portulaca grandiflora*. *Functional Plant Biology,* Vol. 29, pp. 763-773, ISSN 1445-4408

Guy, C.L.; Huber, J.L.A. & Huber, S.C. (1992). Sucrose phosphate synthase and sucrose accumulation at low-temperature. *Plant Physiology,* Vol. 100, pp. 502-508, ISSN 0032-0889

Hamilton, J.G.; Thomas, R.B. & DeLucia, E.H. (2001). Direct and indirect effects of elevated CO_2 on leaf respiration in a forest ecosystem. *Plant, Cell and Environment*, Vol. 24, pp. 975-982, ISSN 0140-7791

Häusler, R.E.; Baur, B., Scharte, J.; Teichmann,T.; Eicks, M.; Fischer, K.L.; Flügge, U-I.; Schubert, S.; Weber, A. & Fischer, K. (2000). Plastidic metabolite transporters and their physiological functions in the induc ible crassulacean acid metabolism plant *Mesembryanthemum crystallinum*. *The Plant Journal,* Vol. 24, pp. 285-296, ISSN 0960-7412

Hobbie, E.A. & Werner, R.A. (2004). Intramolecular, compound-specific and bulk carbon isotope patterns in C_3 and C_4 plants: a review and synthesis. *New Phytologist,* Vol. 161, pp. 371-385, ISSN 0028-646X

Holtum, J.A.M.; Smith, J.A.C. & Neuhaus, H.E. (2005). Intracellular transport and pathways of carbon flow in plants with crassulacean acid metabolism. *Functional Plant Biology,* Vol. 32, pp. 429-449, ISSN 1445-4408

Hopkins, W.G. & Hüner, N.P.A. (2004). Introduction to Plant Physiology. ISBN 0-471-38915-3, John Wiley & Sons, Inc., USA.

Huber, S.C. & Huber, J.L. (1996). Role and Regulation of sucrosephosphate synthase in higher plants. *Annals of Review of Plant Physiology and Plant Molecular Biology,* Vol. 47, pp. 431-444, ISSN

Hunt, S.; Smith, A.M. & Woolhouse, H.W. (1987). Evidence for a lightdependent system for reassimilation of photorespiratory CO_2, which does not include a C_4 cycle, in the C_3-C_4 intermediate species *Moricandia arvensis*. *Planta,* Vol. 171, pp. 227-234, ISSN 0032-0935

Hurry, V.; Strand, Å.; Furbank, R. & Stitt, M. (2000). The role of inorganic phosphate in the development of freezing tolerance and the acclimatization of photosynthesis to low temperature is revealed by the *pho* mutants of *Arabidopsis thaliana. The Plant Journal,* Vol. 24, pp. 383-396, ISSN 0960-7412

Hymus, G.J.; Dijkstra, P.; Baker, N.R.; Drake, B.G. & Long, S.P. (2000). Will rising CO_2 protect plants from the midday sun? A study of photoinhibition of *Quercus myrtifolia* in a scrub-oak community in two seasons. *Plant, Cell and Environment, Vol.* 24, pp. 1361-1368, ISSN 0140-7791

Keeley, J.E. & Rundel, P.W. (2003). Evolution of CAM and C_4 carbon-concentrating mechanisms. *International Journal of Plant Sciences,* Vol. 164, pp. S55-S77, ISSN 1058-5893

Keeley, J.E. (1996). Aquatic CAM photosynthesis. In: *Crassulacean Acid Metabolism. Biochemistry, Ecophysiology and Evolution*, Winter, K. & Smith, J.A.C. (Eds.), pp. 281-295, ISBN 3540581049, Springer, Berlin, Germany.

Kloeckener-Gruissem, B. & Freeling, M. (1995). Transposon-induced promoter scrambling: a mechanism for the evolution of new alleles. *Proceedings of the National Academy of Sciences USA,* Vol. 92, pp. 1836-1840, ISSN

Kluge, M.; Brulfert, J.; Rauh, W.; Ravelomanana, D. & Ziegler, H. (1995). Ecophysiological studies on the vegetation of Madagascar: a $\delta^{13}C$ and δD survey for incidence of Crassulacean acid metabolism (CAM) among orchids from montane forests and succulents from the xerophytic thorn-bush. *Isotopes in Environmental and Health Studies,* Vol. 31, pp. 191-210, ISSN 1025-6016

Kluge, M.; Razanoelisoa, B. & Brulfert, J. (2001). Implications of genotypic diversity and phenotypic plasticity in the ecophysiological success of CAM plants, examined by studies on the vegetation of Madagascar. *Plant Biology,* Vol. 3, pp. 214-222, ISSN 1435-8603

Knight, C.A. & Ackerly, D.D. (2003). Evolution and plasticity of photosynthetic thermal tolerance, specific leaf area and leaf size: congeneric species from desert and coastal environments. *New Phytologist,* Vol. 160, pp. 337-347, ISSN 0028-646X

LaDeau,. SL. & Clark, J.S. (2001). Rising CO_2 levels and the fecundity of forest trees. *Science,* Vol. 292, pp. 95-98, ISSN 0036-8075

Lake, J.A.; Quick, W.P.; Beerling, D.J. & Woodward, F.I. (2001). Signals from mature to new leaves. *Nature*, Vol. 411, p. 154, ISSN 0028-0836

Lawlor, D.W. & Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell and Environment,* Vol. 25, pp. 275–294, ISSN 0140-7791

Lee, H.S.J & Griffiths, H. (1987). Induction and repression of CAM in *Sedum telephium* L. in response to photoperiod and water stress. *Journal of Experimental Botany,* Vol. 38, pp. 834-841, ISSN 0022-0957

Lin, J.; Jach, M.E. & Ceulemans, R. (2001). Stomatal density and needle anatomy of Scots pine (*Pinus sylvestris*) are affected by elevated CO_2. *New Phytol*ogist, Vol. 150, pp. 665-674, ISSN 0028-646X

Long, S.P. (1991). Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? *Plant, Cell and Environment,* Vol. 14, pp. 729-739, ISSN 0140-7791

Long, S.P.; Ainsworth, E.A.; Rogers, A. & Ort, D.R. (2004). Rising atmosphere carbon dioxide: Plants FACE the Future. *Annual Review of Plant Biology,* Vol. 55, pp. 591-628, ISSN 1543-5008

Lüttge, U. (2004). Ecophysiology of crassulacean acid metabolism (CAM). *Annals of Botany,* Vol. 93, pp. 629-652, ISSN 0305-7364

Lüttge, U. (2002). Performance of plants with C_4-carboxylation modes of photosynthesis under salinity. In: *Salinity: Environment – Plants – Molecules*, Läuchli, A. & Lüttge, U. (Eds.), pp 113-135, ISBN 978-90-481-5965-9, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Lynch, M. & Conery, J.S. (2000). The evolutionary fate and consequences of duplicate genes. *Science*, Vol. 290, pp. 1151-1155, ISSN

Markovska, Y.; Tsonev, T. & Kimenov, G. (1997). Regulation of CAM and respiratory recycling by water supply in higher poikilohydric plants *Haberlea rhodopensis* Friv.

and *Ramonda serbica* Pancic at transition from biosis to anabiosis an vice versa. *Botanica Acta,* Vol. 110, pp. 18-24, ISSN 0932-8629

McKown, A.D. & Dengler, N.G. (2007). Key innovations in the evolution of Kranz anatomy and C_4 vein pattern in *Flaveria* (Asteraceae). *American Journal of Botany,* Vol. 94, pp. 382-399, ISSN 0002-9122

McKown, A.D. & Dengler, N.G. (2009). Shifts in leaf vein density through accelerated vein formation in C_4 *Flaveria* (Asteraceae). *Annals of Botany,* Vol. 104, pp. 1085-1098, ISSN 0305-7364

McKown, R.; Kuroki, G. &Warren, G. (1996). Cold responses of *Arabidopsis* mutants impaired in freezing tolerance. *Journal of Experimental Botany*, Vol. 47, pp. 1919-1925, ISSN 0022-0957

McLeod, A.R. & Long, S.P. (1999). Free-air carbon dioxide enrichment (FACE) in Global Change Research: A review. *Advances in Ecological Research,* Vol. 28, pp. 1–55, ISSN 0065-2504

Medlyn, B.E.; Barton, C.V.M.; Broadmeadow, M.S.J.; Ceulemans, R.; De Angelis, P.; Forstreuter, M.; Freeman, M.; Jackson, S.B.; Kellomäki, S.; Laitat, Rey, A.; Robertnz, P.; Sigurdsson, B.D.; Strassemeyer, J.; Wang, K.; Curtis, P.S. & Jarvis, P.J. (2001). Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. *New Phytologist,* Vol. 149, pp. 247-264, ISSN 0028-646X

Monson, R.K. (1989). On the evolutionary pathways resulting in C_4 photosynthesis and crassulacean acid metabolism (CAM). *Advances in Ecological Research,* Vol. 19, pp. 57-101, ISSN 0065-2504

Monson, R.K. (1999). The origins of C_4 genes and evolutionary pattern in the C_4 metabolic phenotype. In: *C_4 Plant Biology*, Sage, R.F. & Monson, R.K. (Eds.), pp. 377–410, ISBN 0126144400, Academic Press, San Diego, CA, USA.

Monson, R.K. (2003). Gene duplication, neofunctionalization, and the evolution of C_4 photosynthesis. *International Journal of Plant Science,* Vol. 164, pp. S43–S54, ISSN 1058-5893

Muhaidat, R. (2007). Diversification of C_4 Photosynthesis in the Eudicots: Anatomical, Biochemical and Physiological Perspectives. PhD Thesis, University of Toronto, Toronto, Canada.

Müller, P.; Li, X.P. & Niyogi, K.K. (2001). Non-photochemical quenching. A response to excess light energy. *Plant Physiology,* Vol. 125, pp. 1558-1566, ISSN 0032-0889

Nielsen, T.H.; Krapp, A.; Roper-Schwarz, U. & Stitt, M. (1998). The sugar-mediated regulation of genes encoding the small subunit of Rubisco and the regulatory subunit of ADP glucose pyrophosphorylase is modified by phosphate and nitrogen. *Plant, Cell and Environment*, Vol. 21, pp. 443-454, ISSN 0140-7791

Osmond, C.B. (1978). Crassulacean acid metabolism: a curiosity in context. *Annual Review of Plant Physiology,* Vol. 29, pp. 379-414, ISSN 0066-4294

Paul, M.J. & Foyer, C.H. (2001). Sink regulation of photosynthesis. *Journal of Experimental Botany*, Vol. 52, pp. 1383-1400, ISSN 0022-0957

Pelleschi, S.; Rocher, J.P. & Prioul, J.-L. (1997). Effect of water restriction on carbohydrate metabolism and photosynthesis in mature leaves. *Plant, Cell and Environment*, Vol. 20, pp. 493-503, ISSN 0140-7791

Preczewski, P.J.; Heckathorn, S.A.; Downs, C.A. & Coleman, J.S. (2000). Photosynthetic thermotolerance is quantitatively and positively associated with production of specific heat-shock proteins among nine genotypes of *Lycopersicon* (tomato). *Photosynthetica,* Vol. 38, pp. 127-134, ISSN 0300-3604

Prentice, C.; Farquhar, G.; Fasham, M.; Goulden, M.; Heimann, M.; Jaramillo, V.; Kheshgi, H.; Le Quéré, C.; Scholes, R. & Wallace, D. (2001). The carbon-cycle and atmospheric carbon dioxide. In: *Climate Change 2001: The Scientific Basis,* Houghton, J.T.; Ding, Y., Griggs, D.J., Noguer, M. & van der Linden P.J. (Eds.), pp. 183–237, ISBN 0521014956, Cambridge University Press, Cambridge, UK.

Raven, J.A. & Spicer, R.A. (1996). The evolution of Crassulacean acid metabolism. In: *Crassulacean Acid Metabolism. Biochemistry, Ecophysiology and Evolution*, Winter, K. & Smith, J.A.C. (Eds.), pp. 360-385, ISBN 3540581049, Springer, Berlin, Germany.

Rawsthorne, S. (1992). C_3-C_4 intermediate photosynthesis: linking physiology to gene expression. *The Plant Journal,* Vol. 2, pp. 267-274, ISSN 0960-7412

Rosche, E. & Westhoff, P. (1995). Genomic structure and expression of the pyruvate, orthophosphate dikinase gene of the dicotyledonous C_4 plant *Flaveria trinervia* (Asteraceae). *Plant Molecular Biology,* Vol. 29, pp. 663-678, ISSN 0167-4412

Sage, R.F. (2004). The evolution of C_4 photosynthesis. *New Phytologist,* Vol. 161, pp. 341-370, ISSN 0028-646X

Sage, R.F. (2001). Environmental and evolutionary preconditions for the origin and diversification of the C_4 photosynthetic syndrome. *Plant Biology,* Vol. 3, pp. 202–213, ISSN 1435-8603

Sage, R.F. (2002)a. Are crassulacean acid metabolism and C_4 photosynthesis incompatible? *Functional Plant Biology,* Vol. 29, pp. 775-785, ISSN 1445-4408

Sage, R.F. (2002)b. C_4 photosynthesis in terrestrial plants does not require Kranz anatomy. *Trends in Plant Science,* Vol. 7, pp. 283-85, ISSN 1360-1385

Sayed, O.H. (2001) Crassulacean acid metabolism 1975-2000 a check list. *Photosynthtica,* Vol. 39, pp. 339-352, ISSN 0300-3604

Sage, R.F.; Kocacinar, F. & Kubien, D. S. (2011). C_4 photosynthesis and temperature. In: *C_4 Photosynthesis and Related Concentrating Mechanisms,* Raghavendra, A. S. & Sage, R. F. (Eds.), pp. 161-195, ISBN 978-90-481-8530-6, Springer, Dordrecht, The Netherlands.

Sipes, D.L. & Ting, I.P. (1985). Crassulacean acid metabolism and crassulacean acid metabolism modifications in *Peperomia camptotricha*. *Plant Physiology,* Vol. 77, pp. 59-63, ISSN 0032-0889

Ślesak, I.; Miszalski, Z.; Karpinska, B.; Niewiadomska, E.; Ratajczak, R. & Karpinski, S. (2002). Redox control of oxidative stress responses in the C_3/CAM intermediate plant *Mesembryanthemum crystallinum*. *Plant Physiology and Biochemistry,* Vol. 40, pp. 669-677, ISSN 0981-9428

Smith, J.A.C. & Winter, K. (1996). Taxonomic distribution of Crassulacean acid metabolism. In: *Crassulacean Acid Metabolism. Biochemistry, Ecophysiology and Evolution*, Winter, K. & Smith, J.A.C. (Eds.), pp. 427–436, ISBN 3540581049, Springer, Berlin, Germany.

Stitt, M. (1996). Metabolic regulation of photosynthesis. In: *Photosynthesis and the Environment,* Baker N.R. (Ed.), pp. 151-190, ISBN 9780792343165, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Strand, Å.; Hurry, V., Gustafsson, P. & Gardeström, P. (1997). Development of *Arabidopsis thaliana* leaves at low temperatures releases the suppression of photosynthesis and photosynthetic gene expression despite the accumulation of soluble carbohydrates. *The Plant Journal*, Vol. 12, pp. 605-614, ISSN 0960-7412

Strand, Å.; Hurry, V.; Henkes, S.; Huner, N.; Gustafsson, P.; Gardeström, P. & Stitt, M. (1999). Acclimation of *Arabidopsis* leaves developing at low temperatures. Increasing cytoplasmic volume accompanies increased activities of enzymes in the Calvin cycle and in the sucrose-biosynthesis pathway. *Plant Physiol*ogy, Vol. 119, pp. 1387-1397, ISSN 0032-0889

Sudderth, E.A.; Muhaidat, R.; McKown, A.D.; Kocacinar, F. & Sage, R.F. (2007). Leaf anatomy, gas exchange and photosynthetic enzyme activity in *Flaveria kochiana. Functional Plant Biology,* Vol. 34, pp. 118-129, ISSN 1445-4408

Tallman, G.; Zhu, J.; Mawson, B.T.; Amodeo, G.; Nouki, Z.; Levy, K. & Zeiger, E. (1997). Induction of CAM in *Mesembryanthemum crystallinum* abolishes the stomatal response to blue light and light-dependent zeaxanthin formation in guard cell chloroplasts. *Plant and Cell Physiology,* Vol. 38, pp. 236-242, ISSN 0032-0781

Tezara, W.; Mitchell, V.J.; Driscoll, S.P. & Lawlor, D.W. (1999). Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature,* Vol. 401, pp. 914–917, ISSN 0028-0836

von Caemmerer, S. & Furbank, R.T. (2003). The C_4 pathway: an efficient CO_2 pump. *Photosynthesis Research,* Vol. 77, pp. 191-207, ISSN 0166-8595

von Caemmerer, S. (2000). Biochemical Models of Leaf Photosynthesis. ISBN 9780643063792, CSIRO Publishing, Collingwood, Australia.

Voznesenskaya, E.V.; Artyusheva, E.G.; Franceschi, V.R.; Pyankov, V.I.; Kiirats, O.; Ku, M.S.B. & Edwards, G.E. (2001). *Salsola arbusculiformis*, a C_3-C_4 intermediate in Salsoleae (Chenopodiaceae). *Annals of Botany,* Vol. 88, pp. 337-348, ISSN 0305-7364

Voznesenskaya, E.V.; Franceschi, V.R.; Kiirats, O.; Freitag, H .& Edwards, G.E. (2001). Kranz anatomy is not essential for terrestrial C_4 plant photosynthesis. *Nature,* Vol. 414, pp. 543–46, ISSN 0028-0836

Walch-Liu, P.; Neumann, G. & Engels, C. (2001). Elevated atmospheric CO_2 concentration favors nitrogen partitioning into roots of tobacco plants under nitrogen deficiency by decreasing nitrogen demand of the shoot. *Journal of Plant Nutrition*, Vol. 24, pp. 835-854, ISSN 0190-4167

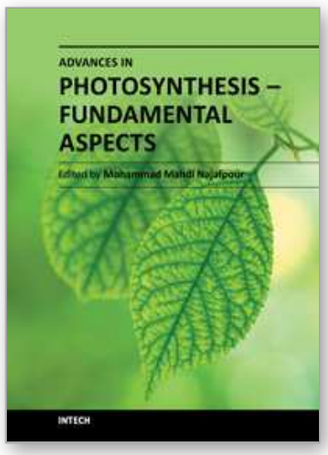
Ward, J. (2005). Evolution and growth of plants in a low CO_2 world. In: *A History of Atmospheric CO_2 and its Effects on Plants, Animals and Ecosystems*, Ehleringer, J.R., Cerling, T.E. & Dearling, D. (Eds.), pp. 232-257, ISBN 978-0-387-22069-7, Springer, Berlin, Germany.

Winter, K. (1985). Crassulacean acid metabolism. In: *Photosynthetic Mechanisms and the Environment*, Barber, J. & Baker, N.R. (Eds.), pp. 329-387, Elsevier, Amsterdam, The Netherlands, ISBN 0444806741

Woodward, F.I. (2002). Potential impacts of global elevated CO_2 concentrations on plants. *Current Opinion in Plant Biology,* Vol. 5, pp. 207-211, ISSN 1369-5266

Yoshimura, Y., Kubota, F. & Ueno, O. (2004). Structural and biochemical bases of photorespiration in C_4 plants: quantification of organelles and glycine decarboxylase. *Planta,* Vol. 220, pp. 307-317, ISSN 0032-0935

Zotz, G. & Hietz, P. (2001). The physiological ecology of vascular epiphytes: current knowledge, open questions. *Journal of Experimental Botany,* Vol. 52, pp. 2067-2078, ISSN 0022-0957



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Photosynthesis is one of the most important reactions on Earth. It is a scientific field that is the topic of many research groups. This book is aimed at providing the fundamental aspects of photosynthesis, and the results collected from different research groups. There are three sections in this book: light and photosynthesis, the path of carbon in photosynthesis, and special topics in photosynthesis. In each section important topics in the subject are discussed and (or) reviewed by experts in each book chapter.

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