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\textsubscript{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\textsubscript{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\textsubscript{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-bisphosphatase 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\textsubscript{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\textsubscript{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\textsubscript{2} from cells that are simultaneously fixing CO\textsubscript{2} by the Calvin cycle. The C\textsubscript{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).

![Diagram of the photosynthetic carbon reduction (PCR) cycle or Calvin cycle.](image)

In normal air (21% O\textsubscript{2}), the rate of photorespiration in sunflower leaves is about 17% of gross photosynthesis. Every photorespired CO\textsubscript{2}, however, requires an input of two molecules of O\textsubscript{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\textsubscript{2} and CO\textsubscript{2} since both gases compete for binding at the active site on Rubisco. Increase in the relative level of O\textsubscript{2} (or decrease in CO\textsubscript{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\textsubscript{2} solubility is less affected than CO\textsubscript{2}. Thus O\textsubscript{2} will inhibit photosynthesis, measured by net CO\textsubscript{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\textsubscript{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\textsubscript{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 Rubisco with lower affinity to $O_2$.

![Fig. 2. The photorespiratory glycolate pathway.](image)

### 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 C4 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 C4 cycle remains to concentrate CO2 in the bundle-sheath cells where the enzymes of the PCR cycle are located. By shuttling the CO2 in the form of organic acids it is possible to build much higher CO2 concentrations in the bundle-sheath cells than would be possible relying on the diffusion of CO2 alone. The concentration of CO2 in bundle-sheath cells may reach 60 mM about tenfold higher than that in C3 plants. Higher CO2 concentrations would suppress photorespiration and support higher rates of photosynthesis. Under optimal conditions, C4 crop species can assimilate CO2 at rates two to three times that of C3 species. All this productivity, however, has an energy cost to building the CO2 concentration in the bundle-sheath cells. For every CO2 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 CO2 by the C4 cycle is five ATP and two NADPH (Bhagwat, 2005; Edwards and Voznesenskaya, 2011).

4. CAM mode of carbon assimilation

Another CO2 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 CO2 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 C4 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 CO2 is fixed by the PCR cycle. As in C4 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).](www.intechopen.com)
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 $\alpha$, is defined as the ratio of carbon isotope ratios in reactant and product:

$$\alpha = R_r / R_p$$

(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 $\Delta$, the deviation of $\alpha$ from unity, as the measure of the carbon isotope discrimination by the plant:

$$\Delta = \alpha - 1 = R_a / R_p - 1$$

(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:

$$\delta^{13} = R_p / R_s - 1$$

(3)

Isotopic composition of plants ($\delta^{13}$) is negative, whereas the process of CO$_2$ diffusion and carboxylation by Rubisco have positive discrimination (i.e. against $^{13}$CO$_2$). Therefore, $\Delta$ values are usually positive while those of $\delta^{13}$ are usually negative. Typically, $\Delta$ and $\delta^{13}$ values are $\sim$10-35×10$^{-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 $\Delta$ (and higher rates of $\delta^{13}$) 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 $\Delta$ values and increase in $\delta^{13}$ 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.
RWC%=(fresh mass – dry mass)/(water saturated mass – dry mass)×100 \hspace{1cm} (4)

Low cell turgor and RWC slow growth and decrease the stomatal conductance for H₂O \( (g_s) \). 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 CO₂ 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 CO₂ supply, determined by \( g_s \) and the ambient CO₂ concentration \( (C_a) \) (Lawlor, 2001). CO₂ supply to the PCR cycle in the chloroplast is determined by \( C_s \) 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 CO₂ concentration within the leaf, \( C_i \), depends on \( A, g_s \) and \( C_a \):

\[
A = g_s \left( C_a - C_i \right) \hspace{1cm} (5)
\]

The CO₂ concentration at the active sites of Rubisco in the chloroplasts \( (C_i) \) is given by:

\[
A = g_m \left( C_i - C_c \right) \hspace{1cm} (6)
\]

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 CO₂ \((C_o, C_i \) and \( C_s \) \) and light in fully hydrated leaves is defined as \( A_{pot} \). To achieve the same \( A_{pot} \) at small RWC as at large RWC, \( C_s \) must saturate Rubisco and so \( C_a \) 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 CO₂ saturation, then metabolism is inhibited (Lawlor and Cornic, 2002).

Experimental studies on CO₂ assimilation of C₃ plants under decreasing RWC show that there are fundamental differences between species in the relative roles of \( g_s \) limitation of CO₂ 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 \( C_s \) 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 CO₂ 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 CO₂. The metabolic phase of limitation is at lower RWC <75% where \( A_{pot} \) is limited by metabolism (Lawlor and Cornic, 2002).

**Type 2:** Elevated CO₂ 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 CO₂ assimilation is decreased (Tezara et al. 1999). Inhibition of \( A \) with 10% or greater \( C_s \) rather than restoration, shows that metabolism is affected by elevated CO₂, 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 C3 species (Pellesschi 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 Rubisco 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 al., 2000).
6.3 Effect of CO₂ concentration

Human activities have caused the concentration of atmospheric CO₂ 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 CO₂ 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 CO₂ 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 CO₂ concentrations partially alleviated the limitation of C₃ (but not C₄) photosynthesis by CO₂ supply and acted as a negative feedback on transpiration in both C₃ and C₄ species (Long, 1991). Subsequent and often longer-term experiments have shown that photosynthesis could acclimate downwards in response to CO₂ enrichment, and there is now some evidence to suggest that photosynthesis is stimulated in C₄ species in response to CO₂ enrichment (Ghannoum et al., 2000). In species with the C₃ photosynthetic pathway, high irradiance can lead to photoinhibition. Field studies have now demonstrated that CO₂ 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 CO₂ enrichment. A detailed analysis of leaf development in Scots pine (Pinus sylvestris) after four years of exposure to CO₂ 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 CO₂ enrichment.

Recently, a gene HIC (HIGH Carbon dioxide) has been identified whose disruption leads to large increases in the number of stomata initiated in response to CO₂ 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 CO₂ concentration is detected by mature leaves (Lake et al., 2001).

Early experiments demonstrated significant reductions in stomatal conductance under CO₂ 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 CO₂, 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 CO₂ enrichment. In a long-term study, trees growing in the enriched CO₂ 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 coordination 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 coordinating 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 C₃/CAM intermediate species change their mode of metabolism in response to stress conditions (Dodd et al. 2002). Facultative or inducible CAM species use the C₃ 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 C₃/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 C₃–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 C₃–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 C₃/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). CO₂ 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 CO₂ concentration was low (Raven and Spicer, 1996). CAM is a CO₂-concentrating mechanism due to the much higher substrate affinity of PEPC for HCO₃⁻ than of the C₃-photosynthesis/Calvin cycle carboxylase Rubisco for CO₂. Thus, during the dark period a concentrated CO₂ pool is built up in the form of vacuolar malic acid accumulation, and during phase III its remobilization in the light leads to internal CO₂ concentrations that may be 2-60 times more than atmospheric CO₂ concentration. For aquatic plants, this CO₂-concentrating mechanism provides a benefit for CO₂ acquisition. For terrestrial plants, the benefit of CO₂-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 (Lütge, 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 (Brulbert et al., 1985). In C₃/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 C₃-state are lost in the CAM-state. In M. crystallinum after the C₃-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 (Lütte, 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 (Lütte, 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 (Lütte, 2002).

7.2 CAM physiotypes
There are some photosynthetic physiotypes for the metabolic cycle of CAM include full CAM, CAM idling, CAM cycling, C₃/CAM and C₄/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 CO₂. In CAM cycling, stomata remain closed during the dark period but some nocturnal synthesis of organic acid fed by respiratory CO₂ occurs, and stomata are open during the light period with uptake of atmospheric CO₂ and direct Calvin-cycle CO₂ reduction (C₃-photosynthesis) in addition to assimilation of CO₂ 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).

<table>
<thead>
<tr>
<th>CAM physiotypes</th>
<th>Phase of CO$_2$ fixation</th>
<th>Phase of stomatal closure</th>
<th>Diel Fluctuation of malate concentration</th>
<th>Diel pH Fluctuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full CAM</td>
<td>I</td>
<td>II, III, IV</td>
<td>&gt;15</td>
<td>High</td>
</tr>
<tr>
<td>CAM idling</td>
<td>---</td>
<td>I, II, III, IV</td>
<td>&gt;15</td>
<td>High</td>
</tr>
<tr>
<td>CAM cycling</td>
<td>II, III, IV</td>
<td>I</td>
<td>&lt;5</td>
<td>Low</td>
</tr>
<tr>
<td>C$_3$/CAM</td>
<td></td>
<td></td>
<td></td>
<td>Intermediate</td>
</tr>
<tr>
<td>C$_4$/CAM</td>
<td></td>
<td></td>
<td></td>
<td>Intermediate</td>
</tr>
</tbody>
</table>

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 isof orm, 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 anaplerotic ‘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 C₃ and C₄ plants have been identified in the genera *Eleucharis* (Cyperaceae), *Panicum* (Poaceae), *Neurachne* (Poaceae), *Mollugo* (Aizoaceae), *Moricandia* (Brassicaceae), *Flaveria* (Asteraceae) *Parthenium* (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 C₃ species and most also include C₄ species.

The intermediate nature of these species is reflected in the isotopic composition (δ¹³C), CO₂ compensation point (Γ) as well as in the differential distribution of organelles in the bundle sheath cells (Table 2).

<table>
<thead>
<tr>
<th>Photosynthetic type</th>
<th>δ¹³C Value (%)</th>
<th>Γ (µmol mol⁻¹)</th>
<th>Organelles in bundle sheath cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₃</td>
<td>~ −30</td>
<td>48–62</td>
<td>Chloroplasts 9-11 8-19 Mitochondria</td>
</tr>
<tr>
<td>C₃–C₄</td>
<td>~ −28</td>
<td>9–17</td>
<td>Chloroplasts 13-25 25-52 Mitochondria</td>
</tr>
<tr>
<td>C₄</td>
<td>~ −15</td>
<td>3–5</td>
<td>Chloroplasts 28-53 30-74 Mitochondria</td>
</tr>
</tbody>
</table>

Table 2. Main characteristics of C₃-C₄ species from various genera showing the intermediate nature of these species.

Intermediate species are also recognized in their CO₂ net assimilation rate as a function of intercellular CO₂ concentration and in the CO₂ compensation point as a function of O₂ concentration in the medium (Fig. 7).

Fig. 7. Generalized curves for net assimilation rate (left) and compensation point (right) of CO₂ in C₃, C₄ and C₃–C₄ intermediate species.

### 8.1 Leaf anatomy

C₃-C₄ species have anatomical characteristics between those of C₃ and C₄. The vascular bundles are surrounded by chlorenchymatous bundle sheath cells reminiscent of the Kranz anatomy of leaves of C₄ plants (Fig. 8). However, the mesophyll cells are not in a concentric
ring around the bundle sheath cells as in a C₄ leaf, but are arranged as in leaves of C₃ 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 C₃-C₄ 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 C₃-C₄ 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 C₃-C₄ 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 C₃-C₄ species, increased partitioning of organelles in bundle sheath cells compared to C₃ 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, C₃-C₄ intermediate species plasmodesmatal densities at the bundle sheath/mesophyll interface approach those of C₄ species and are much greater than those of the C₃ species studied (Brown et al, 1983).

8.2 Leaf gas exchange in C₃-C₄ intermediate species
Photosynthetic rates of C₃ and C₃-C₄ 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 C₃ plants where Ƞ is essentially unaffected by light intensity, Ƞ is strongly light-dependent in C₃-C₄ intermediate species. There is no evidence that the oxygenation reaction of Rubisco was itself being suppressed to any major extent by a C₄-like mechanism. Whereas about 50% of the photorespiratory CO₂ of a C₃ leaf is recaptured before it escapes from the leaf, it was estimated that up to 73% is recaptured in a C₃-C₄ leaf. Clearly, the improved recapture of CO₂ could account for a low Ƞ in C₃-C₄ 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 C₃-C₄ intermediate species

Because of the intermediate nature of Ƞ and the somewhat C₄-like leaf anatomy of the C₃-C₄ species, many researchers attempted to show that these species had a partially functional C₄ cycle which accounted for their low rates of photorespiration and hence Ƞ. However, there is now good evidence that C₃-C₄ intermediates in the genera Alternanthera, Moricandia, Panicum and Parthenium do not have a C₄ cycle which could account for their low rates of photorespiration. Activities of PEPC and the C₄ cycle decarboxylases are far lower than in C₄ leaves, and Rubisco and PEPC are both present in mesophyll and bundle sheath cells. Label from ¹⁴CO₂ is not transferred from C₄ 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 CO₂ 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 C₃-C₄ 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 Ƞ of C₃-C₄ 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, CO₂ 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 C₃-C₄ intermediate species. It seems likely, therefore, that the differential distribution of glycine decarboxylase must contribute to the observed reduction in apparent photorespiration in the C₃-C₄ species (Rawsthorne, 1992; Yoshimura et al., 2004).

9. Evolution of C₄ photosynthesis

C₄ photosynthesis is a series of biochemical and anatomical modifications that concentrate CO₂ around the carboxylating enzyme Rubisco. Many variations of C₄ photosynthesis exist, reflecting at least 45 independent origins in 19 families of higher plants. C₄ photosynthesis is present in about 7500 species of flowering plants, or some 3% of the estimated 250 000 land plant species. Most C₄ plants are grasses (4500 species), followed by sedges (1500 species) and dicots (1200 species). C₄ 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_4 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_3
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, C$_4$
photosynthesis as a functional type should not be threatened by CO\textsubscript{2} rise in the near term (Sage, 2004).

### 9.2 Evolutionary pathways to C\textsubscript{4} photosynthesis

Evolution was not directed towards C\textsubscript{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\textsubscript{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 (Kloeckner-Gruissem and Freeling, 1995; Lynch & Conery, 2000). A model for C\textsubscript{4} evolution has been presented that recognizes seven significant phases (Sage, 2004) (Table 3).

### 10. Single cell C\textsubscript{4} photosynthesis

The term Kranz anatomy is commonly used to describe the dual-cell system associated with C\textsubscript{4} photosynthesis, consisting of mesophyll cells containing PEPC and initial reactions of C\textsubscript{4} biochemistry, and bundle sheath cells containing enzymes for generating CO\textsubscript{2} from C\textsubscript{4} acids and the C\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{4} plants can capture CO\textsubscript{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\textsubscript{2}, even under low atmospheric levels of CO\textsubscript{2}, and their carbon isotope values are the same as in Kranz-type C\textsubscript{4} plants, whereas the values would be more negative if there were leakage of CO\textsubscript{2} and overcycling through the C\textsubscript{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).
<table>
<thead>
<tr>
<th>Stage</th>
<th>Events</th>
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<tr>
<td>General Preconditioning</td>
<td>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.</td>
</tr>
<tr>
<td>Anatomical Preconditioning</td>
<td>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) → C₄ photosynthesis first arose in grasses and is prolific in this family.</td>
</tr>
<tr>
<td>Creating Metabolic Sink for Glycine Metabolism and C₄ Acids</td>
<td>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 CO₂ pump → further increase in organelle number → greater growth and fecundity in high photorespiratory environments → maintaining incremental rise in BSC organelle content → significant reduction in CO₂ compensation points.</td>
</tr>
<tr>
<td>Glycine Shuttles and Photorespiratory CO₂ Pumps</td>
<td>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.</td>
</tr>
<tr>
<td>Efficient Scavenging of CO₂ Escaping from the BSC</td>
<td>Enhancement of PEPC activity in the MC: reorganization of expression pattern of enzymes: specific expression of C₄ 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.</td>
</tr>
<tr>
<td>Integration of C₃ and C₄ Cycles</td>
<td>Avoidance of competition between PEPC and Rubisco in the MC for CO₂ and ATP increase in the phases of C₄ 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 CO₂ between BSC and MC, reduction of MC Rubisco activity in the later stages.</td>
</tr>
<tr>
<td>Optimization and Whole-Plant Coordination</td>
<td>Selection for traits that allow plants to exploit the productive potential of the C₄ 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 C₄ evolution: increase in the specific activity of NADP-ME and reduction of Km for malate. (2) Optimization of PEPC in the final stages of C₄ 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 CO₂ 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.</td>
</tr>
</tbody>
</table>

Table 3. The main evolutionary pathways towards C₄ photosynthesis (Adapted from Sage, 2004).
Fig. 9. Model of proposed function of $\text{C}_4$ 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 $\text{C}_4$ 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 $\text{C}_4$ pathway assimilates atmospheric CO$_2$ into $\text{C}_4$ acids. Two key enzymes in the process are pyruvate-$\text{Pi}$ dikinase (PPDK), located in chloroplasts at the proximal part and PEPC, located in the cytosol. The $\text{C}_4$ 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 $\text{C}_4$ 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 $\text{C}_1$ 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 $\text{C}_4$ pathway functions to convert pyruvate and CO$_2$ into OAA through the combined action of PPDK in the chloroplast and PEPC in the cytosol. $\text{C}_4$ 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 C₃ cycle (Edwards et al., 2004; Edwards and Voznesenskaya, 2011).

Single-cell C₄ photosynthesis could simply be an alternative mechanism to Kranz type C₄ 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 C₄ allows more flexibility in mode of photosynthesis than Kranz-type C₄ plants by, for example, shifting from C₃ to C₄ 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. In 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 C₄ 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


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


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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