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A critical review on the improvement of photosynthetic carbon assimilation in C$_3$ plants using genetic engineering

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Abstract
Global warming is one of the most serious challenges facing us today. It may be linked to the increase in atmospheric CO$_2$ and other greenhouse gases (GHGs), leading to a rise in sea level, notable shifts in ecosystems, and in the frequency and intensity of wild fires. There is a strong interest in stabilizing the atmospheric concentration of CO$_2$ and other GHGs by decreasing carbon emission and/or increasing carbon sequestration. Biotic sequestration is an important and effective strategy to mitigate the effects of rising atmospheric CO$_2$ concentrations by increasing carbon sequestration and storage capacity of ecosystems using plant photosynthesis and by decreasing carbon emission using biofuel rather than fossil fuel. Improvement of photosynthetic carbon assimilation, using transgenic engineering, potentially provides a set of available and effective tools for enhancing plant carbon sequestration. In this review, firstly different biological methods of CO$_2$ assimilation in C$_3$, C$_4$ and CAM plants are introduced and three types of C$_4$ pathways which have high photosynthetic performance and have evolved as CO$_2$ pumps are briefly summarized. Then (i) the improvement of photosynthetic carbon assimilation of C$_3$ plants by transgenic engineering using non-C$_4$ genes, and (ii) the overexpression of individual or multiple C$_4$ cycle photosynthetic genes (*PEPC*, *PPDK*, *PCK*, *NADP–ME* and *NADP-MDH*) in transgenic C$_3$ plants (e.g. tobacco, potato, rice and *Arabidopsis*) are highlighted. Some transgenic C$_3$ plants (e.g. tobacco, rice and *Arabidopsis*) overexpressing the *FBP/SBPase*, *ictB* and cytochrome c$_6$ genes showed positive effects on photosynthetic efficiency and growth characteristics. However, over the last 28 years, efforts to overexpress individual, double or multiple C$_4$ enzymes in C$_3$ plants like tobacco, potato, rice, and *Arabidopsis* have produced mixed results that do not confirm or eliminate the possibility of improving photosynthesis of C$_3$ plants by this approach. Finally, a prospect is provided on the challenges of enhancing carbon assimilation of C$_3$ plants using transgenic engineering in the face of global warming, and the trends of the most promising approaches to improving the photosynthetic performance of C$_3$ plants.

Keywords: Global warming, increase in CO$_2$ concentration, biotic carbon sequestration, C$_3$ plants, C$_4$ plants, CAM plants, overexpression of C$_4$ enzymes, transgenic C$_3$ plants, improvement of photosynthesis

Introduction

The concentration of carbon dioxide (CO$_2$) in the Earth's atmosphere has increased by 31% from 280 parts per million (ppm) in 1850 to 380 ppm in 2005 (IPCC, 2007). It is presently increasing at 1.7 ppm yr^{-1} or 0.46% yr^{-1}, which will lead to 50% higher levels in 2050 than today (Sun et al., 2009). Global surface temperature has increased by 0.88°C since the late 19th century, and 11 out of the 12 warmest years on record have occurred during 1995–2006 (IPCC, 2007). The Earth's mean temperature is projected

to increase by 1.5–5.88°C during the 21st century (IPCC, 2001). In addition to a rise in sea level by 15–23 cm during the 20th century (IPCC, 2007), there have been notable shifts in ecosystems (Greene and Pershing, 2007) and in the frequency and intensity of occurrence of wild fires (Running, 2006; Westerling et al., 2006). The increase in atmospheric CO$_2$ and other greenhouse gases (GHGs) e.g. methane (CH$_4$) and nitrous oxide (N$_2$O), may be the major reasons for climate change (Nowak et al., 2004);

Abbreviations

ATP	adenosine 5′-triphosphate	PEP	phosphoenolpyruvate
CAM	crassulacean acid metabolism	PEPC	phosphoenolpyruvate carboxylase
CH_4	methane	PGA	phosphoglycerate
CO_2	carbon dioxide	PGAL	phosphoglyceraldehyde
DHAP	dihydroxy acetone phosphate	PPDK	pyruvate orthophosphate dikinase
FBPase	fructose-1, 6-bisphosphatase	ppm	parts per million
Fru-6P	fructose 6-phosphate	rca	regulation carboxylation activity or the Rubisco activase gene
G3P/GA-3P	glyceraldehyde 3-phosphate		
GHGs	greenhouse gases	RCA	Rubisco activase
GP	glycerate 3-phosphate	Ru-5P	ribulose 5-phosphate
N_2O	nitrous oxide	Rubisco	ribulose 1,5-bisphosphate carboxylase/oxygenase
NADP	nicotinamide adenine dinucleotide phosphate	RuBP	ribulose-1,5-bisphosphate
NADP-MDH	NADP-malate dehydrogenase	SBPase	sedoheptulose-1,7-bisphosphatase
NADP-ME	NADP-malic enzyme	Sed-7P	sedoheptulose 7-phosphate
OAA	oxaloacetic acid	SPS	sucrose phosphate synthase
PCK	phosphoenolpyruvate carboxykinase	WT	wild type

hence, there is a strong interest in stabilizing the atmospheric abundance of CO_2 and other GHGs to mitigate the risks of global warming (Kerr, 2007; Kintisch, 2007; Kluger, 2007; Mikkelsen et al., 2010). CO_2 is the most prominent GHG in the Earth's atmosphere. Among the different ways to reduce CO_2 emission and increase carbon capture and storage (e.g. chemical transformations, biological conversions, reforming and inorganic transformations, and manipulations of the soil carbon pool) (Lal, 2004; 2008; 2009), biotic carbon sequestration (Figure 1) is recommended by the Kyoto Protocol as one of the important and effective strategies to mitigate the effects of rising atmospheric CO_2 concentrations by enhanced carbon sequestration (Hill et al., 2007). The Kyoto Protocol is the first international environmental agreement to require binding greenhouse gas emission reductions by industrialized nations (http://en.wikipedia.org/wiki/Kyoto_Protocol). Compared to different techniques of carbon sequestration, biotic techniques are immediately applicable, natural, and cost-effective processes, with numerous ancillary benefits but a finite sink capacity (Lal, 2008). The increase in atmospheric CO_2 concentration is mainly from burning fossil fuel, thus, in the future CO_2 mitigation schemes have to match the scale of man-made CO_2 in the atmosphere. For example, if fossil fuel emissions from 1990 to 2100 are limited to 600 PgC, biotic carbon stocks must increase by 120 PgC to stabilize CO_2 concentrations at 450 ppm (Lashof and Hare, 1999); this trend to the utilization of biofuel (bioethanol, biodiesel, CH_4 gas, and H_2 cell) instead of fossil fuel (Ruan et al., 2008).

Biotic sequestration is a process that converts CO_2 into organic compounds, especially sugars, using the energy from sunlight (Smith, 1997), which enhances carbon sequestration and the storage capacity of ecosystems by plant photosynthesis. In plants, there are three types of photosynthetic CO_2 assimilation, and thus three categories of plants (Table 1, Table 2): (i) C_3 plants, in which atmospheric CO_2 is assimilated directly through the C_3 photosynthetic pathway. The majority of terrestrial plants, including many important crops such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum*), soybean (*Glycine max*), and potato (*Solanum tuberosum*), are C_3 plants (Matsuoka et al., 2001); (ii) C_4 plants e.g. maize (*Zea mays*) and sugarcane (*Saccharum officinarum*), which evolved the C_4 photosynthetic pathway (Sage and Pearcy, 1987); and (iii) CAM (crassulacean acid metabolism) plants, in which the stomata are closed during the day and open only at night when the temperature decreases and humidity rises; at night, CO_2 is stored as malate in the large vacuoles and is released for photosynthesis during the day. While C_3 plants grow well in temperate climates, CAM plants such as stonecrops (*Lithops* spp.) and cactus adapt to extreme arid conditions, but their photosynthetic capacity is very low (Black, 1973). In contrast, C_4 plants such as maize and sugarcane adapt to high light, arid, and warm environments and achieve higher photosynthetic capacity and higher water- and nitrogen-use efficiencies compared to C_3 plants (Black, 1973). Both C_4 and CAM plants evolved from an ancestral C_3 species in response to changes in environmental conditions that caused a decrease in CO_2 availability. C_4 plants evolved in response to low atmospheric CO_2 concentrations, while CAM plants evolved either in response to the selection of increased water-use efficiency or for increased carbon gain (Ehleringer and Monson, 1993).

Photosynthesis is limited by multiple factors including photosynthetic electron transport, regeneration of CO_2 acceptor molecules in the reductive pentose phosphate cycle, the activity and substrate specificity of CO_2-fixing enzyme, the associated flow through the photorespiratory pathway, and environmental variables (e.g. temperature, CO_2 concentration and water availability) (Austin, 1989; Raines, 2006; Peterhänsel et al., 2008a). In three distinct phases (carboxylation, reduction, and regeneration) of photosynthesis, several key enzymes directly influence its rate and efficiency (Singh and Malhotra, 2000), such as ribulose-1,5-bisphosphate (RuBP), fructose-1,

6-bisphosphatase (FBPase), and sucrose phosphate synthase (SPS). In C_3 plants, low ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity, photorespiration competing reactions catalysed by Rubisco and resulting in a loss of up to 50% of CO_2 fixation in ambient air, and variation of RuBP activity in stress environments (e.g. high temperature, drought and salinity) limit photosynthesis and carbon assimilation (Häusler et al., 2002). In contrast, C_4 plants, as CO_2 pumps (von Caemmerer and Furbank, 2003), possess a CO_2 concentrating mechanism, in which atmospheric CO_2 is bound to C_4-carbon compounds and is shuttled from the mesophyll cells where the prefixation of bicarbonate occurs via phosphoenolpyruvate carboxylase (PEPC) into the gas-tight bundle-sheath cells in which the bound carbon is released again as CO_2 and enters the Calvin cycle. When this pathway was first discovered, conventional cross plant-breeding approaches were used to try to transfer C_4 traits into C_3 plants, but the results were unsuccessful (Brown and Bouton, 1993).

Recently, rapid development of molecular biology and transgenic technology has provided a set of available and effective tools for improving photosynthetic carbon assimilation in C_3 plants. On the one hand, although enzymes with desirable characteristics are available, they have not resulted in attempts to improve Rubisco enzymes to alter the catalytic properties of Rubisco either by amino acid substitutions in several distinct areas of the Rubisco large subunit (Spreitzer and Salvucci, 2002; Andersson and Taylor, 2003; Parry et al., 2003; Raines, 2006) or by random mutagenesis techniques (e.g., DNA shuffling and 'accelerated evolution' (Stemmer, 1994; Matsumura et al., 2005)). On the other hand, analysis of the photorespiratory pathway of transgenic plants suggests that C_3 photosynthesis is unlikely to be improved by direct manipulation of levels of enzymes in the photorespiratory pathway (Heineke et al., 2001; Winzer et al., 2001; Bauwe and Kolukisaoglu, 2003; Boldt et al., 2005). Hence, over the last 25 years, considerable efforts have been made to try to use genetic engineering and
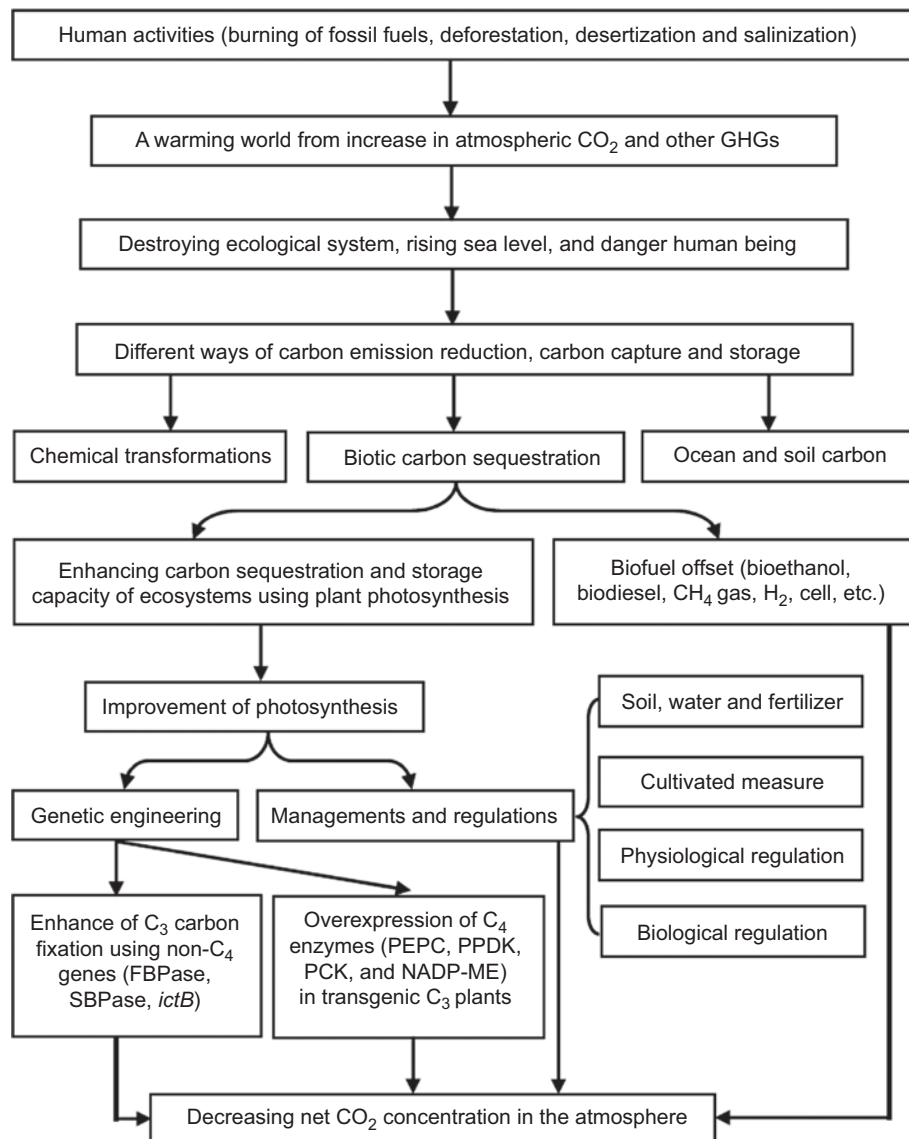

Figure 1. A wide range of processes and technological options for carbon sequestration.

Table 1. Glossary of CO_2 assimilation and photosynthesis, cited from Singh and Malhotra (2000), Firooznia (2007), and Pan (2008).

Term	Definition
ATP	Adenosine triphosphate, an adenine containing nucleotide triphosphate that releases free energy when its phosphate bonds are hydrolyzed. This energy is used by the cell to carry out endergonic reactions.
C_3-plants	Plants that use the Calvin cycle for the initial steps that incorporate CO_2 into organic matter, forming a 3-carbon compound as the first stable intermediate. Most broadleaf plants and plants in the temperate zones are C_3 pathway.
C_4-plants	Plants that preface the Calvin cycle with reactions that incorporate CO_2 into 4-carbon compound. C_4 plants have a distinctive leaf anatomy. This pathway is found mostly in hot regions with intense sunlight. Tropical grasses, such as sugar cane and maize are C_4 plants, but there are many broadleaf plants that are C_4.
CAM-plants	Plants that use crassulacean acid metabolism as an adaptation for arid conditions. CO_2 enters through the stomata during the night and is converted into organic acids, which release CO_2 for use in the Calvin cycle during the day, when the stomata are closed. The jade plant (*Crassula ovata*) and cactus species are typical of CAM plants.
Carbon assimilation	It is any process through which gaseous CO_2 is converted into a solid compound. It mostly refers to the processes found in autotrophs (organisms that produce their own food), usually driven by photosynthesis, whereby carbon dioxide is changed into sugars. Carbon fixation can also be carried out by the process of calcification in marine, calcifying organisms such as *Emiliania huxleyi* and also by heterotrophic organisms in some circumstances.
Carboxylation	The first major step in the Calvin cycle, referring to a chemical reaction through which a carbon-carbon bond is formed between CO_2 and a carbon in an organic compound (RuBP in the Calvin cycle).
Guard cells	Two cells that flank the stoma and regulate its opening and closing.
NADPH	Reduced form of nicotinamide adenine dinucleotide phosphate (NADP), a strong reducing agent, capable of donating energized electrons to reduce an acceptor molecule.
Photosynthesis	It is a process that converts carbon dioxide into organic compounds, especially sugars, using the energy from sunlight.
Reduction	The second major step in the Calvin cycle, referring to the chemical reactions that reduce the 3-carbon organic acids to 3-carbon aldehydes through addition of hydrogen atoms.
Regeneration	The third major step in the Calvin cycle, referring to the chemical reactions that use 3-carbon aldehydes to produce more of the 5-carbon sugar RuBP.
Rubisco	Ribulose bisphosphate carboxylase/oxygenase, the enzyme that carries out the carboxylation step in the Calvin cycle by attaching a CO_2 to the 5-carbon sugar RuBP to form two molecules of 3-phosphoglycerate, a 3-carbon organic acid.
RuBP	Ribulose-1,5-bisphosphate, the 5-carbon sugar that is carboxylated by Rubisco in the first step of the Calvin cycle.

transgenic technologies to (i) increase the efficiency of the Rubisco carboxylase reaction; and (ii) introduce enzymes of the C_4 pathway into transgenic C_3 plants, such as genes encoding enzymes in the C_4 pathway of PEPC, nicotinamide adenine dinucleotide phosphate-malate dehydrogenase (NADP-MDH), and NADP–malic enzyme (NADP–ME) or phosphoenolpyruvate carboxykinase (PCK).

Spreitzer and Salvucci (2002) reviewed the structure of Rubisco, regulatory interactions, and possibilities for a better enzyme. Since then, the requirements of a CO_2-mechanism as found in C_4 plants (Leegood, 2002), the C_4 pathway: an efficient CO_2 pump (von Caemmerer and Furbank, 2003), the biochemical imperatives of the installation of a C_4 pathway in a C_3 plant (Matsuoka et al., 2001; Edwards et al., 2001; Leegood et al., 2002; Häusler et al., 2002; Peterhänsel et al., 2008a), and transgenic approaches to reduce photorespiration (Raines, 2006) were outlined, respectively. However, recently, photosynthetic carbon assimilation using genetic engineering has been developing rapidly, and there is an urgent need to highlight the successes and limitations of attempts to limit increases in atmospheric CO_2 and other GHGs in the field of global warming.

In this review, we first introduce different biological methods of CO_2 assimilation in C_3, C_4, and CAM plants.

In particular, we outline the three types of C_4 pathway and the evolution of C_4 plants as a CO_2 pump. Then, we highlight the improvement of photosynthetic carbon assimilation in C_3 plants using genetic engineering, including (i) the enhancement of C_3 carbon assimilation in normal and stressed conditions (e.g. high temperature and drought) by improving RuBP activation using non-C_4 genes; and (ii) the overexpression of C_4 cycle photosynthetic genes in transgenic C_3 plants (e.g. tobacco (*Nicotiana tabacum*), potato and rice), such as *PEPC*, *NADP–ME* and *PCK*. Finally, we provide a prospect of the challenges for enhancing photosynthetic carbon assimilation of C_3 plants using genetic engineering for tackling global warming.

Carbon assimilation of C_3, C_4, and CAM plants

CO_2 assimilation in C_3 plants

In light-independent or dark reactions the enzyme Rubisco captures CO_2 from the atmosphere, in a process that requires newly formed NADPH, which is the reduced form of $NADP^+$. This is termed the Calvin-Benson Cycle (Figure 2A), in which three-carbon sugars are released, and which combine later to form sucrose and starch. This is the primary carboxylating mechanism in plants and is comprised of 13 reactions catalyzed by

Table 2. Differences in morphological and physiological characters (MPC) and photosynthetic carbon assimilation (PCA) between C_3 and C_4 plants, cited from Luo (2001) and Pan (2008).

MPC	C_3 plants	C_4 plants
Plant type	Typical temperate plants	Typical tropic or subtropical plants
Biomass (kg dry weight/ha·a)	22000 ± 300	39000 ± 17000
Leaf structure	Non Kranz type	Kranz type
Chloroplast (Chl) distribution	Mesophyll cell	Mesophyll cell and bundle sheath cell
Chl structure	Typical Chl	Mesophyll cell is typical Chl, Chl in big and more bundle sheath cell is no thylakold
Chl function	Acting photoreaction and dark reaction	mesophyll cell acts photoreaction, and fixes CO_2 by C_4 pathway; bundle sheath cell acts dark reaction but photoreaction
Chlorophyll a/b	2.8 ± 0.4	3.9 ± 0.6
Activity of PEP carboxylase [pmol/(mg Chl min)]	0.30–0.35	16-18
Location and reaction of photosynthesis	Chl in mesophyll cell: $C_5 + CO_2 \rightarrow 2C_3 + ATP + [H] \rightarrow C_5 + (CH_2O) + H_2O$	Chl in mesophyll cell: $C_3 (PEP) + CO_2 \rightarrow C_4$ Chl in bundle sheath cell: $C_5 + CO_2 \rightarrow 2C_3 + ATP + [H] \rightarrow C_5 + (CH_2O) + H_2O$
Photosynthetic rate [mg CO_2/(dm^2·h)]	15–35	40–80
CO_2 compensation point (mg/L)	30–70	<10
Optimal temperature of photosynthesis (°C)	15–25	30–47
Photorespiration	Strong photosynthesis occurring in mesophyll cell, resulting in loss of 20–40% CO2 assimilation	Weak photosynthesis occurring in bundle sheath cell
Transpiration coefficiency (g H_2O/g dry weight)	450–950	250–350
PCA		
Pathway of CO_2 assimilation	Only Calvin-Benson Cycle (C_3 pathway)	C_4 pathway and Calvin-Benson Cycle (C_3 pathway)
Major enzyme of CO_2 assimilation	RuBP carboxylase	PEP and RuBP carboxylase
Primary receptor of CO_2	RuBP	PEP
Primary product of CO_2 assimilation	PGA with three-carbon	OAA with four-carbon

11 enzymes distributed in three distinct phases namely, carboxylation, reduction, and regeneration (Firooznia, 2007).

The fixation or reduction of CO_2 is a process in which CO_2 combines with a five-carbon sugar, RuBP, to yield two molecules of a three-carbon compound glycerate 3-phosphate (GP), also known as 3-phosphoglycerate (PGA) (Figure 2A). GP, in the presence of ATP (adenosine 5′-triphosphate) and NADPH from the light-dependent stages, is reduced to glyceraldehyde 3-phosphate (G3P). This product is also referred to as 3-phosphoglyceraldehyde (PGAL) or even as triose phosphate. Triose is a 3-carbon sugar. Most (5 out of 6 molecules) of the GA-3P produced is used to regenerate RuBP so the process can continue. The 1 out of 6 molecules of the triose phosphates not "recycled" often condenses to form hexose phosphates, which ultimately yield sucrose, starch and cellulose. The sugars produced during carbon metabolism yield carbon skeletons that can be used for other metabolic reactions like the production of amino acids and lipids (http://en.wikipedia.org/wiki/Photosynthesis).

The pathway of CO_2 assimilation is strictly regulated by the key enzymes of the pathway, which are regulated by metabolites and light (Singh and Malohtra, 2000). Carbon in the form of dihydroxy acetone phosphate

(DHAP) leaves chloroplasts through a phosphate translocator and is converted to sucrose in the cytosol. The major regulatory enzymes in the sucrose biosynthetic pathway include cytosolic FBPase and SPS (Daie, 1993; Chen et al., 2005), both of which catalyze irreversible reactions and have a tight allosteric regulation by cytosolic metabolites and *in vivo* intermediates. The rate of sucrose synthesis is also regulated by a molecular mechanism through changes in the amount of these regulatory proteins and/or post translational modifications of the pre-existing enzyme. Sucrose synthesized in the mesophyll cells is then translocated to various sink tissues through the phloem. Further transport of sucrose from phloem parenchyma cells to the sieve tubes can occur symplastically via plasmodesmata without involving translocators, or apoplastically. From the apoplast to companion cells, sucrose transport proceeds via proton symport driven by a proton gradient (Heldt, 2005).

The primary CO_2 assimilation step in the C_3 pathway is catalysed by Rubisco. However, Rubisco also reacts with O_2 at its catalytic site (oxygenase reaction), leading to photorespiration, which plays a role in protecting photosynthesis from photoinhibition (Osmond and Grace, 1995), but it wastes fixed carbon as released CO_2 and
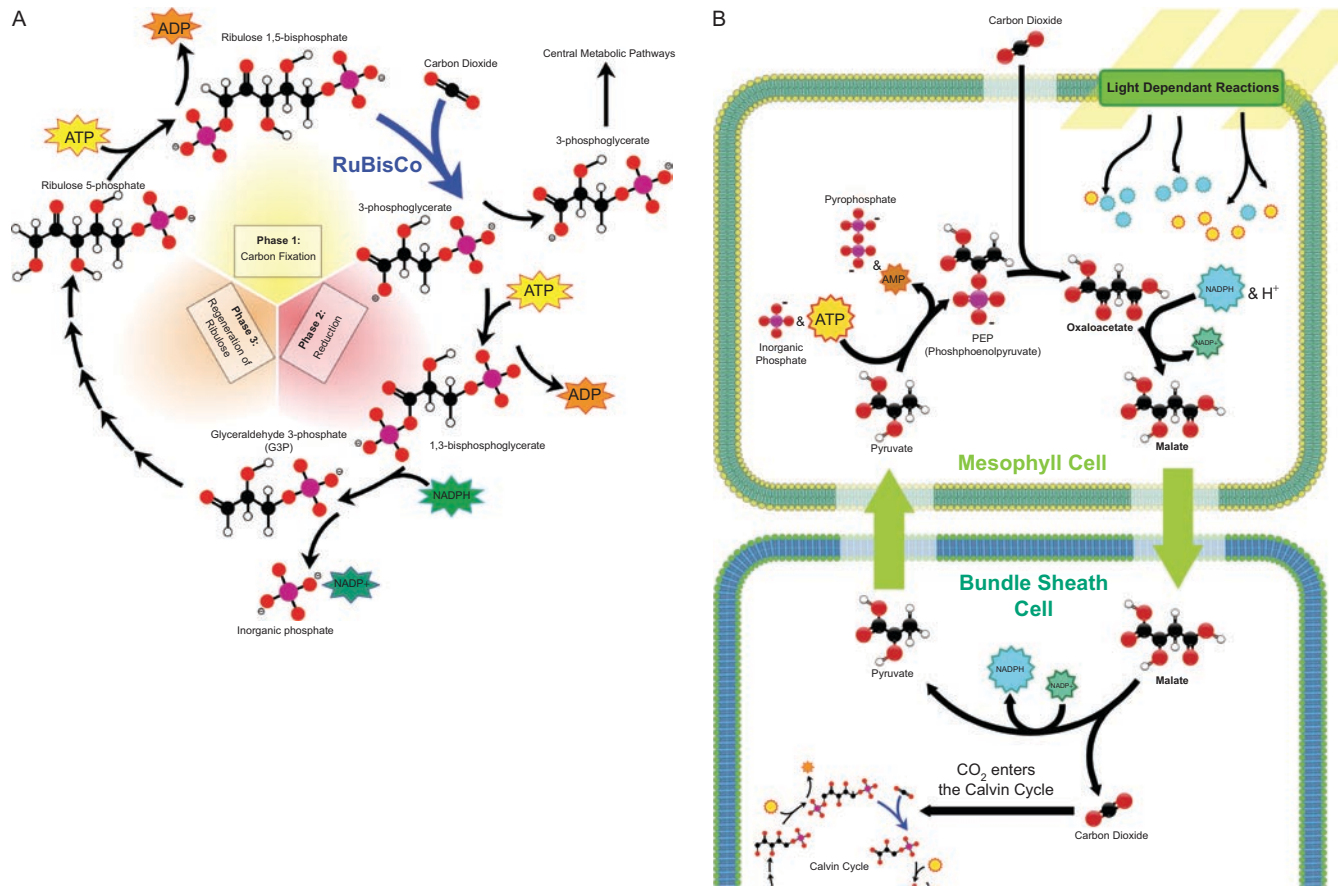
Figure 2. Photosynthetic CO_2 assimilation of C_3 plants (A). (http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/CalvinCycle.html) and C_4 plants (B) (http://en.wikipedia.org/wiki/C_3_carbon_fixation).

decreases the efficiency of photosynthetic CO_2 assimilation in C_3 plants (Leegood et al., 1995). Under current atmospheric conditions (0.036% CO_2, 21% O_2), up to 50% of fixed carbon is lost by photorespiration (Ogren, 1984; Häusler et al., 2002; Miyao, 2003), and the loss of photosynthetic carbon fixation from photorespiratory processes increases substantially with high temperatures and in drought conditions (Ogren, 1984; Brooks and Farquhar, 1985; Sharkey, 1988).

CO_2 assimilation in C_4 plants

The C_4 pathway evolved in hot, arid regions in response to increasing O_2 levels as a mechanism to increase the CO_2 concentration at the site of Rubisco (Sage, 2004). This allows Rubisco to operate closer to its maximal carboxylation rate, reducing the oxygenation reaction and thereby reducing the carbon losses caused by photorespiration (Hatch, 1987). C_4 plants chemically fix CO_2 in the cells of the mesophyll by adding it to the three-carbon molecule phosphoenolpyruvate (PEP), a reaction catalyzed by an enzyme called PEPC (Cooper and Wood, 1971; O'Leary, 1982) and which creates the four-carbon organic acid, oxaloacetic acid (OAA) (Figure 2B). OAA or malate synthesized by this process is then translocated to specialized bundle sheath cells where the enzyme, Rubisco, and other Calvin cycle enzymes

are located, and where CO_2 released by decarboxylation of the four-carbon acids is then fixed by Rubisco activity to the three-carbon sugar 3-phosphoglyceric acids. The physical separation of Rubisco from oxygen-generating light reactions reduces photorespiration and increases CO_2 fixation and thus the photosynthetic capacity of the leaf (Taiz and Zeiger, 2006). For C_4 plants, the location of the site of decarboxylation, its distance from the mesophyll interface, and the physical arrangement of chloroplasts and mitochondria in the bundle sheath cell are as important to the efficiency of the process as the properties of the bundle sheath cell wall (von Caemmerer and Furbank, 2003). C_4 plants can produce more sugar than C_3 plants in high light and temperature conditions. Many important crop plants are C_4 plants including maize, sorghum, sugarcane, and millet.

According to the use of different C_4-acid decarboxylases (Hatch et al., 1975), three sub-groups of C_4 plants have been characterized: the NADP-ME type carries out C_4 acid decarboxylation in the chloroplast, while the NAD-ME type decarboxylates in the mitochondria and the PCK type decarboxylates predominantly in the cytosol (e.g. *Urochloa panicoides*, Finnegan et al., 1999) (Table 3, Figure 3). The classification of species into these 3 sub-types is not as straightforward as originally thought, and particularly in the NADP-ME type there is

Table 3. Three types of C_4 pathway.

| Type	C_4 acid entering bundle sheath cells	Decarboxylate location	Decarboxylase	C_3 acid returning to mesophyll cell	Plant species
NADP-ME	Malate	Chloroplast	NADP-ME	Pyruvate	Maize, sugar cane, sorghum, etc.
NAD-ME	Aspartate	Mitochondria	NAD-ME	Alanine	Bristlegrasses, *Portulaca oleracea*, etc.
PCK	Aspartate	Cytosol	PCK	Pyruvate and Alanine	*Leymus chinensis, Sporobolus virginicus*, etc.
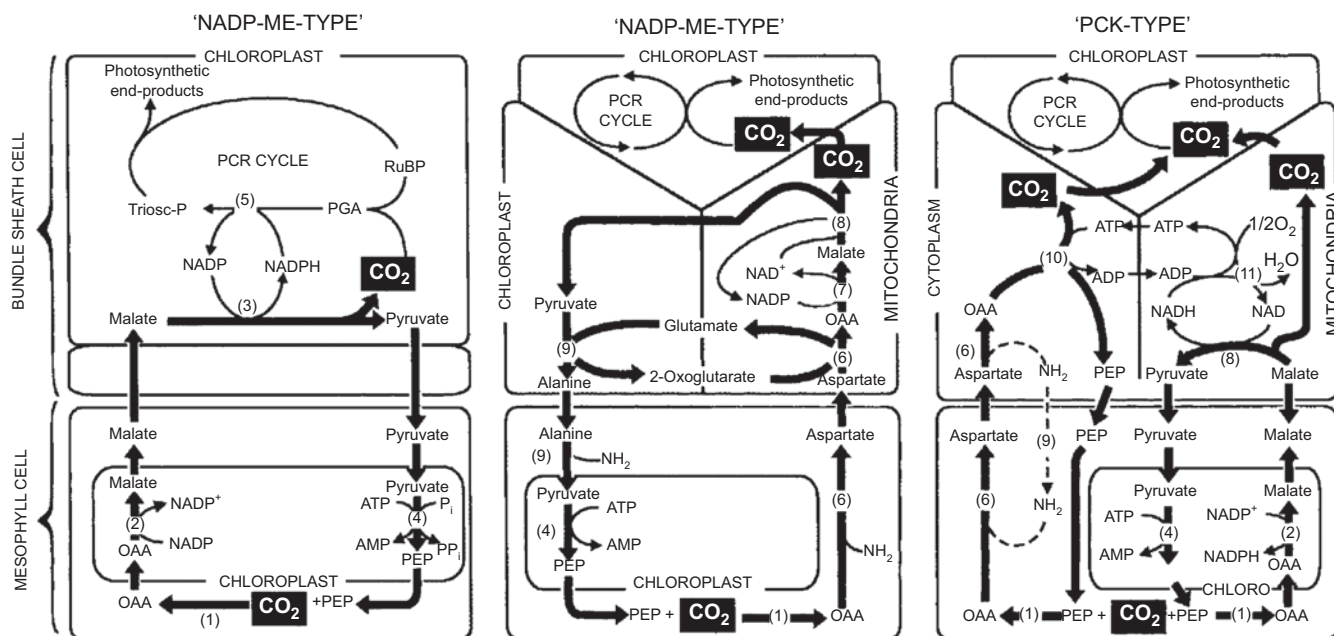
Figure 3. Three decarboxylation types C_4 photosynthesis [von Caemmerer and Furbank (2003) adapted from Furbank et al. (2000)]. The path of carbon flow and the location of the site of CO_2 release in the decarboxylation reaction is shown in the three biochemical sub-groups of C_4 plants. Metabolite flow between cells is denoted by heavy arrows. Key enzymes are numbered as follows: 1, phosphoenolpyruvate carboxylase; 2, NADP malate dehydrogenase; 3, NADP malic enzyme; 4, pyruvate Pi dikinase; 5, 3-phosphoglycerate kinase/glyceraldehyde 3-phosphate dehydrogenase; 6, aspartate aminotransferase; 7, NAD malate dehydrogenase; 8, NAD malic enzyme; 9, alanine aminotransferase; 10, phosphoenolpyruvate carboxykinase; 11, mitochondrial NADH oxidation.

some flexibility in the identity of the C_4 acid transported and the decarboxylase itself (Repo and Hatch, 1976; Meister et al., 1996; Wingler et al., 1999). In the NAD-ME type, there are also examples of diversity in biochemistry and structure. For example, some species have NAD-ME type biochemistry but a "classical" PCK-type anatomy (Prendergast et al., 1987).

The spatial separation between the mesophyll cells and bundle sheath cells has been thought to be a prerequisite for an efficient CO_2-concentrating mechanism. However, single cell C_4 photosynthesis was found in some aquatic plants, which lack a "Kranz"-anatomy typical of C_4 plants, and were identified as being capable of inducing a C_4-like metabolism, including (probably) *Elodea canadensis* (de Groote and Kennedy, 1977), *Hydrilla verticillata* (Holaday and Bowes, 1980; Salvucci and Bowes, 1981, 1983; Spencer et al., 1996; Magnin et al., 1997), and *Egeria densa* (Casati et al., 2000). In addition, the terrestrial chenopod *Borszczowia aralocaspica*, a halophyte with succulent leaves adapted to a semi-dry environment, is likely to carry out C_4 metabolism in only one cell type (Freitag and Stichler, 2000).

CO_2 assimilation of CAM plants

Crassulacean acid metabolism (CAM) is performed by about 6% of vascular plant species (Winter and Smith, 1996), such as *Crassula ovata*, cactus, and most succulents, mainly growing in habitats that are affected by drought, such as deserts and tropical forest canopies (Wild et al., 2010). In CAM plants, CO_2 uptake occurs at night when relative humidity is high, thus reducing water loss from open stomata and enabling the plants to survive in dry conditions (Figure 4A). The diurnal cycle of CAM can be divided into four phases (Osmond, 1978) (Figure 4B): The period of night-time CO_2 fixation catalysed by PEPC and the accumulation of malate in the vacuole is denoted as phase I. With the onset of light, phase II follows, characterized by a decline in PEPC fixation and, eventually, a certain amount of direct C_3-like fixation by Rubisco. Phase III starts with stomatal closure in late morning, when malate is decarboxylated and the released CO_2 is refixed by Rubisco. In the afternoon, stomata may re-open for direct CO_2 fixation by Rubisco in phase IV. The duration of the phases and amplitudes of CO_2 uptake differ widely between species and environmental
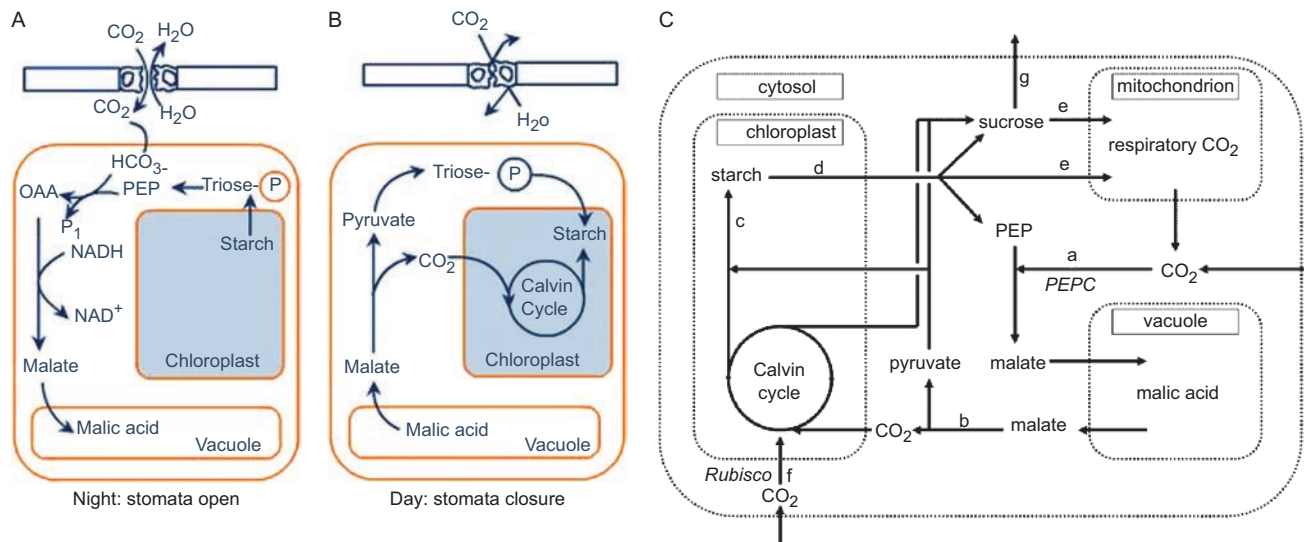
Figure 4. Crassulacean acid metabolism (A and B). (http://www.tutorvista.com/content/biology/biology-iv/photosynthesis/carbon-pathway.php) and C: proposed scheme of cellular carbon flow in *Kalanchoe daigremontiana* (Wild et al. (2010) modified from Dodd et al. (2002)), focusing on the sucrose pool. In C, a: PEPC fixation; b: malate decarboxylation; c: starch accumulation; d: starch degradation; e: respiration; f: Rubisco fixation; g: export.

conditions and, in particular, additional direct fixation of CO_2 by Rubisco (phases II and IV) is highly variable (Dodd et al., 2002).

In phase I, CO_2 is attached to the C_3 molecule PEP derived from the breakdown of storage carbohydrates (usually starch or soluble sugars), which results in the formation of the C_4 molecule malate; however, in contrast to C_4 metabolism, CAM physically separates CO_2 fixation to PEP from the Calvin cycle, and only temporally separates these two processes. CAM plants need to maintain a high pool of easily available carbohydrates for photosynthesis. A low level of carbohydrates for PEP generation may lead to limited fixation of CO_2 by PEPC (Dodd et al., 2003), which, in turn, would further reduce carbohydrate stocks. Thus, the maintenance of CAM, and, in consequence, the ability to cope with unfavorable environmental conditions, depends on the availability of these carbohydrates. This necessity to maintain large carbohydrate stocks, together with the high energy demand caused by conversions between metabolites and intracellular transport processes, limits the productivity of CAM plants (Wild, 2010). However, some CAM plants exhibit growth rates close to C_3 plants (Nobel, 1991), therefore, there are highly coordinated control mechanisms that regulate the competition between carbohydrate storage and growth. By investigating the flux of carbon from PEPC and direct Rubisco fixation to different leaf carbon pools and to phloem sap over the diurnal cycle in CAM plant *Kalanchoe daigremontiana*, Wild (2010) showed (i) a high sucrose turnover, fed by nocturnal starch degradation and direct Rubisco fixation during the day; and (ii) a detailed dissection of the carbon fixation and mobilization pattern, revealed that direct fixation of Rubisco during light accounted for 30% of phloem sucrose, but only 15% of fixed carbon, which indicates that carbon from direct Rubisco fixation was preferentially used for leaf export.

Evolution of C_4 photosynthetic genes

C_4 photosynthetic genes may be specific for C_4 plants (Hatch, 1987), because the activities of the corresponding enzymes are low in C_3 plants and their kinetic properties are usually different from those of C_3 enzymes (Svensson et al., 1997; Dong et al., 1998). However, recent studies revealed that C_3 plants have at least two different types of genes, one encoding enzymes of "housekeeping" function and the other very similar to the C_4 genes of C_4 plants, though expression of the latter is very low or even undetectable in C_3 plants (Miyao, 2003). Hence, the C_4 genes may have evolved from a set of preexisting counterpart genes in ancestral C_3 plants, with modifications in the expression level in the leaves and kinetic properties of the enzymes (Ku et al., 1996). The C_4 genes in C_4 plants and their homologues in C_3 plants are designated as C_4-specific and C_4-like genes, respectively (Miyao, 2003); in addition to C_4-specific or C_4-like genes, both C_3 and C_4 plants have other homologous genes for the housekeeping function, which are designated as C_3-specific genes. Among C_4 enzymes and plant species, the number of homologous genes and the evolutionary origins of C_4-specific genes are different (Monson, 1999). Modifications of the C_4-like genes required for functioning in the C_4 pathway probably share common features in all the C_4-specific genes examined so far (Miyao, 2003).

Improvement of photosynthetic carbon assimilation in C_3 plants using genes from the non-C_4 pathway

In C_3 plants, the following features of Rubisco place it centre stage in attempts to improve their photosynthetic performance. First, low Rubisco activity limits light-saturated photosynthesis under present atmospheric conditions (Chen and Xu, 2007; Suzuki et al., 2009).

Second, the limitation to photosynthetic CO_2 assimilation in hot and/or dry environments is dominated by Rubisco because CO_2 availability is restricted and photorespiration is stimulated (Raines, 2006). Finally, Rubisco is not only an inefficient enzyme with a low turnover number (Raines, 2006), but it also catalyses two competing reactions: carboxylation and oxygenation. The oxygenation reaction directs the flow of carbon through the photorespiratory pathway (Figure 5), which can result in a loss of between 30–50% of photosynthetic carbon fixation (Ogren, 1984; Häusler et al., 2002; Miyao, 2003).

The low activity and the competing reactions catalysed by Rubisco are major limitations to photosynthetic carbon assimilation in C_3 plants, these limitations can be overcome by introducing Rubisco with a higher catalytic rate and/or better able to discriminate between gaseous substrates (Parry et al., 2007). However, the results of approaches that have tried to improve catalytic properties of Rubisco and reduce photorespiration using transgenic engineering have been modest (Heineke et al., 2001; Winzer et al., 2001; Bauwe and Kolukisaoglu, 2003; Boldt et al., 2005; Matsumura et al., 2005; Raines, 2006). In contrast, many studies have been conducted to improve photosynthetic carbon assimilation in C_3 plants using genes from non-C_4 plants (Table 4) (Chen and Xu, 2007; Feng et al., 2007; Feng et al., 2009; Kumar et al., 2009; Suzuki et al., 2009), some of which expressed the FBP/SBPase (Miyagawa et al., 2001; Tamoi and Shigeoka, 2005), *ictB* (Lieman-Hurwitz et al., 2003; 2005), and cytochrome c_6 genes (Chida et al., 2007); all showed positive effects on photosynthesis in transgenic C_3 plants.

Miyagawa et al. (2001) first reported the expression of a single plastid-targeted enzyme that improved carbon assimilation and growth in transgenic tobacco plants. Transgenic plants expressing a cyanobacterial FBP/SBPase targeted to chloroplasts showed enhanced photosynthetic efficiency and growth characteristics under atmospheric conditions (360 ppm CO_2). Compared to wild type (WT) tobacco, final dry matter, and photosynthetic CO_2 fixation of the transgenic plants were 1.5- and 1.24-fold higher, respectively. Transgenic tobacco also showed a 1.2-fold increase in initial activity of Rubisco compared to WT plants; levels of intermediates in the Calvin cycle and the accumulation of carbohydrates were also higher than those in WT plants.

Under many environmental conditions plant photosynthesis and growth are limited by the availability of CO_2 at the site of Rubisco (Austin et al., 1989). Lieman-Hurwitz et al. (2003; 2005) expressed *ictB*, a gene involved in HCO_3-accumulation in *Synechococcus* sp. PCC7942, in *Arabidopsis thaliana* and tobacco plants. Transgenic plants exhibited significantly faster photosynthetic rates than the WTs under limiting, but not under saturating CO_2 concentrations. Growth of transgenic *A. thaliana* plants under low humidity was considerably faster than that of the WTs. There was no difference in the amount of Rubisco or the activity of the enzyme activated *in vitro* between the WTs and transgenic plants. In contrast, the *in vivo* Rubisco activity, without prior activation of the enzyme, in plants grown under low humidity was considerably higher in *ictB*-expressing plants than in their WTs. The CO_2 compensation point in transgenic plants was lower than in WTs, suggesting a higher CO_2 concentration in close proximity to Rubisco. This may explain the higher activation level of Rubisco and enhanced photosynthesis and growth in the transgenic plants. These data indicate the potential use of *ictB* for stimulation of crop yield. Nelson (1988) and Austin (1989) reviewed there are no generally known instances where genetic improvement in crop yield has been attributed by improved photosynthesis. For example, in a few cases, notably maize, where selection for high photosynthetic rate has been made (Crosbie et al., 1981), there has been a considerable response to selection (about 1.5% per generation);
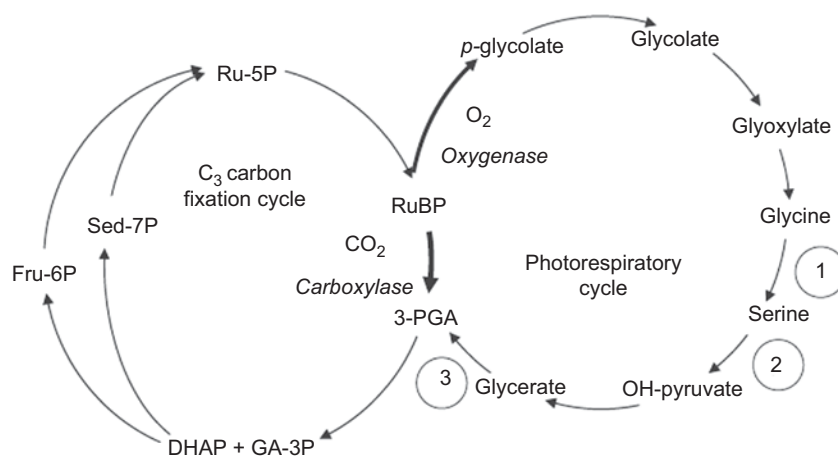
Figure 5. The relationship between the C_3 cycle and the photorespiratory cycle (Raines, 2006). Ribulose is the initiating enzyme in both of these pathways, carrying out the carboxylase reaction to form 3-PGA, which then enters the C_3 cycle; alternatively, the oxygenase reaction takes place forming p-glycolate, which is then metabolized via the photorespiratory pathway. The activities of three enzymes in this cycle have been reduced via either antisense studies: (1) glycine decarboxylase, (2) serine hydroxymethyl transferase (SHMT) or an insertional mutant and (3) glycerate kinase. Ru-5P, ribulose 5-phosphate; Sed-7P, sedoheptulose 7-phosphate; Fru-6P, fructose 6-phosphate; DHAP, dihydroxyacetonephosphate; GA-3P, glyceraldehyde 3-phosphate; RuBP, ribulose bisphosphate.

Table 4. Improvement of photosynthetic carbon fixation in C_3 plants using genes from non-C_4 plants.

Target C_3 plants	Gene introduced	Results	References
Tobacco	FBP/SBPase	Compared with wild-type tobacco, final dry matter and photosynthetic CO_2 fixation of the transgenic plants were 1.5-fold and 1.24-fold higher, respectively. Transgenic tobacco also showed a 1.2-fold increase in initial activity of Rubisco compared with wild-type plants.	Miyagawa et al., 2001
Arabidopsis thaliana and *Nicotiana tabacum*	*ictB*, a gene involved in HCO_3-accumulation in *Synechococcus* sp. PCC7942	Transgenic plants exhibited significantly faster photosynthetic rates than the wild-types under limiting (*Arabidopsis*: 7 µmol CO_2 $m^{-2} s^{-1}$ for transformants *vs*. 5.5 µmol CO_2 $m^{-2} s^{-1}$ for non-transformants; tobacco: 11 µmol CO_2 $m^{-2} s^{-1}$ for transformants *vs*. 9 µmol CO_2 $m^{-2} s^{-1}$ for non-transformants) but not under saturating CO_2 concentrations. The CO_2 compensation point in the transgenic plants that express *ictB* was lower than in the wild-types (*Arabidopsis*: 40 µL/L for transformants *vs*. 46 µL/L for non-transformants; tobacco: 48 µL/L for transfomants *vs*. 57 µL/L for non-transformants), suggesting that the concentration of CO_2 in close proximity to Rubisco was higher.	Lieman-Hurwitz et al., 2003; 2005
Tobacco	FBP/SBPase	The photosynthetic CO_2 fixation and the final dry matter under atmospheric conditions in the transgenic plants were higher than that in the WT plants. Their results suggest that both FBPase and SBPase involved in the regeneration of RuBP seem to be one of the limiting factors that participate in the regulation of the carbon flow through the Calvin cycle and the determination of the partitioning of carbon to end products.	Tamoi and Shigeoka, 2005
Rice	*OsBP-73*, a rice gene, encoding a novel DNA-binding protein with a SAP-like domain	The 29.3% decrease of leaf net photosynthetic rate in the transgenic rice with silenced *OsBP-73* gene is related to both RuBP carboxylation and RuBP regeneration limitations, and the latter is a predominant limitation to photosynthesis.	Chen and Xu, 2007
Arabidopsis thaliana	An algal cytochrome c_6	At 60 d after planting, the plant height, leaf length and root length of the transformants were 1.3-, 1.1- and 1.3-fold those in the wild-type plants, respectively; the CO_2 assimilation capacity of the transgenic plants was 1.3-fold that of the WT. The results demonstrate that the growth and photosynthesis of *Arabidopsis* plants could be enhanced by the expression of the algal cytochrome c_6 gene.	Chida et al., 2007
Rice	SBPase	Transgenic plants accumulating SBPase in chloroplasts resulted in enhanced tolerance to salt stress at the young seedlings stage. Moreover, CO_2 assimilation in transgenic rice plants was significantly more tolerant to salt stress than in wild-type plants. Under salt stress, SBPase maintained the activation of Rubisco by providing more regeneration of the acceptor molecule RuBp in the soluble stroma and by preventing the sequestration of Rubisco activase to the thylakoid membrane from the soluble stroma, and, thus, enhanced the tolerance of photosynthesis to salt stress.	Feng et al., 2007
Rice	SBPase antisense gene under the control of the maize ubiquitin promoter.	Low N in addition to a 34% decrease in SBPase activity was sufficient to diminish photosynthesis and limit biomass production. Decreased SBPase activity may have reduced the N use efficiency of photosynthesis and growth and alter biomass allocation.	Feng et al., 2009
Arabidopsis thaliana	A chimeric activase comprised of the tobacco activase, but containing an *Arabidopsis* Rubisco recognition domain	Transgenic lines of *rca* mutant expressing a chimeric activase showed higher rates of photosynthesis (6.9 µmol CO_2 $m^{-2} s^{-1}$) than the WT (6.4 µmol CO_2 $m^{-2} s^{-1}$), and after a short exposure to higher temperatures (38°C for 2 h) and they also recovered better, when they were returned to the normal temperature (22°C for 2 h). Moreover, under extended exposure to moderately elevated temperature, the transgenic lines had higher biomass (6 g) and seed yield (202 mg) when compared with the WT plants (4.1 g and 50 mg, respectively).	Kumar et al., 2009
Rice	An overexpression of *RBCS*-sense	Although the overexpression of *RBCS*-sense led to an enhancement of Rubisco protein content in the uppermost (5.5 µmol m^{-2} for transgenic plants *vs*. 4.5 µmol m^{-2} for WT), fully expanded leaves, it does not result in increased photosynthetic rates or plant biomass, because of an apparent down-regulation in its activation state.	Suzuki et al., 2009

however, after five generations of selection, the lines with a high photosynthetic rate gave similar yields to the base population (Crosbie and Pearce, 1982). Similar conclusions have been reached in studies on soybean (Secor et al., 1982; Ford et al., 1983), and peas (Hobbs, 1986). In contrast, in *Populus*, high photosynthetic rate, estimated by a ^{14}C method, was positively related to tree biomass (Isebrands et al., 1988). In contrast, there are some evidences of the positive effects of overexpressing C_4 genes in C_3 plants on photosynthetic rate and yield (Kurek et al., 2007; Kershanskaya and Teixeira da Silva, 2010). For example, transgenic wheat plants exhibited a higher photosynthetic capacity (up to 40%) and a higher grain yield (up to 20–50%), compared to untransformed plants (Zhang et al., 2010).

Feng et al. (2007) showed that overexpression of SBPase was an effective method for enhancing salt tolerance in rice. This is because transgenic rice plants accumulating SBPase in chloroplasts resulted in enhanced tolerance to salt stress at the young-seedling stage. Moreover, CO_2 assimilation in transgenic rice plants was significantly more tolerant to salt stress than in WT plants. Under salt stress, SBPase maintained the activation of Rubisco by providing more regeneration of the acceptor molecule RuBp in the soluble stroma and by preventing the sequestration of Rubisco activase (RCA) to the thylakoid membrane from the soluble stroma, and, thus, enhanced the tolerance of photosynthesis to salt stress.

The limitation imposed by Rubisco on C_3 carbon fixation is exacerbated in high light and temperature conditions (Krapp et al., 1994; Stitt and Schulze, 1994), although growth in elevated CO2 can have a compensatory effect (Masle et al., 1993). Plant photosynthesis declines when the temperature exceeds its optimum range, particularly in temperate species like *A. thaliana*. Recent evidence (Kurek et al., 2007) indicates that the reduction in photosynthesis is linked to Rubisco deactivation due to the inhibition of RCA under moderately elevated temperature. To test the hypothesis that thermostable RCA can improve photosynthesis under elevated temperature, Kurek et al. (2007) used gene shuffling technology to generate several *A. thaliana* RCA1 (short isoform) variants exhibiting improved thermostability. WT RCA1 and selected thermostable RCA1 variants were introduced into an *Arabidopsis* RCA deletion line. In a long-term growth test at either constant 26°C or daily 4 h 30°C exposure, the transgenic lines with the thermostable RCA1 variants exhibited higher photosynthetic rates, improved development patterns, higher biomass, and increased seed yields compared to the lines expressing WT RCA1 and a slight improvement compared to untransformed *Arabidopsis* plants. Their results provide clear evidence that RCA is a major limiting factor in plant photosynthesis under moderately elevated temperature and a potential target for genetic manipulation to improve crop plant productivity under heat stress conditions.

On the other hand, at moderately high temperature, the decrease in photosynthetic rate may be attributed to a reduced ability of RCA to achieve optimum activation of Rubisco, leading to reduced RCA. In order to overcome this problem, Kumar et al. (2009) transformed the *Arabidopsis rca* (regulation carboxylation activity or RCA gene) mutant with a more thermostable, chimeric activase where a Rubisco recognition domain in the more thermostable tobacco activase was replaced with that from *Arabidopsis*. Transgenic lines expressing this activase showed higher photosynthetic rate than the WT after a short exposure to higher temperature and they also recovered more efficiently when they were returned to normal temperature. Moreover, under extended exposure to moderately elevated temperature of 30°C, the transgenic lines had higher biomass and seed yield, compared to WT plants.

Overexpression of C_4 cycle photosynthetic genes in transgenic C_3 plants

Based on (i) the limited factors of photosynthesis in C_3 plants and (ii) high photosynthesis efficiency, high rates of biomass accumulation, and high water and N-use efficiency in C_4 plants, biotechnologists have long been intrigued by the overexpression of different enzymes of the C_4 pathway in C_3 plants (Edwards et al., 2001; Leegood, 2002; Häusler et al., 2002; von Caemmerer and Furbank, 2003). Hence, individual or multiple enzymes (PEPC, PPDK, PCK, NADP-ME and NADP-MDH) of the C_4 pathway have been overexpressed in different C_3 plants (e.g. tobacco, potato, rice and *Arabidopsis*) (Table 5, Table 6). In this section, we highlight the success and limitations by transformation of individual or multiple genes, respectively.

Single gene transformation

Overexpression of a single enzyme (PEPC, PPDK, PCK and NADP-ME) of the C_4 pathway has been reported in C_3 plants of tobacco, potato, wheat, rice and *Arabidopsis* (Table 5). The efficient fixation of atmospheric CO_2 by PEPC is a prerequisite for the insertion of a single cell C_4 cycle in C_3 plants. The cDNAs of the C_4-type PEPC sequences for cloning were first obtained from maize (Izui et al., 1986) and *Flaveria trinervia* (Poetsch et al., 1991). Hudspeth et al. (1992) expressed the PEPC gene of maize in transgenic tobacco (*N. tabacum*). Where transcription was under the control of a maize PEPC gene promoter, a low level of aberrantly large PPC transcript was detected. Higher levels of maize PEPC transcript of the correct size were obtained with a chimeric gene construct containing a tobacco (*Nicotiana plumbaginifolia*) chlorophyll *a/b* binding protein gene promoter. The PEPC activity in the leaves of these transgenic plants was up to 2-fold higher than that of non-transformed plants. Biochemical analyses of these plants indicated that the transgenic plants had significantly elevated levels of titratable acidity and malic acid. However, these biochemical differences did

Table 5. Introduction single C_4 enzymes into C_3 plants.

Target C_3 plants	Enzyme genes induced (C_4 plants)	Results	References
Tobacco	PEPC (Maize)	Transgenic plants showed 2-fold higher PEPC activities than that of control plants (97 *vs.* 48 Chl·h), significantly elevated levels of titratable acidity (16 *vs.* 10 µeq/g fresh wt) and malic acid (8.9 vs. 4.5 µmol/mg Chl), but no physiological changes with respect to photosynthetic rate or CO_2 compensation point.	Hudspeth et al., 1992
	PPDK (*Mesembryanthemum crystallinum*)	Transgenic plants have 125–166% of PPDK activity relative to WT, produced more seeds per seed capsule (149 for transformants *vs.* 100 for WT) and heavier seed capsules than the WT (13.21 g /100 seed capsules for transformants *vs.* 11.99 g/100 seed capsules for WT).	Sheriff et al., 1998
Potato	PPDK (Maize)	PPDK activity in the leaves of transgenic potatoes was up to 5.4-fold higher than that of control potato plants (WT and treated control plants), but only a small effect on the total photosynthetic characteristics of the transgenic plants.	Ishimaru et al., 1998
	PEPC (*Corynebacterium glutamicum*)	Elevated CO_2/O_2 ratios in the mesophyll are concomitant with a more favoured carboxylation/oxygenation ratio of Rubisco.	Häusler et al., 1999
	PEPC (*Orynebacterium glutamicum*)	Overexpression of PEPC gene resulted in a 3- to 6-fold induction of endogenous cytosolic NADP-ME and an increase in the activities of NAD-ME (3-fold).	Häusler et al., 2001
	NADP-ME (*Flaveria pringlei*)	There were no changes in enzyme activity.	Häusler et al., 2001
Rice	PEPC (Maize)	Most transgenic rice plants showed high-level expression of the maize gene; the activities of PEPC in leaves of some transgenic plants (0.04 to 6.76 µmol/mg/min) were 2- to 3-fold higher than those in maize (2.25 µmol/mg/min). Under atmospheric conditions, the photosynthetic rates of transgenic plants were comparable to those of the two untransformed rice cultivars.	Ku et al., 1999; 2000
	PPDK (Maize)	Transgenic plants have higher photosynthetic rates (up to 35%) than untransformed plants, which is at least in part due to an enhanced stomatal conductance and a higher internal CO_2 concentration.	Ku et al., 2000
	PCK (*Urochloa panicoides*)	20% of the radioactivity was incorporated into 4C compounds (malate, oxaloacetate, and aspartate) in excised leaves of transgenic plants, as compared with about 1% in excised leaves of control plants, indicating that the ectopic expression of PCK in rice chloroplasts was able to change the carbon flow in mesophyll cells into a C_4-like photosynthetic pathway.	Suzuki et al., 2000
	NADP-ME (Maize)	A 20- to 70-fold increase in NADP-ME activity may have caused abnormal chloroplasts with agranal thylakoid membranes. They postulate a high level of chloroplastic NADP-ME activity could strongly a.ect the development of chloroplasts.	Takeuchi et al., 2000
	PPDK (Maize)	The intron (s) or the terminator sequence of the maize gene, or a combination of both, was necessary for high-level expression.	Fukayama et al., 2001
	NADP-ME (Maize)	In transformants, the accumulation of the maize C_4-specific NADP-ME led to bleaching of leaf color and growth hindrance in rice plants under natural light. These deteriorative effects resulted from enhanced photoinhibition of photosynthesis due to 30-fold increase of NADP-ME activity relative to non-transformants inside the chloroplast by the action of the maize enzyme.	Tsuchida et al., 2001
	PEPC (Maize)	PEPC did not contribute significantly to the photosynthetic CO_2 fixation of transgenic rice plants. Rather, it slightly lowered the CO_2 assimilation rate (7.3%). Overproduction of PEPC did not directly affect photosynthesis significantly, but it suppressed photosynthesis indirectly by stimulating respiration in the light.	Fukayama et al., 2003
	PEPC (Maize)	The transgenic rice (0.25 U/mg protein) showed 14- to 60-fold higher PEPC activity than control plants (0.02 U/mg protein), which facilitates provision of carbon to the anaplerotic pathway, biosynthesis of amino acids and prevents the accumulation of starch.	Suzuki et al., 2006

Table 5. Continued.

Target C$_3$ plants	Enzyme genes induced (C$_4$ plants)	Results	References
	PEPC (Maize)	The transformants showed enhanced photosynthesis rate (24.2–25.4 µmol m^{-2} s^{-1} vs. 19.6 of control plants) at high temperature (35–41°C) but without any improvement of yield.	Bandyopadhyay et al., 2007
	PEPC (Maize)	PEPC transgenic plants exhibited a 25-fold increase of PEPC activity and the enhanced activity of carbonic anhydrase (more than a 2-fold increase). Furthermore, PEPC transgenic rice produced 22% more grains than the WT plants.	Jiao, 2008
	PPDK (*Echinochloa*)	Enzyme activity was elevated 1- to 11-fold. However, no appreciable change demonstrated in carbon assimilation of the transgenic upland rice though increased photoinhibition was noted under high light intensity.	Wang and Li, 2008
	PPDK (Maize)	Compare to WT (21.22 µmol CO$_2$ m^{-2} s^{-1}), the photosynthesis rate of most of transgenic lines (21.26–26.80 µmol CO$_2$ m^{-2} s^{-1}) was increased.	Zhang et al., 2010
Arabidopsis thaliana	NADP-ME	The transformants did not show any differences in morphology and development when grown in long days; however, dark-induced senescence progressed more rapidly in transgenic plants compared to the WT; malate and fumarate are important forms of fixed carbon that can be rapidly metabolized under stress conditions in *Arabidopsis*.	Fahnenstich et al., 2007
Wheat	PEPC (Maize)	Transgenic plants exhibit a higher photosynthetic capacity (up to 40%) than untransformed plants; high grain yield increased up to 25–50% in transgenic plants in comparison with WTs.	Kershanskaya and Teixeira da Silva, 2010

not produce any significant physiological changes with respect to the photosynthetic rate or CO$_2$ compensation point.

Ishimaru et al. (1998) overexpressed a C$_4$ maize PPDK gene in C$_3$ transgenic potato. PPDK activity in the leaves of transgenic potatoes was up to 5.4-fold higher than that of the control plants (WT and treated control plants). In the transgenic plants, PPDK activity in the leaves was negatively correlated with pyruvate content and was positively correlated with malate content. A significant increase in the δ^{13}C value was observed in the transgenic plants, suggesting a certain contribution of PEPC as the initial acceptor of atmospheric CO$_2$. Their results suggested that elevated PPDK activity may alter carbon metabolism and lead to a partial operation of C$_4$-type carbon metabolism. However, since parameters associated with CO$_2$ gas exchange were not affected, the altered carbon metabolism had only a small effect on the total photosynthetic characteristics of the transgenic plants.

Fukayama et al. (2001) introduced the C$_4$-*Pdk* gene encoding the C$_4$ enzyme PPDK of maize (*Zea mays* cv Golden Cross Bantam) into the C$_3$ plant, rice (*Oryza sativa* cv Kitaake). When the intact maize C$_4$-*Pdk* gene, containing its own promoter and terminator sequences and exon/intron structure, was introduced, the PPDK activity in the leaves of some transgenic lines was greatly increased, in one line reaching 40-fold more than that of the WT plants. In a homozygous line, the PPDK protein accounted for 35% of total leaf-soluble protein or 16% of total leaf nitrogen. In contrast, introduction of a chimeric gene containing the full-length cDNA of the maize PPDK fused to the maize C$_4$-*Pdk* promoter or the rice *Cab* promoter only increased PPDK activity and protein level slightly (not exceeding 5-fold). Their results indicate that

the intron(s) or the terminator sequence of the maize gene, or a combination of both, is necessary for high-level expression.

Zhang et al. (2010) also introduced the intact maize C$_4$-*Pdk* gene into rice (*Oryza sativa* L. *indica* "IR64"). Expression of C$_4$-*Pdk* in most transgenic rice lines resulted in the increase of CO$_2$ assimilation rates compared to untransformed control plants. Most of the transformants showed higher photosynthetic activities than the WT plants. In addition, the photosynthesis rates of some transgenic IR64 lines also increased in the greenhouse. Molecular analysis revealed that the intact maize C$_4$-*Pdk* gene integrated in the rice genome and affected the phenotypes of plants, particularly tillers, and enhanced the yield of transgenic IR64 rice plants in the greenhouse.

Kershanskaya and Teixeira da Silva (2010) introduced the maize C$_4$-specific PEPC gene into wheat. Their results showed that transgenic plants exhibited a higher photosynthetic capacity (up to 40%), an increase in PEPC-enzyme activity (up to 6-fold), the appearance of a C$_4$ Kranz leaf anatomy structure, and a higher grain yield (up to 20–50%), compared to untransformed plants.

So far, there has been significant progress in the overexpression of the key enzymes of C$_4$-type biochemistry in transgenic C$_3$ plants, but the above efforts to overexpress the individual enzymes of C$_4$ photosynthesis in C$_3$ plants (rice, tobacco and potato) have produced modest results, and it is still uncertain whether this approach will be sufficient to improve the photosynthetic rate.

Double transformation

Although overproduction of a single C$_4$ enzyme can alter the carbon metabolism in C$_3$ plants, only few examples

Table 6. Introduction multiple C_4 enzymes into C_3 plants.

C_3 plants	Enzyme genes induced (C_4 pathway)	Results	References
Tobacco	PEPC (*Orynebacterium glutamicum*) and NADP-ME (*Flaveria pringlei*),	Photorespiration in transgenic tobacco plants appeared to be diminished most in single PEPC or NADP-ME overexpressors rather than in double transformants (PEPC and NADP-ME).	Häusler et al., 2001
Potato	PEPC and NADP-ME	Double transformants with an additional 3-5-fold over-expression of *Flaveria pringlei* NADP-malic enzyme in the chloroplast showed a temperature-dependent decrease in the electron requirement for CO_2 assimilation, suggesting a slight suppression of photorespiration.	Lipka et al., 1999
		In double transformants, activities of cytosolic NADP-ME was increased (89 mU mg^{-1} of transformants *vs*. 63 mU mg^{-1} of non-transformants), as well as the increase of PEPC activities (40 mU mg^{-1} of transformants *vs*. 23 mU mg^{-1} of non-transformants).	Häusler et al., 2001
	PCK and PEPC	Transformants of two enzymes genes resulted in improved photosynthetic properties and relief of pleiotropic effects.	Peterhänsel et al., 2008b
Rice	Maize PEPC and PPDK	The photosynthetic rates of plants with both maize enzymes are up to 35% higher than those of untransformed plants. The grain yield is about 10–30% higher in PEPC and 30–35% higher in PPDK transgenic rice plants relative to untransformed plants. These results suggest that introduction of C_4 photosynthesis enzymes into rice has a good potential for enhancing the crop's photosynthetic capacity and yield.	Ku et al., 2001
		The transgenic rice plants expressing both PEPC and PCK showed up to 55-fold higher PEPC activity than the control plants and PCK activity comparable to the transgenic rice plants expressing only PCK, but elevated PEPC activity in combination with PCK activity contributed little to C_4-like carbon flow.	Suzuki et al., 2006
		The photosynthesis rate and PEPC enzyme activity of transgenic homozygous lines increased by 19–40% and 1. 5 to 4. 9-fold, respectively compared with those of the WT, indicating that these transgenic rice lines are of the great potential values in increasing photosynthesis for hybrid rice breeding.	Yuan et al., 2007
		The activities of PEPC and PPDK increased up to 110- and 40-fold more, respectively, than those of nontransgenic rice. High expression of C_4 enzymes did not result solely from the high expression activity of the maize gene, since the introduction of a maize PPDK cDNA fused to the maize Pdk promoter or rice *Cab* promoter did not lead to high expression of PPDK. In some transgenic rice plants carrying the intact maize gene, the level of PPDK protein amounted to 35% of total leaf-soluble protein. The high expression of each C_4 enzyme altered metabolism slightly but did not seem to increase the photosynthetic efficiency of transgenic rice leaves.	Matsuoka et al., 2000
		Untransformed rice possessed all the C_4 photosynthetic enzymes but their activities are very low. PCR indicated that the PEPC and PPDK genes from maize were transformed into common rice. The PEPC and PPDK activities of the C_4 enzymes in PEPC+PPDK transgenic rice were 18 and 3-fold of non-transformants.	Zhang et al., 2008
	PPEC, PPDK, NADP- MDH, NADP- ME	Overproduction individually of PPDK, MDH or ME did not affect the rate of photosynthetic CO_2 assimilation. The reduction in CO_2 assimilation in PEPC overproduction lines remained unaffected by overproduction of PPDK, ME or a combination of both; however, it was significantly restored by the combined overproduction of PPDK, ME, and MDH. Transgenic rice plants overproducing the four enzymes showed slight stunting. Comparison of transformants overproducing different combinations of enzymes indicated that overproduction of PEPC together with ME was responsible for stunting, and that overproduction of MDH had some mitigating effects.	Taniguchi et al., 2008

in the greenhouse showed positive effects on photosynthesis. Transgenic C_3 plants overproducing multiple enzymes are now being produced for improving their photosynthetic performance (Table 6), including the combinations of PEPC and NADP-ME (Lipka et al., 1999; Häusler et al., 2001), as well as PEPC and PPDK (Streatfield et al., 1999; Matsuoka et al., 2000; Ku et al., 2001; Yuan et al., 2007; Jiao, 2008; Zhang et al., 2008).

Lipka et al. (1999) transformed two potato lines using NADP-ME-cDNA constructs, one of which already overexpressed the PEPC from *Corynebacterium glutamicum*. Both genes were under the control of the constitutive 35S CaMV promoter. Increased levels of NADP-ME were found in chloroplasts of transformants. Expression of both genes led to a significantly reduced electron requirement for apparent CO_2 assimilation (e/A) at higher temperature. At low temperatures (15°C) 11 electrons per CO_2 were assimilated (e/A) in controls, single (PEPC or NADP-ME) and double (PEPC and NADP-ME) transformation. However, when the leaf temperature was raised to 36°C, the electron requirement of the double transformation (15 e/A) was 65% of controls or single transformation (23 e/A). Thus, the temperature-dependent increase in electron requirement was reduced in the double transformation, suggesting a suppression in the oxygenation reaction of Rubisco.

The introduction of a fully operational C_4 cycle might ultimately provide a clue as to whether C_3 photosynthesis can be improved or not (Häusler et al., 2002; Yuan et al., 2007). In transgenic rice plants overexpressing *Urochloa* PCK in combination with maize PEPC, PEPC added little to the effects already obtained with PCK alone (Suzuki et al., 2006). Transgenic lines of hybrid rice expressing PEPC and PPDK genes from maize showed that the photosynthesis rate and the PEPC enzyme activity of the transgenic homozygous lines increased by 19–40% and 1.5 to 4.9-fold, respectively, compared with those of the WT plants, indicating that the transgenic rice lines have great potential value in increasing photosynthesis for hybrid rice breeding (Yuan et al., 2007).

Four enzymes, namely, the maize C_4-specific PEPC, PPDK, the sorghum NADP-MDH, and the rice C_3-specific NADP-ME, were overexpressed in the mesophyll cells of rice plants independently or in combinations (Taniguchi et al., 2008). Overproduction individually of PPDK, NADP-MDH or NADP-ME did not affect the rate of photosynthetic CO_2 assimilation, while in the case of PEPC it was slightly reduced. The reduction in CO_2 assimilation in lines overexpressing PEPC remained by overexpression of PPDK, NADP-ME, or their combination, although it was significantly restored to levels comparable to or slightly higher than those of non-transgenic rice by the combined overexpression of PPDK, NADP-ME, and NADP-MDH. The extent of the restoration of CO_2 assimilation was more marked at higher CO_2 concentrations; however, overexpression of the four enzymes in combination did not act to concentrate CO_2 inside the chloroplast, and transgenic rice plants overproducing the four enzymes showed slight stunting. A comparison of transformants overexpressing different combinations of enzymes indicated that overexpression of PEPC together with NADP-ME was responsible for the stunting, and that overexpression of NADP-MDH had some mitigating effects.

Factors that affect the expression levels and efficiency of C_4 enzymes in C_3 plants

Expression of a transgene is hampered by many mechanisms including positional effects (Gelvin, 1998), silencing (Chandler and Vaucheret, 2001) and rearrangement (Hiei et al., 1994) of the transgene or factors associated with the transformation protocol (Karami, 2008). For example, rearrangements occurred frequently during gene transfer mediated by *Agrobacterium tumefaciens* in a study in which C_4 enzymes were overproduced (Miyao, 2003). In the next section, we discuss the factors influencing the expression levels and efficiency of C_4 enzymes in C_3 plants.

First, phylogenetic distance may hamper the expression of genes from C_4 plants in the leaves of C_3 plants (Häusler et al., 2002). Not only incorrect initiation and termination of transcription, but also incorrect splicing could occur when genes from monocots are introduced into dicots (Goodall and Filipowicz, 1991). For example, the intact maize C_4-specific PEPC gene was not expressed at high levels in tobacco leaves, because of incorrect transcription initiation (Hudspeth et al., 1992). A major problem has been in generating transgenic lines with enzyme activities sufficient to support the high fluxes necessary to sustain the C_4 pathway (Häusler et al., 2002).

Second, conventional techniques for overproduction of transgenes, namely, the introduction of a chimeric gene containing cDNA for a C_4 enzyme, fused to a strong promoter alone or together with enhancer sequences, only can increase the several fold activity of C_4 enzymes in the leaves of C_3 plants exceeding that of non-transformants (Matsuoka et al., 2001).

Finally, at present, it still remains obscure if foreign PEPC is expressed in guard cells of these transformants (Miyao et al., 2003). A research group claims that the photosynthetic rate under saturating light can be greatly increased by overexpression of the maize PEPC in transgenic rice plants (Jiao et al., 2001); however, their results need to be reconsidered, because the correlation between the photosynthetic rate and the level of the PEPC protein in transgenic rice leaves has not yet been confirmed. In contrast, some groups reported negative effects on photosynthesis, and the photosynthetic rate was slightly lowered by overexpression (Agarie et al., 2002). It is more likely that the suppression of photosynthesis results from the enhanced respiration by elevated PEPC (Miyao et al., 2003).

Challenges and prospects

Challenges of improving carbon assimilation of C_3 plants using genetic engineering

To improve the photosynthetic performance of C_3 plants for global warming, botanists and plant breeders have been interested in enhancing the low activity of Rubisco, decreasing photorespiration, in which up to 50% of the fixed carbon may be lost, and introducing enzymes of the C_4 pathway into C_3 plants.

Overexpression of the non-C_4 genes FBP/SBPase, *ictB* and cytochrome c_6 showed positive effects on photosynthetic efficiency and growth characteristics in C_3 plants. However, over the last 28 years, efforts to overexpress individual or multiple C_4 enzymes in C_3 plants, specifically rice, tobacco, potato, and *Arabidopsis*, have produced mixed results, and it is still uncertain whether this approach will be sufficient to improve the photosynthetic rate (Sheehy et al., 2000). In addition, it seems unlikely that attempts to introduce single cell CO_2-concentrating mechanisms will be successful without simultaneously introducing some of the structural characteristics of C_4 photosynthesis (Matsuoka et al., 2001), that is, a compartment in which CO_2 could be concentrated (Leegood, 2002).

Under saturating CO_2 conditions, Rubisco does not limit photosynthesis (Masle et al., 1993; Makino et al., 2000; Long et al., 2004), and the limitation of photosynthesis is shifted to the RuBP regeneration capacity of the C_3 cycle (Harrison et al., 1998; Haake et al., 1999; Henkes et al., 2001; Raines, 2003; Miyagawa et al., 2001; Lefebvre et al., 2005). Hence, in the future, in a high CO_2 world, Rubisco will not be limiting, and it is likely to be enzymes of the regenerative phase of the cycle that will be targets for improving C_3 carbon fixation (Raines, 2006). FBP/SBPase enzymes of the C_3 cycle have been identified as being important control points, and two examples of over-expression of these enzymes have demonstrated that photosynthesis can be increased in these plants, leading to increased yield. However, what has not yet been tested is the impact of these manipulations on the photosynthesis and yield of plants grown in elevated CO_2 conditions.

Overproduction of C_4 enzymes in C_3 plants can be achieved by introducing appropriate gene constructs (Mann, 1999; Surridge, 2002; Miyao, 2003). So far, most approaches have aimed at introducing the highest possible activities of the respective enzymes in transgenic C_3 plants (Häusler et al., 2002). This is reasonable because these enzymes are expressed to very high levels in the leaves of C_4 plants, for example, PEPC activity in maize is 1186 Chl·h, but only 48 Chl·h in tobacco (Hudspeth et al., 1992); Total NADP-ME activity in *Flaveria trinervia* (C_4) is 737.9 mU mg^{-1}, but only 14.3 mU mg^{-1} for *Flaveria aangustifolium* (C_3) and 13.1 mU mg^{-1} for *Flaveria pringlei* (C_3) (Häusler et al., 2002). It is also necessary to screen a number of transgenic plants to obtain the desired expression level of a C_4 enzyme and to confirm the enzyme location in the leaves of C_3 plants. However, whether this pathway can operate with desirable effects on C_3 photosynthesis or not is a matter of controversy (Edwards, 1999; Häusler et al., 2002; Leegood, 2002), and a high expression of the introduced enzyme is not necessarily linked with high *in vivo* activities. Considering the C_4 pathway operating in a single cell found in some aquatic organisms (for a review see Leegood, 2002), it might be possible that the C_4-like pathway could support C_3 photosynthesis under

some stress conditions such as drought, in which CO_2 availability is limited. It appears to be useful and perhaps even necessary to engineer more of the C_4-cycle enzymes and/or the respective promoters to be better adapted to the specific requirements of a C_4-like cycle in a 'C_3 environment'. Thus, it raises further questions as to whether the C_4 genes encoding the PPDK, NADP-ME and PCK enzymes should be introduced in one genome in addition to PEPC either by transgene-breeding or by multigene transformation to achieve an overall increase in yield under natural field conditions (Bandyopadhyay et al., 2007). However, Taniguchi et al. (2008) considered that rice is not a good plant material for an approach to introduce the C_4-like pathway with respect to increased production, because (i) overproduction of a single C_4 enzyme (PEPC, PPDK, NADP-MDH or NADP-ME) did not improve photosynthesis of rice; (ii) overproduction of the maize PEPC inhibited photosynthesis through stimulation of respiration in the light and reduction of Rubisco activity (Fukayama et al., 2003); (iii) the combinations of C_4 enzymes examined, only overproduction of all the four enzymes had some positive effects on photosynthesis, and only to a limited extent; and (v) transgenic rice plants overproducing the four enzymes showed stunting.

Hence, the mixed results of overexpression of C_4 enzymes in C_3 plants do not confirm or eliminate the possibility of improving photosynthesis of C_3 plants by this approach. Furthermore, it is important for the improvement of photosynthetic carbon assimilation in C_3 plants to (i) find an alternative approach to reducing photorespiration, such as the introduction of pyrenoids or carboxysomes into the chloroplasts of C_3 plants (Leegood, 2002), the movement of the location of photorespiratory glycine metabolism to the bundle-sheath (Monson and Rawsthorne, 2000; Winzer et al., 2001), and the improvement of forms of Rubisco, notably those from rhodophyte algae in which the relative specificity for CO_2 compared to O_2 is higher than that of the higher plant Rubisco (Whitney et al., 2001); (ii) understand the complex factors that regulate the development of the different cell types in C_4 plants, in which anatomical characteristics should be incorporated, although this may only be controlled by a few genes, which have not yet been identified; (iii) determine whether photosynthesis could be substantially improved by changing the relative level of expression of the introduced enzymes; (iv) develop an efficient PEPC system towards increasing the productivity of C_3 plants; (v) conduct more field studies to evaluate whether there will be differences in productivity with increasing photosynthesis rate under normal planting densities and natural field conditions.

In addition, improvement of photosynthetic carbon assimilation of C_3 plants using genetic engineering has only been reported in tobacco, potato, rice, and *Arabidopsis*. For global warming, drying and salinization (Rozema and Flowers, 2008), scientists have long been developing xero-halophytes used for desertic or

marginal lands where no other plants are able to grow and to save good quality water for human consumption, provide human nutrition and green biofuel, reduce CO_2 emission, and assimilate and sequester carbon into biomass. Several tolerant plants with potential interest for agriculture and environmental management have already been identified, such as *Atriplex halimus* (Lutts et al., 2004), *Kosteletzkya virginica* (Ruan et al., 2008), and *Salicornia bigelovii* (Zerai et al., 2010). However, most orphan xero-halophyte plants have relatively no genetic information compared to conventional crops with known genetic information (e.g. genetic transformation system, sequences, QTLs, etc.). Hence, to improve photosynthetic carbon assimilation of xero-halophytes, sometime in the future, as an alternative to improvement of carbon assimilation using genetic engineering, enhance carbon fixation by environmental, physiological, and biological regulations will have great potential and we will highlight this topic in another review.

Declaration of interest

References

Agarie S, Miura A, Sumikura R, Tsukamoto S, Nose A, Arima S, Matsuoka M, Miyao-Tokutomi M. (2002). Overexpression of C_4 PEPC caused O_2-insensitive photosynthesis in transgenic rice plants. Plant Sci, 162, 257–265.

Andersson I, Taylor TC. (2003). Structural framework for catalysis and regulation in ribulose-1,5-bisphosphate carboxylase/oxygenase. Arch Biochem Biophys, 414, 130–140.

Austin RB. (1989). Genetic variation in photosynthesis. J Agric Sci, 112, 287–294.

Bandyopadhyay A, Datta K, Zhang J, Yang W, Raychaudhuri S, Miyao M, Datta SK. (2007). Enhanced photosynthesis rate in genetically engineered *indica* rice expressing *pepc* gene cloned from maize. Plant Sci, 172, 1204–1209.

Bauwe H, Kolukisaoglu U. (2003). Genetic manipulation of glycine decarboxylation. J Exp Bot, 54, 1523–1535.

Black Jr CC. (1973). Photosynthetic carbon fixation in relation to net CO_2 uptake. Ann Rev Plant Physiol, 24, 253–286.

Boldt R, Edner C, Kolukisaoglu U, Hagemann M, Weckwerth W, Wienkoop S, Morgenthal K, Bauwe H. (2005). D-Glycerate 3-kinase, the last unknown enzyme in the photorespiratory cycle in *Arabidopsis*, belongs to a novel kinase family. Plant Cell, 17, 2413–2420.

Brooks A, Farquhar GD. (1985). Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase oxygenase and the rate of respiration in the light – estimates from gas-exchange measurements on spinach. Planta, 165, 397–406.

Brown RH, Bouton JH. (1993). Physiology and genetics of interspecific hybrids between photosynthetic types. Ann Rev Plant Physiol Plant Mol Biol, 44, 435–456.

Casati P, Lara MV, Andreo CS. (2000). Induction of a C_4-like mechanism of CO_2 fixation in *Egeria densa*, a submerged aquatic species. Plant Physiol, 123, 1611–1621.

Chandler VL, Vaucheret H. (2001). Gene activation and gene silencing. Plant Physiol, 125, 145–148.

Chen S, Hajirezaei M, Peisker M, Tschiersch H, Sonnewald U, Börnke F. (2005). Decreased sucrose-6-phosphate phosphatase level in transgenic tobacco inhibits photosynthesis, alters carbohydrate partitioning, and reduces growth. Planta, 221, 479–492.

Chen Y, Xu DQ. (2007). Changes in leaf photosynthesis of transgenic rice with silenced *OsBP-73* gene. Photosynthetica, 45, 419–425.

Chida H, Nakazawa A, Akazaki H, Hirano T, Suruga K, Ogawa M, Satoh T, Kadokura K, Yamada S, Hakamata W, Isobe K, Ito T, Ishii R, Nishio T, Sonoike K, Oku T. (2007). Expression of the algal cytochrome c_6 gene in *Arabidopsis* enhances photosynthesis and growth. Plant Cell Physiol, 48, 948–957.

Cooper TG, Wood HG (1971). The carboxylation of phosphoenolpyruvate and pyruvate. II. The active species of 'CO_2' utilized by phosphoenolpyruvate carboxylase and pyruvate carboxylase. J Biol Chem, 246, 5488–5490.

Crosbie TM, Pearce RB. (1982). Effects of recurrent phenotypic selection for high and low photosynthesis on agronomic traits in two maize populations. Crop Sci, 22, 809–813.

Crosbie TM, Pearce RB, Mock JJ. (1981). Recurrent phenotypic selection for high and low photosynthesis in two maize populations. Crop Sci 21, 736–740.

Daie J. (1993). Cytosolic fructose-1,6-bisphosphatase: A key enzyme in the sucrose biosynthetic pathway. Photosynth Res, 38, 5–14.

de Groote D, Kennedy RA. (1977). Photosynthesis in *Elodea canadensis* Michx. Plant Physiol, 59, 1133–1135.

Dodd AN, Borland AM, Haslam RP, Griffiths H, Maxwell K. (2002). Crassulacean acid metabolism: plastic, fantastic. J Exp Bot, 53, 569–580.

Dodd AN, Griffiths H, Taybi T, Cushman JC, Borland AM. (2003). Integrating diel starch metabolism with the circadian and environmental regulation of crassulacean acid metabolism in *Mesembryanthemum crystallinum*. Planta, 216, 789–797.

Dong LY, Masuda T, Kawamura T, Hata S, Izui K. (1998). Cloning, expression, and characterization of a root-form phosphoenolpyruvate carboxylase from *Zea mays*: comparison with the C_4-form enzyme. Plant Cell Physiol, 39, 865–873.

Edwards G. (1999). Tuning up crop photosynthesis. Nat Biotechnol, 17, 22–23.

Edwards GE, Furbank RT, Hatch MD, Osmond CB. (2001). What does it take to be C_4? Lessons from the evolution of C_4 photosynthesis. Plant Physiol, 125, 46–49.

Ehleringer JR, Monson RK. (1993). Evolutionary and ecological aspects of photosynthetic pathway variation. Ann Rev Ecol Syst, 24, 411–439.

Fahnenstich H, Saigo M, Niessen M, Zanor MI, Andreo CS, Fernie AR, Drincovich MF, Flügge UI, Maurino VG. (2007). Alteration of organic acid metabolism in *Arabidopsis* overexpressing the maize C_4 NADP-malic enzyme causes accelerated senescence during extended darkness. Plant Physiol, 145, 640–652.

Feng LL, Han YJ, Liu G, An BG, Yang J, Yang GH, Li YS, Zhu YG. (2007). Overexpression of sedoheptulose-1,7-bisphosphatase enhances photosynthesis and growth under salt stress in transgenic rice plants. Funct Plant Biol, 34, 822–834.

Feng LL, Li H, Jiao JM, Li D, Zhou L, Wan J, Li YS. (2009). Reduction in SBPase activity by antisense RNA in transgenic rice plants: effect on photosynthesis, growth, and biomass allocation at different nitrogen levels. J Plant Biol, 52, 382–393.

Finnegan PM, Suzuki S, Ludwig M, Burnell JN. (1999). Phosphoenolpyruvate carboxykinase in the C_4 monocot *Urochloa panicoides* is encoded by four differentially expressed genes. Plant Physiol, 120, 1033–1041.

Firooznia F. (2007). The story of the Calvin cycle: bringing carbon fixation to life. Am Biol Teach, 69: 364–367.

Ford DM, Shibles R, Green DE. (1983). Growth and yield of soybean lines selected for divergent leaf photosynthetic ability. Crop Sci, 23, 517–520.

Freitag H, Stichler W. (2000). A remarkable new leaf type with unusual photosynthetic tissue in a central *Asiatic* genus of Chenopodiacea. Plant Biol, 2, 154–160.

Fukayama H, Hatch MD, Tamai T, Tsuchida H, Sudoh S, Furbank RT, Miyao M. (2003). Activity regulation and physiological impacts of maize C_4-specific phosphoenolpyruvate carboxylase overproduced in transgenic rice plants. Photosyn Res, 77, 227–239.

Fukayama H, Tsuchida H, Agarie S, Nomura M, Onodera H, Ono K, Lee B-H, Hirose S, Toki S, Ku MSB, Makino A, Matsuoka M, Miyao M. (2001). Significant accumulation of C_4-specific pyruvate, orthophosphate dikinase in a C_3 plant, rice. Plant Physiol, 127, 1136–1146.

Furbank RT, Jenkins, CLD, Hatch, MD. (2000). Regulation of C_4 photosynthesis. In: Leegood RC, Sharkey TD, von Caemmerer S, eds. Photosynthesis: Physiology and Metabolism (Advances in Photosynthesis). Dordrecht, The Netherlands: Kluwer Academic Publishers, 435–457.

Gelvin SB. (1998). The introduction and expression of transgenes in plants. Curr Opin Biotechnol, 9, 227–232.

Goodall GJ, Filipowicz W. (1991). Different effects of intron nucleotide composition and secondary structure on pre-mRNA splicing in monocot and dicot plants. EMBO Journal, 10, 2625–2644.

Greene CH, Pershing AJ. (2007). Climate drives sea change. Science, 315, 1084–1085.

Haake V, Geiger M, Walch-Liu P, Engels C, Zrenner R, Stitt M. (1999). Changes in aldolase activity in wild-type potato plants are important for acclimation to growth irradiance and carbon dioxide concentration, because plastid aldolase exerts control over the ambient rate of photosynthesis across a range of growth conditions. Plant J, 17, 479–489.

Harrison EP, Willingham NM, Lloyd JC, Raines CA. (1998). Reduced sedoheptulose-1,7-bisphosphatase levels in transgenic tobacco lead to decreased photosynthetic capacity and altered carbohydrate accumulation. Planta, 204, 27–36.

Hatch MD, Kagawa T, Craig S. (1975). Subdivision of C_4-pathway species based on differing C_4 acid decarboxylating systems and ultrastructural features. Aust J Plant Physiol, 2, 111–128.

Hatch MD. (1987). C_4 photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. Biochim Biophy Acta, 895, 81–106.

Häusler RE, Hirsch HJ, Kreuzaler F, Peterhänsel C. (2002). Overexpression of C_4-cycle enzymes in transgenic C_3 plants: a biotechnological approach to improve C_3-photosynthesis. J Exp Bot, 53, 591–607.

Häusler RE, Kleines M, Uhrig H, Hirsch HJ, Smets H. (1999). Overexpression of phosphoenolpyruvate carboxylase from *Corynebacterium glutamicum* lowers the CO_2 compensation point (Γ*) and enhances dark and light respiration in transgenic potato. J Exp Bot, 50, 1231–1242.

Häusler RE, Rademacher T, Li J, Lipka V, Fischer KL, Schubert S, Kreuzaler F, Hirsch HJ. (2001). Single and double overexpression of C_4-cycle genes had differential effects on the pattern of endogenous enzymes, attenuation of photorespiration and on contents of UV protectants in transgenic potato and tobacco plants. J Exp Bot, 52, 1785–1803.

Heineke D, Bykova N, Gardestrom P, Bauwe H. (2001). Metabolic response of potato plants to an antisense reduction of the P-protein of glycine decarboxylase. Planta, 212, 880–887.

Heldt HW. (2005). Plant Biochemistry (3rd ed). San. Diego, CA: Elsevier Academic Press.

Henkes S, Sonnewald U, Badur R, Flachmann R, Stitt M. (2001). A small decrease of plastid transketolase activity in antisense tobacco transformants has dramatic effects on photosynthesis and phenylpropanoid metabolism. Plant Cell, 13, 535–551.

Hiei Y, Ohta S, Komari T, Kumashiro T. (1994). Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J, 6, 271–282.

Hill PW, Marshall C, Williams GG, Blum H, Harmens H, Jones DL, Farrar JF. (2007). The fate of photosynthetically-fixed carbon in *Lolium perenne* grassland as modified by elevated CO_2 and sward management. New Phytol 173, 766–777.

Hobbs SLA. (1986). Relationships between carbon dioxide exchange rate, photosynthetic area and biomass in pea. Can J Plant Sci, 66, 465–472.

Holaday AS, Bowes G. (1980). C_4 acid metabolism and dark CO_2 fixation in a submerged aquatic macrophyte (*Hydrilla verticillata*). Plant Physiol, 65, 331–335.

Hudspeth RL, Grula JW, Dai Z, Edwards GE, Ku MSB. (1992). Expression of maize phosphoenolpyruvate carboxylase in transgenic tobacco. Effects on biochemistry and physiology. Plant Physiol, 98, 458–464.

IPCC. (2001). Climate change 2001: the scientific basis. Intergovernment panel on climate change. Cambridge, UK: Cambridge University Press.

IPCC. (2007). Climate change 2007: climate change impacts, adaptation and vulnerability. Working Group II. Geneva, Switzerland: IPCC.

Isebrands JG, Ceulemans R, Wiard B. (1988). Genetic variation in photosynthetic traits among *Populus* clones in relation to yield. Plant Physiol Biochem, 26, 427–437.

Ishimaru K, Ohkawa Y, Ishige T, Tobias DJ, Ohsugi R. (1998). Elevated pyruvate, orthophosphate dikinase (PPDK) activity alters carbon metabolism in C_3 transgenic potato with a C_4 maize PPDK gene. Physiol Plantarum, 103, 340–346.

Izui K, Ishijima S, Yamagushi Y, Katagiri F, Murata T, Shigesada K, Sugiyama T, Katsuki H. (1986). Cloning and sequence analysis of cDNA encoding active phosphoenolpyruvate carboxylase of the C_4-pathway of maize. Nucl Acids Res, 14, 1615–1628.

Jiao D, Li X, Huang X, Chi W, Kuang T, Ku MSB. (2001). The characteristics of CO_2 assimilation of photosynthesis and chlorophyll fluorescence in transgenic PEPC rice. Chinese Sci Bull, 46, 1080–1084.

Jiao DM. (2008). Redesigning C_4 rice from limited rice C_4 photosynthesis. In: Sheehy JE, Mitchell PL, Hardy B, ed. Charting new pathways to C_4 rice. Singapore: World Scientific Publishing Co. Pte. Ltd., 145–162.

Karami O. (2008). Factors affecting *Agrobacterium*-mediated transformation of plants. Transgenic Plant J, 3, 127–137.

Kerr RA. (2007). Scientists tell policy makers we're all warming the world. Science, 315, 754–757.

Kershanskaya OI, Teixeira da Silva JA. (2010). Photosynthetic basis for wheat crop improvement: Genetic modification of photosynthesis. In: Yerlan T, Ed. Khazakistan Plant Science and Biotechnology. Asian Austral J Plant Sci Biotech, 4 (Special Issue 1), 27–34.

Kintisch E. (2007). New congress may be warming up to plans for capping emissions. Science, 315, 444.

Kluger J. (2007). Global warming: what now? Our feverish planet badly needs a cure. Time Mag, 9, 50–109.

Krapp A, Chaves MM, David MM, Rodriques ML, Pereira JS, Stitt M. (1994). Decreased ribulose-1,5-bisphosphate carboxylase/oxygenase in transgenic tobacco transformed with antisense rbcS. 8. Impact on photosynthesis and growth in tobacco growing under extreme high irradiance and high temperature. Plant Cell Environ, 17, 945–953.

Ku MSB, Agarie S, Nomura M, Fukayama H, Tsuchida H, Ono K, Hirose S, Toki S, Miyao M, Matsuoka M. (1999). High-level expression of maize phosphoenolpyruvate carboxylase in transgenic rice plants. Nat Biotechnol, 17, 76–80.

Ku MSB, Cho D, Ranade U, Hsu T-P, Li X, Jiao D-M, Ehleringer J, Miyao M, Matsuoka M. (2000). Photosynthetic performance of transgenic rice plants overexpressing maize C_4 photosynthesis enzymes. In: Sheehy JE, Mitchell PL, Hardy B, eds. Redesigning rice photosynthesis to increase yield. Philippines, and Amsterdam: Elsevier, 193–204.

Ku MSB, Cho DH, Li X, Jiao DM, Pinto M, Miyao M, Matsuoka M. (2001). Introduction of genes encoding C_4 photosynthesis enzymes into rice plants: physiological consequences. In: Goode JA, Chadwich DC, ed. Rice Biotechnology: Improving Yield, Stress Tolerance and Grain Quality. NY, USA: John Wiley & Sons, 100–116.

Ku MSB, Kano-Murakami Y, Matsuoka M. (1996). Evolution and expression of C_4 photosynthesis genes. Plant Physiol, 111, 949–957.

Kumar A, Li C, Portis Jr AR. (2009). Arabidopsis thaliana expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. Photosynth Res, 100, 143–153.

Kurek I, Chang TK, Bertain SM, Madrigal A, Liu L, Lassner MW, Zhu G. (2007). Enhanced thermostability of Arabidopsis Rubisco activase improves photosynthesis and growth rates under moderate heat stress. Plant Cell, 19, 3230–3241.

Lal R. (2004) Soil carbon sequestration impacts on global climate change and food security. Science, 304, 1624–1627.

Lal R. (2008). Carbon sequestration. Phil Trans R Soc B, 363, 815–830.

Lal R. (2009). Challenges and opportunities in soil organic matter research. Eur J Soil Sci, 60, 158–160.

Lashof D, Hare B. (1999). The role of biotic carbon stocks in stabilizing greenhouse gas concentrations at safe levels. Environ Sci Policy, 2, 101–109.

Leegood RC, Lea PJ, Adcock MD, Haeusler RE. (1995). The regulation and control of photorespiration. J Exp Bot, 46, 1397–1414.

Leegood RC. (2002). C_4 photosynthesis: principles of CO_2 concentration and prospects for its introduction into C_3 plants. J Exp Bot, 53, 581–590.

Lefebvre S, Lawson T, Zakhleniuk OV, Lloyd JC, Raines CA. (2005). Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. Plant Physiol, 138, 451–460.

Lieman-Hurwitz J, Asipov L, Rachmilevitch S, Marcus Y, Kaplan A. (2005). Expression of cyanobacterial *ictB* in higher plants enhanced photosynthesis and growth. In: Omasa K, Nouchi I, de Kok LJ, eds. Plant Responses to Air Pollution and Global Change. New York: Springer Verlag Tokyo Berlin Heidelberg, 133–139.

Lieman-Hurwitz J, Rachmilevitch S, Mittler R, Marcus Y, Kaplan A. (2003). Enhanced photosynthesis and growth of transgenic plants that express *ictB*, a gene involved in HCO_3–accumulation in cyanobacteria. Plant Biotechnol, J 1, 43–50.

Lipka V, Häusler RE, Rademacher T, Li J, Hirsch H-J, Kreuzaler F. (1999). Solanum tuberosum double transgenic expressing phosphoenolpyruvate carboxylase and NADP-malic enzyme display reduced electron requirement for CO_2 fixation. Plant Sci, 144, 93–105.

Long SP, Ainsworth EA, Rogers A, Ort DR. (2004). Rising atmospheric carbon dioxide: plants face the future. Ann Rev Plant Biol, 55, 591–628.

Luo HY. (2001). Comparison on C_3, C_4 and CAM plants. J Higher Correspondence Education (Nat Sci), 14, 35–38 (in Chinese).

Lutts S, Lefèvre I, Delpérée C, Kivits S, Dechamps C, Robledo A, Correal E. (2004). Heavy metal accumulation by the halophyte species Mediterranean saltbush. J Environ Qual, 33, 1271–1279.

Magnin NC, Cooley BA, Reiskind JB, Bowes G. (1997). Regulation and localization of key enzymes during the induction of Kranz-less, C_4-type photosynthesis in *Hydrilla verticillata*. Plant Physiol, 115, 1681–1689.

Makino A, Nakano H, Mae T, Shimada T, Yamamoto N. (2000). Photosynthesis, plant growth and N allocation in transgenic rice plants with decreased Rubisco under CO_2 enrichment. J Exp Bot, 51, 383–389.

Mann CC. (1999). Genetic engineers aim to soup up crop photosynthesis. Science, 283, 314–316.

Masle J, Hudson GS, Badger MR. (1993). Effects of ambient CO_2 concentration on growth and nitrogen use in tobacco (Nicotiana tabacum) plants transformed with an antisense gene to the small-subunit of ribulose-1,5-bisphosphate carboxylase oxygenase. Plant Physiol, 103, 1075–1088.

Matsumura I., Patel M., Greene D. (2005). Directed evolution of Rubisco through genetic selections of metabolically engineered *Escherichia coli*. FASEB J, 19, A292–A292.

Matsuoka M, Fukayama H, Tsuchida H, Nomura M, Agarie S, Ku MSB, Miyao M. (2000). How to express some C_4 photosynthesis genes at high levels in rice. Stud Plant Sci, 7, 167–175.

Matsuoka M, Furbank RT, Fukayama H, Miyao M. (2001). Molecular engineering of C_4 photosynthesis. Ann Rev Plant Physiol Plant Mol Biol, 52: 297–314.

Meister M, Agostino A, Hatch MD. (1996). The roles of malate and aspartate in C_4 photosynthetic metabolism in *Flaveria bidentis* (L.). Planta, 199, 262–269.

Mikkelsen M, Jorgensen M, Krebs FC. (2010). The teraton challenge. A review of fixation and transformation of carbon dioxide. Energy Environ Sci, 3, 43–81.

Miyagawa Y, Tamoi M, Shigeoka S. (2001). Overexpression of a cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase in tobacco enhances photosynthesis and growth. Nat Biotechnol, 19, 965–969.

Miyao M. (2003). Molecular evolution and genetic engineering of C_4 photosynthetic enzymes. J Exp Bot 54, 179–189.

Monson RK, Rawsthorne S. (2000). CO_2 assimilation in C_3–C_4 intermediate plants. In: Leegood RC, Sharkey TD, von Caemmerer S, eds. Photosynthesis: physiology and metabolism. Advances in photosynthesis, Vol. 9. Dordrecht: Kluwer Academic Publishers, 533–550.

Monson RK. (1999). The origins C_4 genes and evolutionary pattern in the C_4 metabolic phenotype. In: Sage RF, Monson RK, eds. C_4 plant biology. San Diego: Academic Press, 377–410.

Nelson CJ. (1988). Genetic associations between photosynthetic characteristics and yield: review of the evidence. Plant Physiol Biochem, 26, 543–554.

Nobel PS. (1991). Achievable productivities of certain CAM plants: basis for high values compared with C_3 and C_4 plants. New Phytol, 119, 183–205.

Nowak R, Ellsworth DS, Smith SD. (2004). Functional responses of plants to elevated atmospheric CO_2-do photosynthetic and productivity data from FACE experiments support early predictions? New Phytol, 162, 253–280.

O'Leary MH. (1982). Phosphoenolpyruvate carboxylase: an enzymologist's view. Ann Rev Plant Physiol, 33, 297–315.

Ogren WL. (1984) Photorespiration – pathways, regulation and modification. Ann Rev Plant Physiol Mol Biol, 35, 415–442.

Osmond CB, Grace SC. (1995). Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reactions of photosynthesis? J Exp Bot, 46, 1351–1362.

Osmond CB. (1978). Crassulacean acid metabolism: curiosity in context. Ann Rev Plant Physiol Plant Mol Biol, 29, 379–414.

Pan RZ. (2008). Plant Physiology (6 rd). Beijing: Higher Education Press.

Parry MAJ, Andralojc PJ, Mitchell RAC, Madgwick PJ, Keys AJ. (2003). Manipulation of Rubisco: the amount, activity, function and regulation. J Exp Bot, 54, 1321–1333.

Parry MAJ, Madgwick PJ, Carvalho JFC, Andralojc PJ. (2007). Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. J Agric Sci, 145, 31–43.

Peterhänsel C, Hirsch HJ, Kreazaaler F. (2008b). Overexpression of C_4 pathway genes in the C_3 dicots potato, tobacco, and *Arabidopsis*: experiences and future challenges. In: Sheehy JE, Mitchell PL, Hardy B, eds. Charting new pathways to C_4 rice. Singapore: World Scientific Publishing Co. Pte. Ltd., 163–170.

Peterhänsel C, Niessen M, Kebeish RM. (2008a). Metabolic engineering towards the enhancement of photosynthesis. Photochem Photobiol, 84, 1317–323.

Poetsch W, Hermans J, Westhoff P. (1991). Multiple cDNAs of phosphoenolpyruvate carboxylase in the C_4 dicot *Flaveria trinervia*. FEBS Lett, 292, 133–136.

Prendergast HDV, Hattersley PW, Stone NE. (1987). New structural/biochemical associations in leaf blades of C_4 grasses (Poaceae). Aust J Plant Physiol, 14, 403–420.

Raines CA. (2003). The Calvin cycle revisited. Photosynth Res, 75, 1–10.

Raines CA. (2006). Transgenic approaches to manipulate the environmental responses of the C_3 carbon fixation cycle. Plant Cell Environ, 29, 331–339.

Repo E, Hatch MD. (1976). Photosynthesis in *Gomphrena celosioides* and its classification among C_4 pathway plants. Aust J Plant Physiol, 3, 863–876.

Rozema J, Flowers T. (2008). Crops for a salinized world. Science, 322, 1478–1480.

Ruan CJ, Li H, Guo YQ, Qin P, Gallagher JL, Seliskar DM, Lutts S, Mahy G. (2008). *Kosteletzkya virginica*, an agroecoengineering halophytic species for alternative agricultural production in China's east coast: ecological adaptation and benefits, seed yield, oil content, fatty acid and biodiesel properties. Ecol Eng, 32, 320–328.

Running SM. (2006). Is global warming causing more large wildfires? Science, 313, 927–928.

Sage RF, Pearcy RW. (1987). The nitrogen use efficiency of C_3 and C_4 plants. I. leaf nitrogen, growth and biomass partitioning in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). Plant Physiol, 84, 954–958.

Sage RF. (2004). The evolution of C_4 photosynthesis. New Phytol, 161, 341–370.

Salvucci ME, Bowes G. (1981). Induction of reduced photorespiratory activity in submerged and amphibious aquatic macrophytes. Plant Physiol, 67, 335–340.

Salvucci ME, Bowes G. (1983). Two photosynthetic mechanisms mediating the low photorespiratory state in submerged aquatic angiosperm. Plant Physiol, 73, 488–496.

Secor J, McCarty DR, Shibles R, Green DE. (1982). Variability and selection for leaf photosynthesis in advanced generations of soybeans. Crop Sci, 22, 255–259.

Sharkey TD. (1988). Estimating the rate of photorespiration in leaves. Physiol Plantarum, 73, 147–152.

Sheehy JE, Mitchell PL, Hardy B. (2000). Redesigning rice photosynthesis to increase yield. Philippines: International Rice Research Institute, Amsterdam: Elsevier.

Sheriff A, Meyer H, Riedel E, Schmitt JM, Lapke C. (1998). The influence of plant pyruvate, orthophosphate dikinase on a C_3 plant with respect to the intracellular location of the enzyme. Plant Sci, 136, 43–57.

Singh R, Malhotra SP. (2000). Carbon fixation, sucrose synthesis and its transport to storage tissues. Dev Crop Sci, 26, 1–34.

Smith AL. (1997). Oxford dictionary of biochemistry and molecular biology. Oxford: Oxford University Press.

Spencer WE, Wetzel RG, Teeri J. (1996). Photosynthetic phenotype plasticity and the role of phosphoenolpyruvate carboxylase in *Hydrilla verticillata*. Plant Sci, 118, 1–9.

Spreitzer RJ, Salvucci ME. (2002). Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. Ann Rev Plant Biol, 53, 449–475.

Stemmer WPC. (1994). Rapid evolution of a protein *in-vitro* by DNA shuffling. Nature, 370, 389–391.

Stitt M, Schulze D. (1994). Does Rubisco control the rate of photosynthesis and plant growth – an exercise in molecular ecophysiology. Plant Cell Environ, 17, 465–487.

Streatfield SJ, Weber A, Kinsman EA, Häusler RE, Li J, Post-Beitenmiller D, Kaiser WM, Pyke KA, Flügge UI, Chory J. (1999). The phosphoenolpyruvate/phosphate translocator is required for phenolic metabolism, palisade cell development and plastid-dependent nuclear gene expression. Plant Cell, 11, 1609–1621.

Sun JD, Yang LX, Wang YL, Ort DR. (2009). Facing the global change: Opportunities for improvement in photosynthetic radiation use efficiency and crop yield. Plant Sci, 177, 511–522.

Surridge C. 2002. The rice squad. Nature, 416, 576–578.

Suzuki S, Murai N, Burnell JN, Arai M. (2000). Changes in photosynthetic carbon flow in transgenic rice plants that express C_4-type phosphoenolpyruvate carboxykinase from *Urochloa panicoides*. Plant Physiol, 124, 163–172.

Suzuki S, Murai N, Kasaoka K, Hiyoshi T, Imaseki H, Burnell JN, Arai M. (2006). Carbon metabolism in transgenic rice plants that express phosphoenolpyruvate carboxylase and/or phosphoenolpyruvate carboxykinase. Plant Sci, 170, 1010–1019.

Suzuki Y, Miyamoto T, Yoshizawa R, Mae T, Makino A. (2009). Rubisco content and photosynthesis of leaves at different positions in transgenic rice with an overexpression of RBCS. Plant Cell Environ, 32, 417–427.

Svensson P, Bläsing O, Westhoff P. (1997). Evolution of the enzymatic characteristics of C_4 phosphoenolpyruvate carboxylase. A comparison of the orthologous PPCA phosphoenolpyruvate carboxylases of *Flaveria trinervia* (C_4) and *Flaveria pringlei* (C_3). Eur J Biochem, 246, 452–460.

Taiz L, Zeiger E. (2006). Plant Physiology (4th ed.). Sunderland MA: Sinauer Associates Inc.

Takeuchi K, Akagi H, Kamasawa N, Osumi M, Honda H. (2000). Aberrant chloroplasts in transgenic rice plants expressing a high level of maize NADP-dependent malic enzyme. Planta 211, 265–274.

Tamoi M, Shigeoka S. (2005). Improvement of photosynthesis in higher plants. In: Omasa K, Nouchi I, DeKok LJ, ed. Plant Responses to Air Pollution and Global Change. New York: Springer-Verlag, 141–147.

Taniguchi Y, Ohkawa H, Masumoto C, Fukuda T, Tamai T, Lee K, Sudoh S, Tsuchida H, Sasaki H, Fukayama H, Miyao M. (2008). Overproduction of C_4 photosynthetic enzymes in transgenic rice plants: an approach to introduce the C4-like photosynthetic pathway into rice. J Exp Bot, 59, 1799–1809.

Tsuchida H, Tamai T, Fukayama H, Agarie S, Nomura M, Onodera H, Ono K, Nishizawa Y, Lee BH, Hirose S, Toki S, Ku MSB, Matsuoka M, Miyao M. (2001). High level expression of C_4-specific NADP–malic enzyme in leaves and impairment of photoautotrophic growth in a C_3 plant, rice. Plant Cell Physiol, 42, 138–145.

von Caemmerer S, Furbank RT. (2003). The C_4 pathway: an efficient CO_2 pump. Photosynth Res, 77, 191–207.

Wang JM, Li RZ. (2008). Integration of C_4-specific ppdk gene of *Echinochloa* to C_3 upland rice and its photosynthesis characteristics analysis. Afr J Biotechnol, 7, 783–787.

Westerling AL, Hidalgo HG, Cayan DR, Swetnam TW. (2006). Warming and earlier spring increase western U.S. forest wildfire activity. Science, 313, 940–943.

Whitney SM, Baldet P, Hudson GS, Andrews TJ. (2001). Form 1 Rubiscos from non-green algae are expressed abundantly but not assembled in tobacco chloroplasts. Plant J, 26, 535–547.

Wild B, Wanek W, Postl W, Richter A. (2010). Contribution of carbon fixed by Rubisco and PEPC to phloem export in the Crassulacean acid metabolism plant *Kalanchoe daigremontiana*. J Exp Bot, 61, 1375–1383.

Wingler A, Walker RP, Chen ZH, Leegood RC. (1999). Phosphoenolpyruvate carboxykinase is involved in the decarboxylation of aspartate in the bundle sheath of maize. Plant Physiol, 120, 539–545.

Winter K, Smith JAC. (1996). An introduction to Crassulacean acid metabolism. Biochemical principles and ecological diversity. In: Winter K, Smith JAC, eds. Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution. Berlin: Springer, 1–13.

Winzer T, Heineke D, Bauwe H. (2001). Growth and phenotype of potato plants expressing an antisense gene of P-protein of glycine decarboxylase under control of a promoter with preference for the mesophyll. Ann Appl Biol, 138, 9–15.

Yuan DY, Duan MJ, Tan YN, Yi ZL, Yuan LP, Sun SS. (2007). Generating marker-free transgenic homozygous rice restorer lines with PEPC and PPDK genes by *Agrobacterium*-mediated transformation using super binary vector. Hybrid Rice, 22, 57–63 (in Chinese with English abstract).

Zerai DB, Glenn EP, Chatervedi R, Lu Z, Mamood AN, Nelson SG, Ray DT. (2010). Potential for improvement of *Salicornia bigelovii* through selective breeding. Ecol Eng, 36, 730–739.

Zhang BJ, Hua C, Zhou F, Zhou QC, Chen QZ, Wang RF, Jiao DM. (2008). Photosynthetic characteristics of transgenic rice with PEPC+PPDK gene. Sci Agric Sin, 41, 3008–3014 (in Chinese with English abstract).

Zhang JF, Bandyopadhyay A, Sellappan K, Wang GY, Xie HA, Datta K, Datta SK. (2010). Characterization of a C_4 maize pyruvate orthophosphate dikinase expressed in C_3 transgenic rice plants. Afr J Biotechnol, 9, 234–242.