PLANT PHYSIOLOGY
Dark Reactions in C3 Plants

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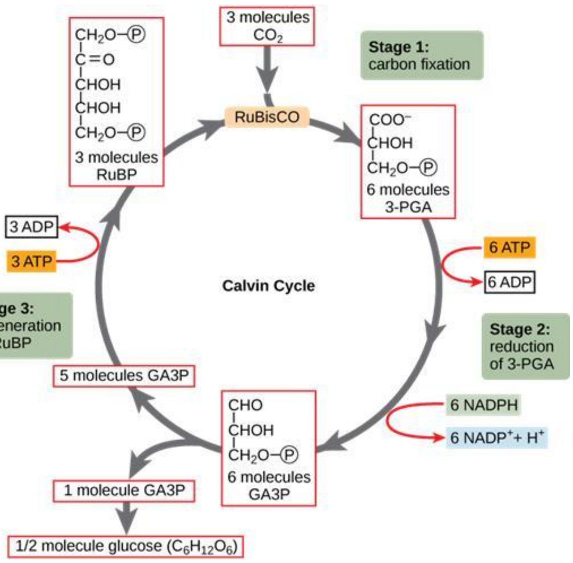
SELF-PROPAGATING ENTREPRENEURIAL EDUCATION DEVELOPMENT



Siklus Calvin (Calvin cycle) adalah lintasan utama redukti CO_2 menjadi karbohidrat pada tanaman yang dapat dibagi tiga tahapan reaksi: karboxilasi (fixasi CO_2), Reduksi (3-PGA), dan Regenerasi ribulose bisphosphate (RuBP). Ini membutuhkan energy (ATP) dab reduktan (NADPH) hasil reaksi terang.

LECTURE OUTCOMES

After the completion of this lecture and mastering the lecture materials, students should be able;

1. To explain the mechanism of CO_2 assimilation to be carbohydrate (sugars) in C3 Plants
2. To explain the diffusion of CO_2 from the atmosphere into the site of CO_2 assimilation in the chloroplasts
3. To explain reactions, enzymes and products involved in the reduction of CO_2 to be carbohydrate in C3 Plants

LECTURE OUTLINE

1. INTRODUCTION Definition CO_2 Diffusion 2. CALVIN CYCLE Discovery Calvin Cycle Stages Labelling Study	CO_2 Fixation Cost Light and CO_2 Compensation Point 3. PHOTORESPIRATION Rubisco Actvities Energetic Consequence of Photorespiration

1. INTRODUCTION

1.1 Definition

1. Dark reactions are reactions that convert inorganic CO_2 into carbohydrate and are not dependent directly on light and so may occur during the night and during the day.
2. However, the reactions are driven by NADPH and ATP which are the products of light reactions, and hence the name of carbon reactions of photosynthesis is suggested.
3. The solar energy (ca. 3 x 10^{21} J/year) is converted via endergonic reactions in plants into carbohydrates (ca. 2 x 10^{11} tonnes of carbon/year).

1.2 CO_2 Diffusion

1. Sufficient CO_2 surround the site of CO_2 fixation in the chloroplasts must be available to sustain the CO_2 assimilation.
2. The liquid phase of chloroplasts, stoma, is the site of CO_2 assimilation to be carbohydrate.
3. As the atmosphere is the source CO_2, it must be transported through diffusion process into the chloroplasts.
4. The process of CO_2 diffusion from the atmosphere into chloroplast has to overcome a series of resistances which are generally divided into boundary layer resistance (r_a), stomatal resistance (r_s) and mesophyll resistance (r_m).
5. Carbon dioxide diffuses through the pore into the substomatal cavity and into the intercellular spaces between mesophyll cells.
6. This portion of the diffusion path of CO_2 into the chloroplast is a gaseous phase.
7. The remainder of the diffusion path to the chloroplast is a liquid phase, which begins at the water layer that wets the walls of the mesophyll cells and continue through the plasma membrane, the cytosol, and the chloroplast (**Fig. 2.19**).
   - In air of high relative humidity, the diffusion gradient that drives water loss is about 50 times larger than the gradient that drives CO_2 uptake. In drier air, this difference can be even larger. Therefore, a decrease in stomatal resistance through the opening of stomata facilitates higher CO_2 uptake but is unavoidably accompanied by substantial water loss.

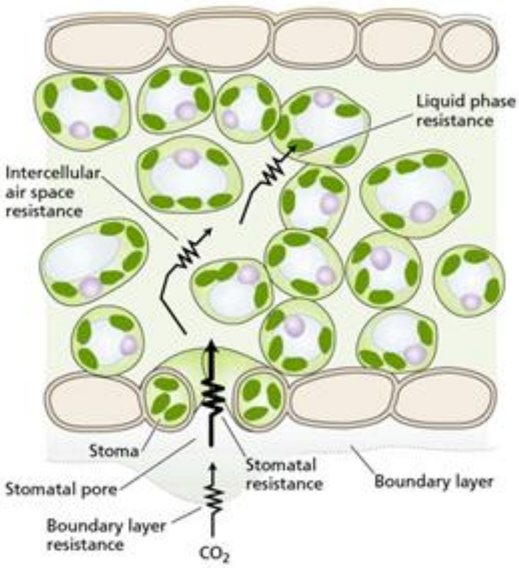
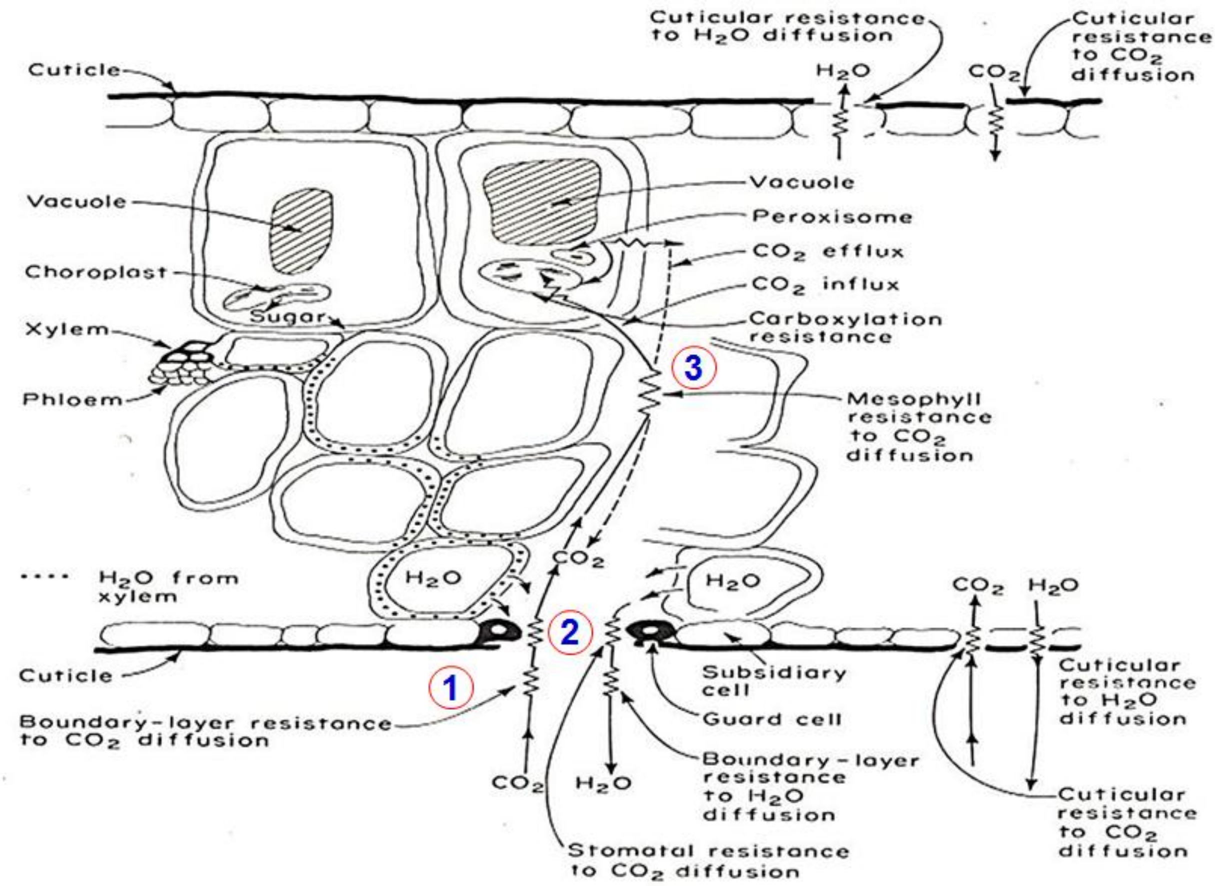


Fig. 2.19 Points of resistance to the diffusion of CO_2 from outside the leaf to the chloroplasts (*source: Taiz L., Zeiger E., 2010*)



Difusi CO_2

- Analisis Fluks CO_2 dengan pendekatan difusi

$$F = -D\frac{\partial C}{\partial x}$$

- Analisis Fluks CO_2 dengan pendekatan resisten (tahanan)

$$F = \frac{C_A - C_C}{r_m + r_s + r_a}$$

Ilustrasi

Soal : Diketahui r_m = 100 m.s^{-1}, r_a = 500 m.s^{-1} & r_s = 800 m.s^{-1}, dan [CO_2] = 400 μl.l^{-1} di atmosfir pada suhu 25^{0}C. Dalam khloroplast, [CO_2] dianggap nol. Berapakah flux CO_2 ke dalam khloroplast dari atmosfir

Jawab

Konversi satuan volume konsentrasi CO_2 perlu dilakukan ke satuan standar internasional (liter/m^3)

[CO_2] = 400 μl.l^{-1}= (400/10^6)*1000 liter.m^{-3} = 0,4 liter.m^{-3}

Satuan konsentrasi CO_2 diatas perlu juga dikonversi ke satuan massa seperti berikut:

$$[CO_2] = \frac{0.4}{[(273+25)/273]22{,}414} * 44 = 0{,}719 \text{ g.m}^{-3}$$

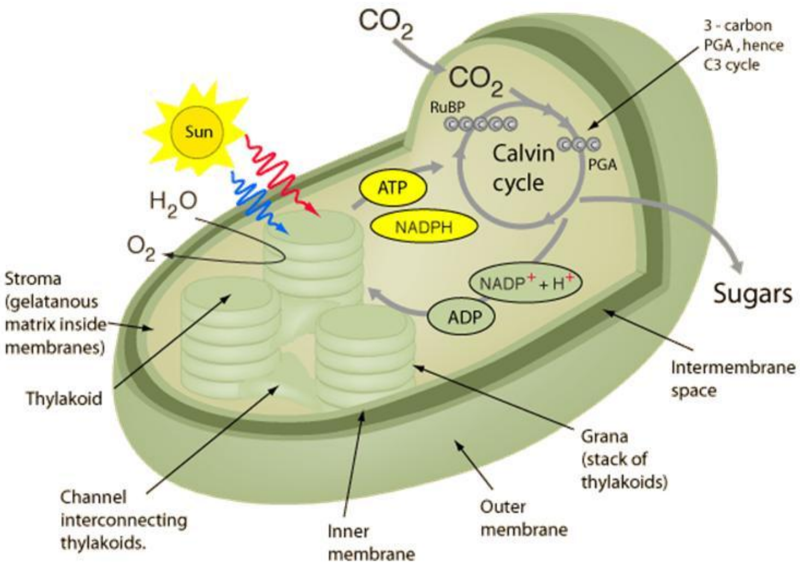
Konversi diatas melibatkan pengaruh suhu pada volume gas (1 mol gas menempati 22, 414 liter pada suhu/273^{0}K dan tekanan standard/1 atm)

$$F(CO_2) = \frac{(0{,}719-0)\text{g.m}^{-3}}{(100+500+800)\text{m.s}^{-1}} = 0{,}000514 \text{ g.m}^{-2}\text{s}^{-1} = 0{,}514 \text{ mg.m}^{-2}\text{s}^{-1}$$

2. CALVIN CYCLE

1. Discovery

1. The C_3 carbon reduction cycle (Calvin Cycle) is the primary pathway of carbon fixation in plants.
2. This cycle was elucidated in a series of elegant experiments by M. Calvin, A. Benson, J. A. Bassham, and their colleagues in the 1950s (Benson 1951).
3. It is found in many prokaryotes and in all photosynthetic eukaryotes, from the most primitive algae to the most advanced angiosperms.



2. Calvin Cycle Stages

1. The Calvin—Benson cycle proceeds in **three stages** that are highly coordinated in the chloroplast (Fig. 8.2)
   - ***Carboxylation*** of the CO_2 acceptor molecule. The first committed enzymatic step of the cycle is the reaction of CO_2 and water with ribulose 1,5-bisphosphate to generate two molecules of a 3-carbon intermediate (3-phosphoglycerate).
   - ***Reduction*** of 3-phosphoglycerate. The 3-phosphoglycerate is reduced to 3-carbon carbohydrates (triose phosphates) by two enzymatic reactions driven by photochemically generated ATP and NADPH.
   - ***Regeneration*** of the CO_2 acceptor ribulose 1,5-bisphosphate. The cycle is completed by regeneration of

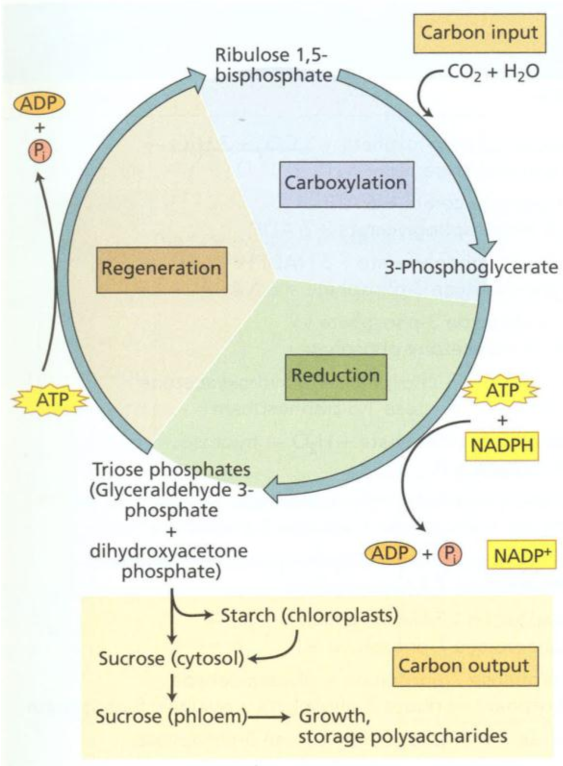


Fig. 8.2 The Calvin—Benson cycle proceeds in three stages

ribulose 1,5-bisphosphate through a series of ten enzyme-catalyzed reactions, one requiring ATP.

2. In the first step of the Calvin—Benson cycle, three molecules of CO_2 and three molecules of H_2O react with three molecules of ribulose 1,5-bisphosphate to yield six molecules of 3—phosphoglycerate.
   - This reaction is catalyzed by the chloroplast enzyme ribulose-1,5—bisphosphate carboxylase/oxygenase, referred to as **rubisco**.
3. In the first partial reaction, a H^+ is extracted from carbon 3 of ribulose 1,5-bisphosphate (Fig. 8.4).
4. The addition of gaseous CO_2 to the unstable rubisco-bound enediol intermediate drives the second partial reaction to the irreversible formation of 2-carboxy-3-ketoarabinitol 1,5-bisphosphate.
5. Finally, the hydration of the resulting intermediate yields two molecules of 3-phosphoglycerate.
6. The CO_2 from the atmosphere is bound at C2 of ribulose.

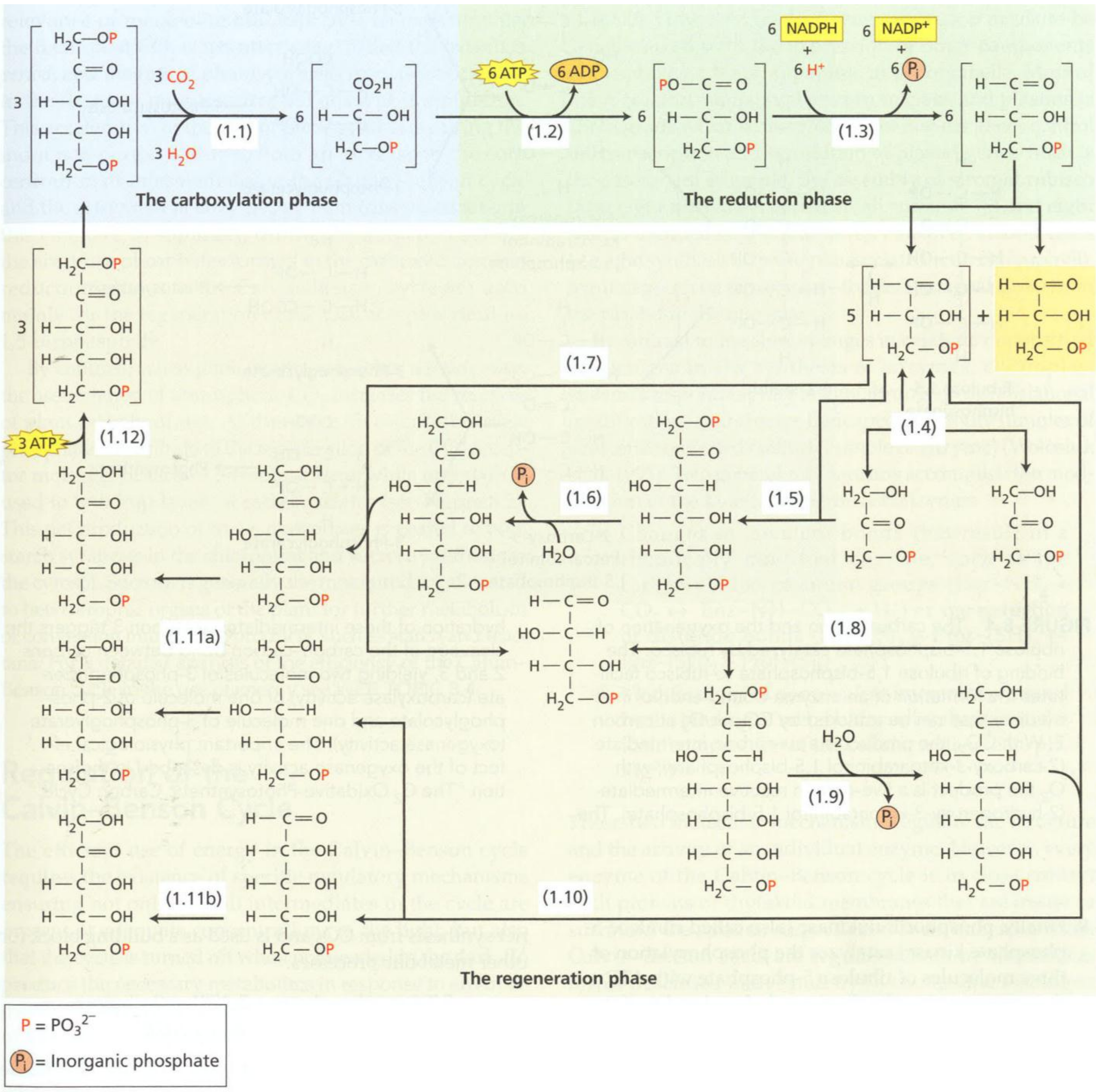


Fig. 8.3 The Calvin—Benson cycle

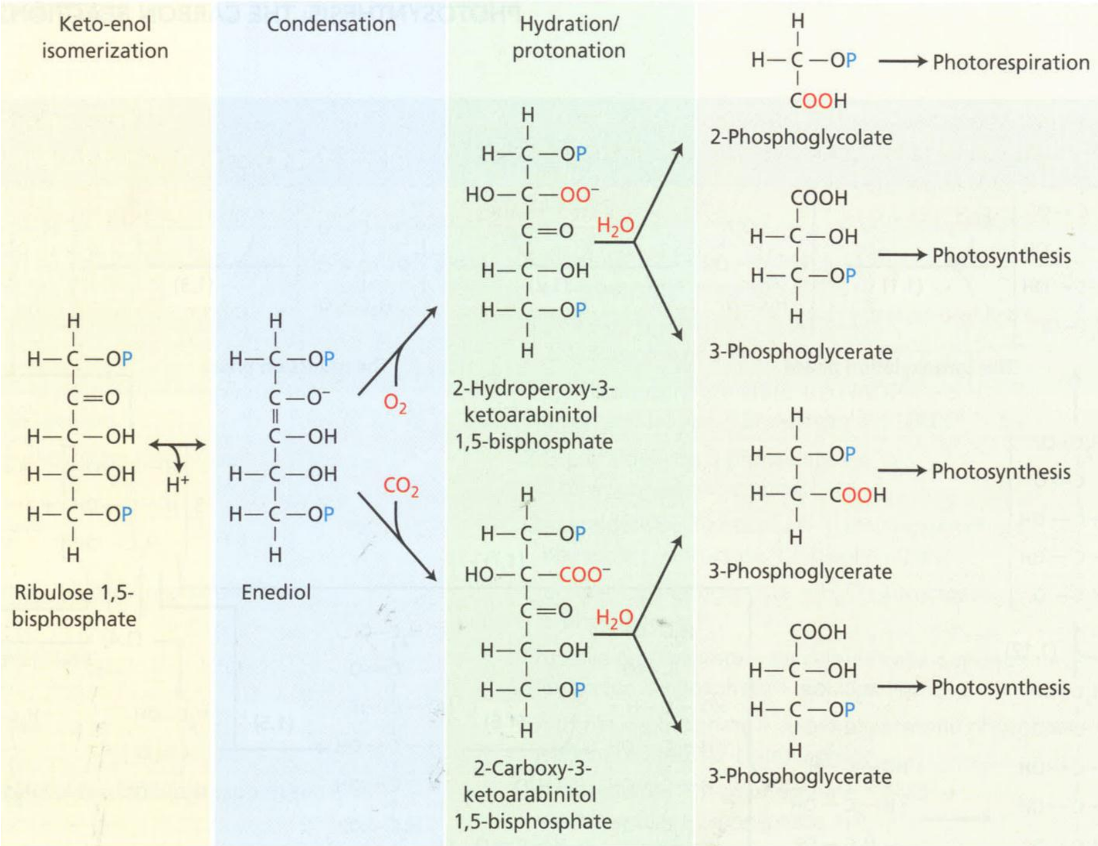
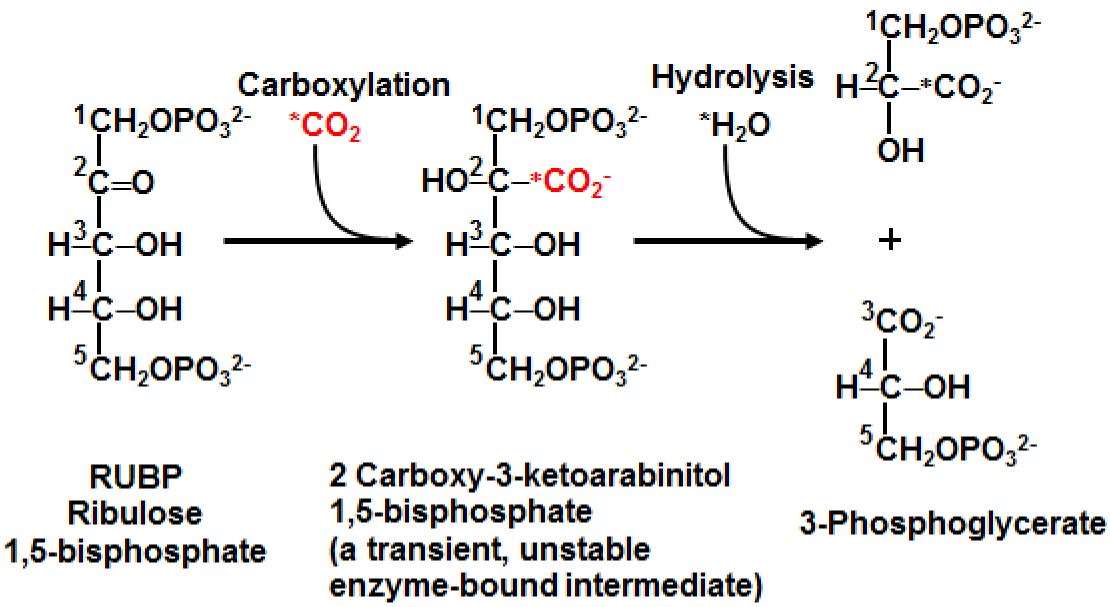


Fig. 8.4 The carboxylation and the oxygenation of ribulose 1,5-bisphosphate catalyzed by rubisco



3. Labelling Study

1. Labeling study of carbon compounds in the alga Chorella after exposure to $^{14}CO_2$ was used to determine the **compound formed in photosynthesis.**

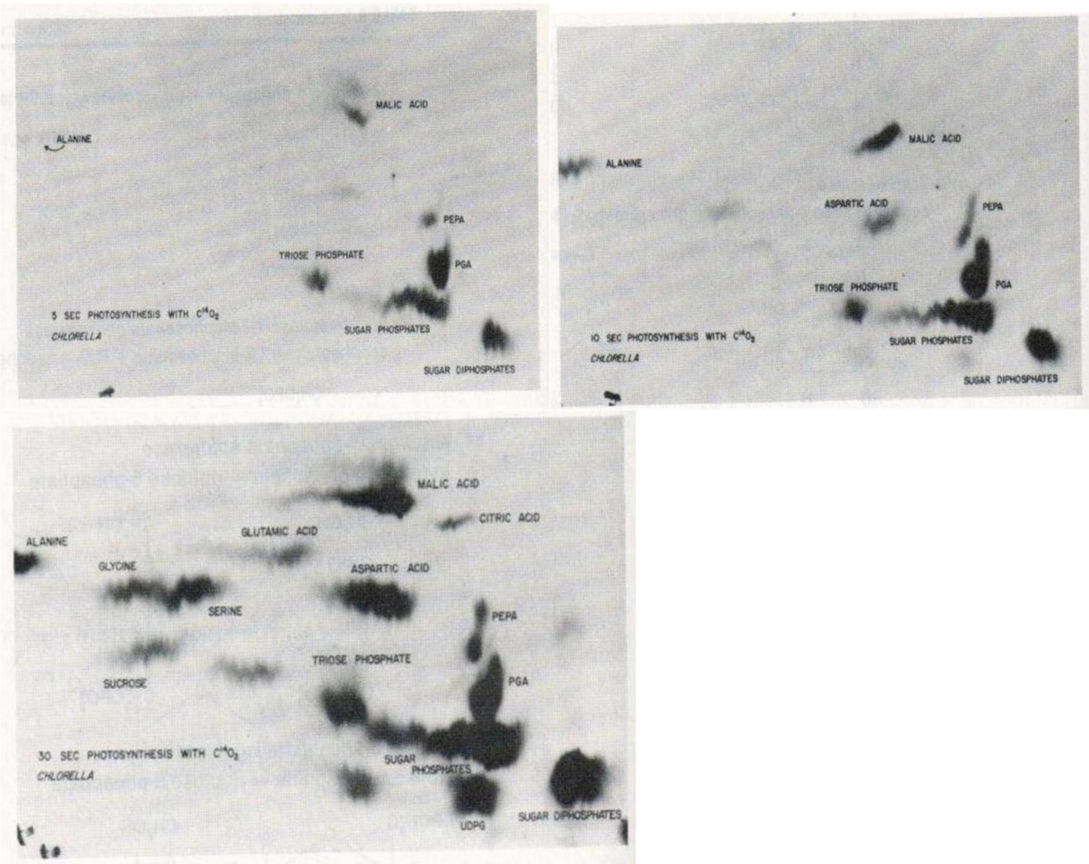
   - For instance, the $^{13}CO_2$ labeling of a soybean leaf using a compact-disc case as a labeling chamber.

- The labeling gas (21% O_2, either 200 or 300-ppm $^{13}CO_2$, and the balance N_2) entered at the bottom left through a copper pipe closed at the end and with multiple exit holes along the sides.
- At the end of the labeling period, the leaf was cut from its stem, immersed in liquid nitrogen, and subsequently lyophilized.

Labeling Study

21% O_2 + $^{13}CO_2$ (200 or 300-ppm) + balance N_2)

1. It was found that 3-phosphoglyceric acid (PGA) was the heavily labeled compound after shortest exposure.
2. This indicates that it is the first stable intermediate of the PCR cycle (From Bassham, 1965).



4. CO_2 Fixation Cost

6 (ribulose 1,5 bisP) + $6CO_2$ + $6H_2O$ → 12 (3-P-glycerate) + $12H^+$
12 (3-P-glycerate) + 12ATP → 12 (1,3-bisP-glycerate) + 12ADP
12 (1,3-bisP-glycerate) + 12 (NADPH + H^+) → 12 (triose-P) + 12 $NADP^+$ + 12 Pi
6 (triose-P) → 3 (fructose 1,6-bisP)
3 (fructose 1,6-bisP) + 3 H_2O → 3 (fructose 6-P) + 3 Pi
2 (fructose 6-P) + 2 (triose-P) → 2 (xylulose 5-P) + 2 (erythrose 4-P)
2 (erythrose 4-P) + 2 (triose-P) → 2 (sedoheptulose 1,7-bisP)
2 (sedoheptulose 1,7-bisP) + 2 (triose-P) → 2 (xylulose 5-P) + 2 (ribulose 5-P)
4 (xylulose 5-P) → 4 (ribulose 5-P)
2 (ribose 5-P) → 2 (ribulose 5-P)
6 (ribulose 5-P) + 6ATP → 6 (ribulose 1,5-bisP) + 6ADP

Net:

$6CO_2$ + $11H_2O$ + 12NADPH + 18ATP →
fructose 6-P + 12 $NADP^+$ + $6H^+$ + 18ADP + 17Pi

The above equation is divided by 6

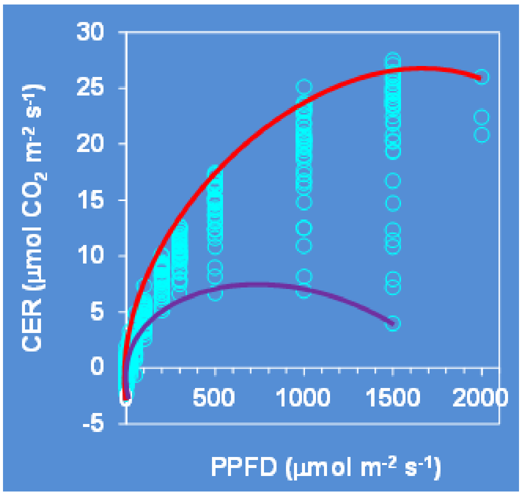
CO_2+(11/6)H_2O+2NADPH+3ATP→ 1/6 F 6-P+ 2$NADP^+$+H^++3ADP+(17/6)Pi

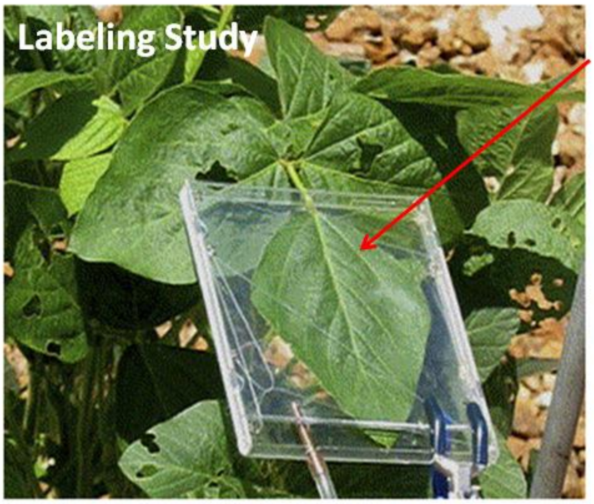
The reduction cost of 1 mol CO_2 : 2 mol NADPH + 3 mol ATP

5. Light and CO_2 Compensation Point

1. The rate of CO_2 assimilation, measured by carbon exchange rate (CER), is strongly influenced by light and the CO_2 concentration.
2. Light or CO_2 compensation point is the level of light or CO_2 at which the rate of CO_2 fixation is equal to that of respiration.

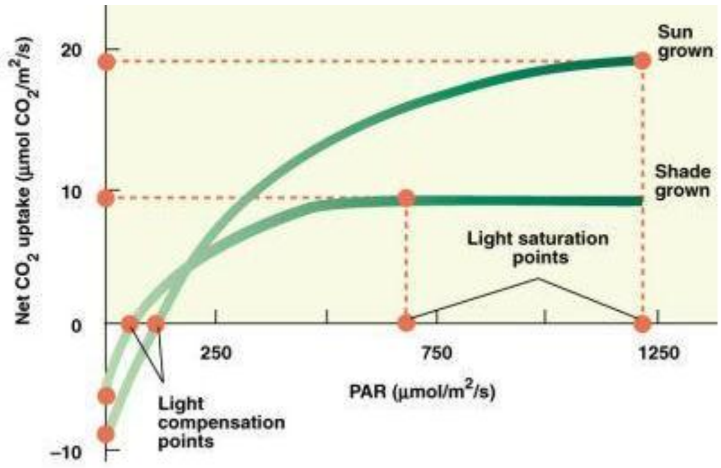
   This is CER of several soybean lines measured in Malang. PPFD = Photosynthetic Photon Flux Density. CER = CO_2 Exchange Rate (net photosynthesis)

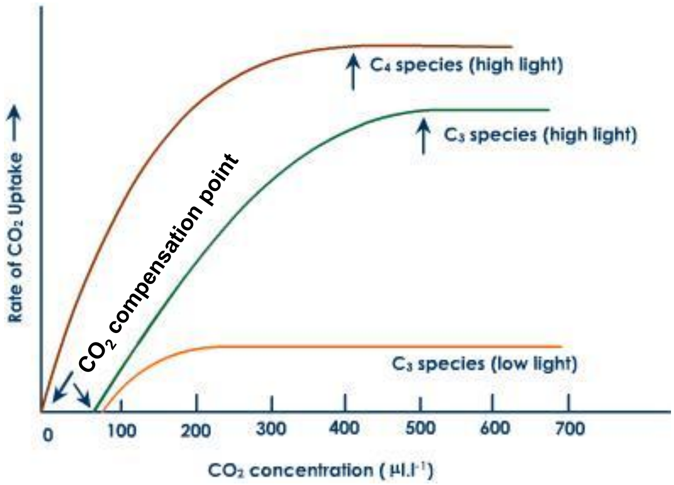


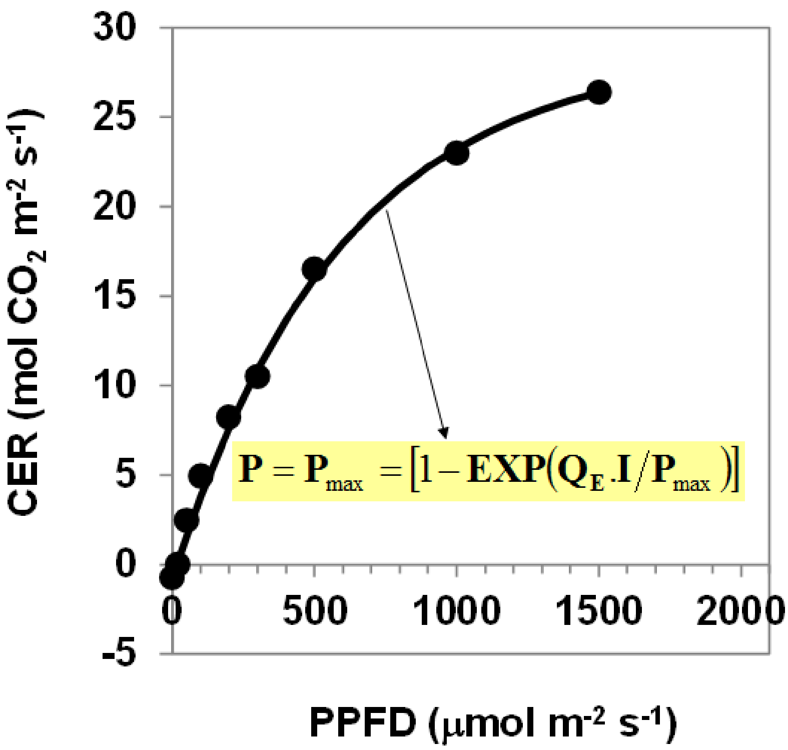


21% O_2 + $^{13}CO_2$ (200 or 300-ppm) + balance N_2)

The $^{13}CO_2$ labeling of a soybean leaf using a compact-disc case as a labeling chamber. The labeling gas (21% O_2, either 200 or 300-ppm $^{13}CO_2$, and the balance N_2) entered at the bottom left through a copper pipe closed at the end and with multiple exit holes along the sides. At the end of the labeling period, the leaf was cut from its stem, immersed in liquid nitrogen, and subsequently lyophilized.







Pmax = 29.5 μmol CO_2 m^{-2} s^{-1}
Q_E = 0.0494 μmol CO_2 m^{-2} s^{-1}/(μmol photon m^{-2} s^{-1})
Q_E = quantum efficiency

3. PHOTORESPIRATION

1. Rubisco Activities

1. Rubisco has the capacity to catalyze both the carboxylation and oxygenation of ribulose 1,5-bisphosphate (Miziorko and Lorimer 1983).
   Carboxylation: RUBP + $CO_2 \rightarrow$ 2 (3-phosphoglycerate)
   Oxygenation: RUBP + $O_2 \rightarrow$ 3-phosphoglycerate + 2-phosphoglycolate
2. Carboxylation yields two molecules of 3-phosphoglycerate, while oxygenation ("Catalysis" in Fig. 8.5) produces one molecule each of 3-phosphoglycerate and 2-phosphoglycolate ("Products" in Figure 8.5).
3. The oxygenation of ribulose 1,5-bisphosphate catalyzed by rubisco initiates a coordinated network of enzymatic reactions that are compartmentalized in chloroplasts, leaf peroxisomes, and mitochondria.

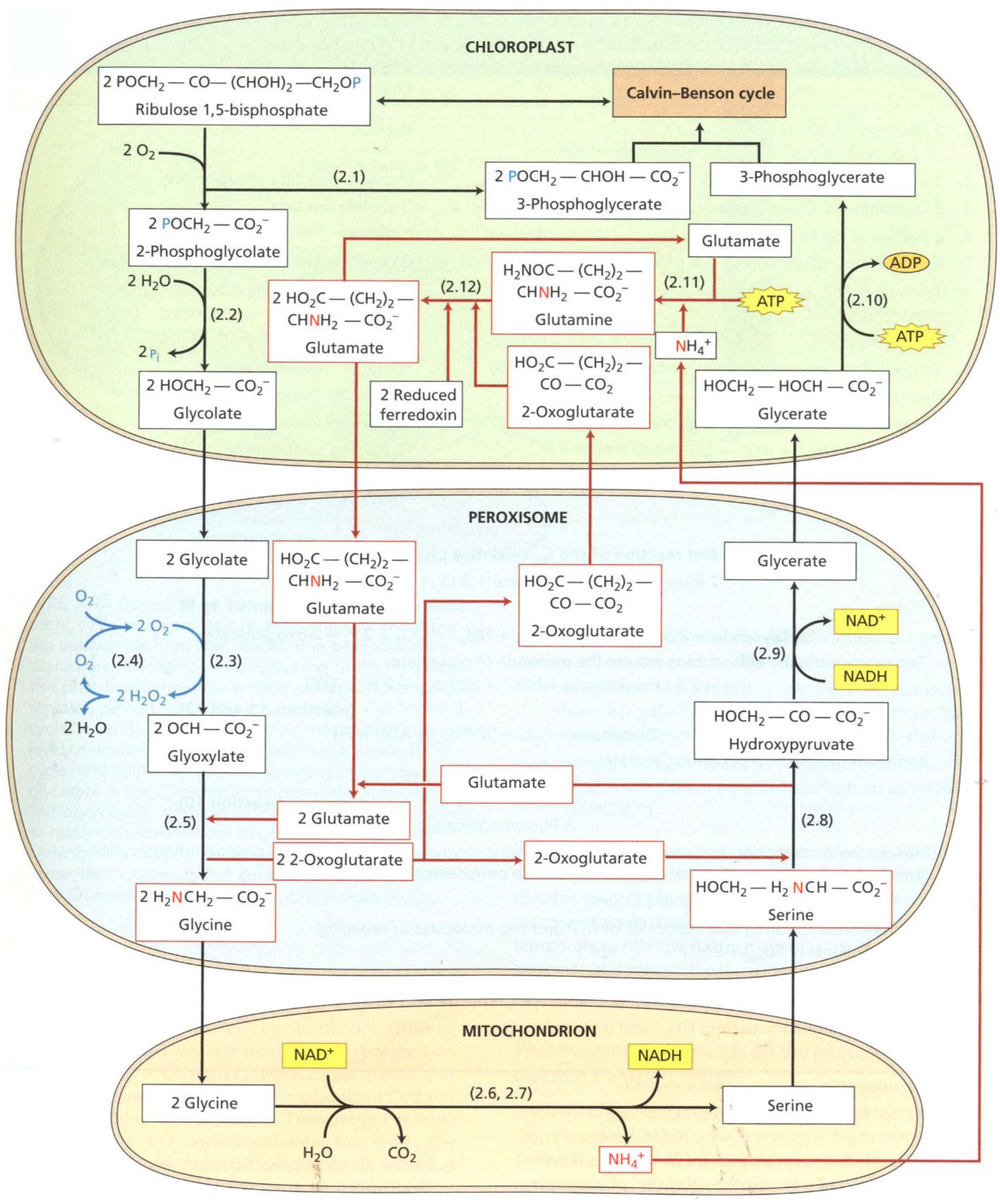


Fig. 8.8 Operation of the C2 oxidative photosynthetic cycle (**photorespiration**) involves cooperation among three organelles; chloroplasts, peroxisomes, and mitochondria.

4. This process, known as **photorespiration**, causes the partial loss of CO_2 fixed by the Calvin—Benson cycle and the concurrent uptake of oxygen in photosynthetically active leaves.
5. The negative impact of these competing reactions on plant growth has been demonstrated with a variety of photorespiratory mutants of Arabidopsis that exhibit retarded growth, precocious senescence, and cell death at the usual atmospheric CO_2 concentration (0.03%), but are normal in a high-CO_2 environment (0.3% or more).
6. Moreover, several crops show a dramatic increase in yield when grown in greenhouses with elevated levels of CO_2.

2. Energetic Conseqence of Photorespiration

Energetic consequence of carboxylating RuBP

$3CO_2+3H_2O+9ATP+6[NAD(P)H+H^+] \rightarrow$ triose-P$+9ADP+6[NAD(P)^+]$

Cost of fixing one CO_2 : **$3ATP+2[NAD(P)H+H^+]$**

Energetic consequence of oxygenating RuBP

$R\text{-}5P+O_2+2ATP+2.5[NAD(P)H+H+] \rightarrow 0.9(R\text{-}5P)+0.5CO_2+2ADP+2.5[NAD(P)^+]+2Pi$

Cost of fixing one O_2 : **$2ATP+2.5[NAD(P)H+H^+]$**

Total cost ($3CO_2/O_2$): **$11ATP+8.5[NAD(P)H+H^+]$**

Quiz

1. What is the first event in the formation of carbohydrate from CO_2 ?
2. What is the first reaction in the reduction of CO_2 to be $(CH_2O)n$?
3. What is the molecule or compound that binds or reacts with CO_2 ?
4. What is the first product of CO_2 reduction in photosynthesis ?
5. How was it known that a particular product was the first compound formed in photosynthesis
6. What is the enzyme catalyzing the first reaction of CO_2 reduction ?
7. How many RUBP required to reduce 1 mol CO_2 in photosynthesis
8. How many are ATP and NADPH required to reduce 1 mol CO_2 in photosynthesis
9. Based on ATP and NADP requirement, how much is light required to reduce 1 mol CO_2 in photosynthesis
10. How many mol are CO_2 reduced per mol H_2O broken down in the photolysis of photosynthesis

REFERENCES

Taiz, L. and Zeiger, E., 2010. Plant Physiology Chapter 7: Light Reactions. Benjamin/Cummings, Company, Inc., Redwood City, California