Siklus Calvin (Calvin cycle) adalah lintasan utama reduksi CO₂ menjadi karbohidrat pada tanaman yang dapat dibagi tiga tahapan reaksi: karboxilasi (fixasi CO₂), Reduksi (3-PGA), dan Regenerasi ribulose bisphosphate (RuBP). Ini membutuhkan energy (ATP) dan 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 CO₂ assimilation to be carbohydrate (sugars) in C3 Plants
2. To explain the diffusion of CO₂ from the atmosphere into the site of CO₂ assimilation in the chloroplasts
3. To explain reactions, enzymes and products involved in the reduction of CO₂ to be carbohydrate in C3 Plants

**LECTURE OUTLINE**

1. **INTRODUCTION**
   - Definition
   - CO₂ Diffusion
2. **CALVIN CYCLE**
   - Discovery
   - Calvin Cycle Stages
   - Labelling Study
3. **PHOTORESPiration**
   - Rubisco Activities
   - Energetic Consequence of Photorespiration

**CO₂ Fixation Cost**
- Light and CO₂ Compensation Point
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. $3 \times 10^{21}$ J/year) is converted via endergonic reactions in plants into carbohydrates (ca. $2 \times 10^{11}$ 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)](image-url)
Difusi CO₂
- Analisis Fluks CO₂ dengan pendekatan difusi
  \[ F = -D \frac{\partial C}{\partial x} \]
- Analisis Fluks CO₂ dengan pendekatan resisten (tahanan)
  \[ F = \frac{C_A - C_C}{r_m + r_a + r_s} \]

Ilustrasi
Soal: Diketahui \( r_m = 100 \text{ m.s}^{-1} \), \( r_a = 500 \text{ m.s}^{-1} \) & \( r_s = 800 \text{ m.s}^{-1} \), dan \([CO_2] = 400 \mu\text{l.l}^{-1}\) di atmosfir pada suhu 25°C. Dalam kloroplast, \([CO_2]\) dianggap nol. Berapakah flux CO₂ ke dalam kloroplast dari atmosfir

Jawab
Konversi satuan volume konsentrasi CO₂ perlu dilakukan ke satuan standar internasional (liter/m³)
\[[CO_2] = 400 \mu\text{l.l}^{-1} = (400/10^6)\times1000 \text{ liter.m}^{-3} = 0,4 \text{ liter.m}^{-3}\]

Satuan konsentrasi CO₂ diatas perlu juga dikonversi ke satuan massa seperti berikut:
\[[CO_2]=0.4\frac{g}{[(273+25)/273]22,414}\times44=0.719\ \text{g.m}^{-3}\]

Konversi diatas melibatkan pengaruh suhu pada volume gas (1 mol gas menempati 22, 414 liter pada suhu/273°K dan tekanan standard/1 atm)
\[ F(CO_2)=\frac{(0.719-0)g.m^{-3}}{(100+500+800)\text{m.s}^{-1}}=0,000514 \text{ g.m}^{-2}\text{s}^{-1}=0,514 \text{ mg.m}^{-2}\text{s}^{-1}\]
2. CALVIN CYCLE

1. Discovery
   1. The C₃ 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 CO₂ acceptor molecule. The first committed enzymatic step of the cycle is the reaction of CO₂ 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 CO₂ acceptor ribulose 1,5-bisphosphate. The cycle is completed by regeneration of Fig. 8.2 The Calvin—Benson cycle proceeds in three stages
riboflavin 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$_2$O 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% O₂, either 200 or 300-ppm ¹³CO₂, and the balance N₂) 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% O₂ + ¹³CO₂ (200 or 300-ppm) + balance N₂)

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. CO₂ Fixation Cost

\[
6 \text{(ribulose 1,5 bisP)} + 6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow 12 \text{(3-P-glycerate)} + 12\text{H}^+ \\
12 \text{(3-P-glycerate)} + 12\text{ATP} \rightarrow 12 \text{(1,3-bisP-glycerate)} + 12\text{ADP} \\
12 \text{(1,3-bisP-glycerate)} + 12 \text{(NADPH + H⁺)} \rightarrow 12 \text{(triose-P)} + 12 \text{NADP⁺} + 12 \text{Pi} \\
6 \text{(triose-P)} \rightarrow 3 \text{(fructose 1,6-bisP)} \\
3 \text{(fructose 1,6-bisP)} + 3 \text{H}_2\text{O} \rightarrow 3 \text{(fructose 6-P)} + 3 \text{Pi} \\
2 \text{(fructose 6-P)} + 2 \text{(triose-P)} \rightarrow 2 \text{(xylulose 5-P)} + 2 \text{(erythrose 4-P)} \\
2 \text{(erythrose 4-P)} + 2 \text{(triose-P)} \rightarrow 2 \text{(sedoheptulose 1,7-bisP)} \\
2 \text{(sedoheptulose 1,7-bisP)} + 2 \text{(triose-P)} \rightarrow 2 \text{(xylulose 5-P)} + 2 \text{(ribulose 5-P)} \\
4 \text{(xylulose 5-P)} \rightarrow 4 \text{(ribulose 5-P)} \\
2 \text{(ribose 5-P)} \rightarrow 2 \text{(ribulose 5-P)} \\
6 \text{(ribulose 5-P)} + 6\text{ATP} \rightarrow 6 \text{(ribulose 1,5-bisP)} + 6\text{ADP}
\]

**Net:**

\[
6\text{CO}_2 + 11\text{H}_2\text{O} + 12\text{NADPH} + 18\text{ATP} \rightarrow \\
\text{fructose 6-P} + 12 \text{NADP⁺} + 6\text{H}^+ + 18\text{ADP} + 17\text{Pi}
\]

*The above equation is divided by 6*

\[
\text{CO}_2 + (11/6)\text{H}_2\text{O} + 2\text{NADPH} + 3\text{ATP} \rightarrow \frac{1}{6} \text{F 6-P} + 2\text{NADP⁺} + \text{H}^+ + 3\text{ADP} + (17/6)\text{Pi}
\]

**The reduction cost of 1 mol CO₂:** 2 mol NADPH + 3 mol ATP

5. Light and CO₂ Compensation Point

1. The rate of CO₂ assimilation, measured by carbon exchange rate (CER), is strongly influenced by light and the CO₂ concentration.
2. Light or CO₂ compensation point is the level of light or CO₂ at which the rate of CO₂ fixation is equal to that of respiration.

This is CER of several soybean lines measured in Malang. PPFD = Photosynthetic Photon Flux Density. CER = CO₂ Exchange Rate (net photosynthesis)

![Labeling Study](image)

21% O₂ + ^13^CO₂ (200 or 300-ppm) + balance N₂)

The ^13^CO₂ labeling of a soybean leaf using a compact-disc case as a labeling chamber. The labeling gas (21% O₂, either 200 or 300-ppm ^13^CO₂, and the balance N₂) 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.
$$P = P_{max} = \left[1 - \text{EXP}(Q_{E} I / P_{max})\right]$$

$P_{max} = 29.5 \text{ \mu mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$

$Q_{E} = 0.0494 \text{ \mu mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / (\text{\mu mol photon m}^{-2} \text{ s}^{-1})$

$Q_{E}$ = quantum efficiency
3. PHOTORESPIRATION

1. Rubisco Activities
   1. Rubisco has the capacity to catalyze both the carboxylation and oxygenation of ribulose 1,5-bisphosphate (Mizirok and Lorimer 1983).
   
   Carboxylation: \( \text{RUBP} + \text{CO}_2 \rightarrow 2 \text{ (3-phosphoglycerate)} \)
   
   Oxygenation: \( \text{RUBP} + \text{O}_2 \rightarrow 3\text{-phosphoglycerate} + 2\text{-phosphoglycolate} \)
   
   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 (photorepiration) involves cooperation among three organelles: chloroplasts, peroxisomes, and mitochondria.
4. This process, known as **photorespiration**, causes the partial loss of CO₂ 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 CO₂ concentration (0.03%), but are normal in a high-CO₂ environment (0.3% or more).

6. Moreover, several crops show a dramatic increase in yield when grown in greenhouses with elevated levels of CO₂.

### 2. Energetic Consequence of Photorespiration

**Energetic consequence of carboxylating RuBP**

\[
3\text{CO}_2 + 3\text{H}_2\text{O} + 9\text{ATP} + 6[\text{NAD(P)H} + \text{H}^+] \rightarrow \text{triose-P} + 9\text{ADP} + 6[\text{NAD(P)}^+] 
\]

Cost of fixing one CO₂: **3ATP + 2[NAD(P)H+H+]**

**Energetic consequence of oxygenating RuBP**

\[
\text{R-5P} + \text{O}_2 + 2\text{ATP} + 2.5[\text{NAD(P)H} + \text{H}^+] \rightarrow 0.9(\text{R-5P}) + 0.5\text{CO}_2 + 2\text{ADP} + 2.5[\text{NAD(P)}^+] + 2\text{Pi} 
\]

Cost of fixing one O₂: **2ATP + 2.5[NAD(P)H+H+]**

Total cost (3CO₂/O₂): **11ATP + 8.5[NAD(P)H+H+]**

### Quiz

1. What is the first event in the formation of carbohydrate from CO₂?
2. What is the first reaction in the reduction of CO₂ to be (CH₂O)n?
3. What is the molecule or compound that binds or reacts with CO₂?
4. What is the first product of CO₂ 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 CO₂ reduction?
7. How many RUBP required to reduce 1 mol CO₂ in photosynthesis?
8. How many are ATP and NADPH required to reduce 1 mol CO₂ in photosynthesis?
9. Based on ATP and NADP requirement, how much is light required to reduce 1 mol CO₂ in photosynthesis?
10. How many mol are CO₂ reduced per mol H₂O broken down in the photolysis of photosynthesis?

### REFERENCES