

11. PHOTOSYNTHETIC PATHWAYS - C₃, C₄ AND CAM

Dark reaction or Blackman's reaction or Path of carbon in photosynthesis

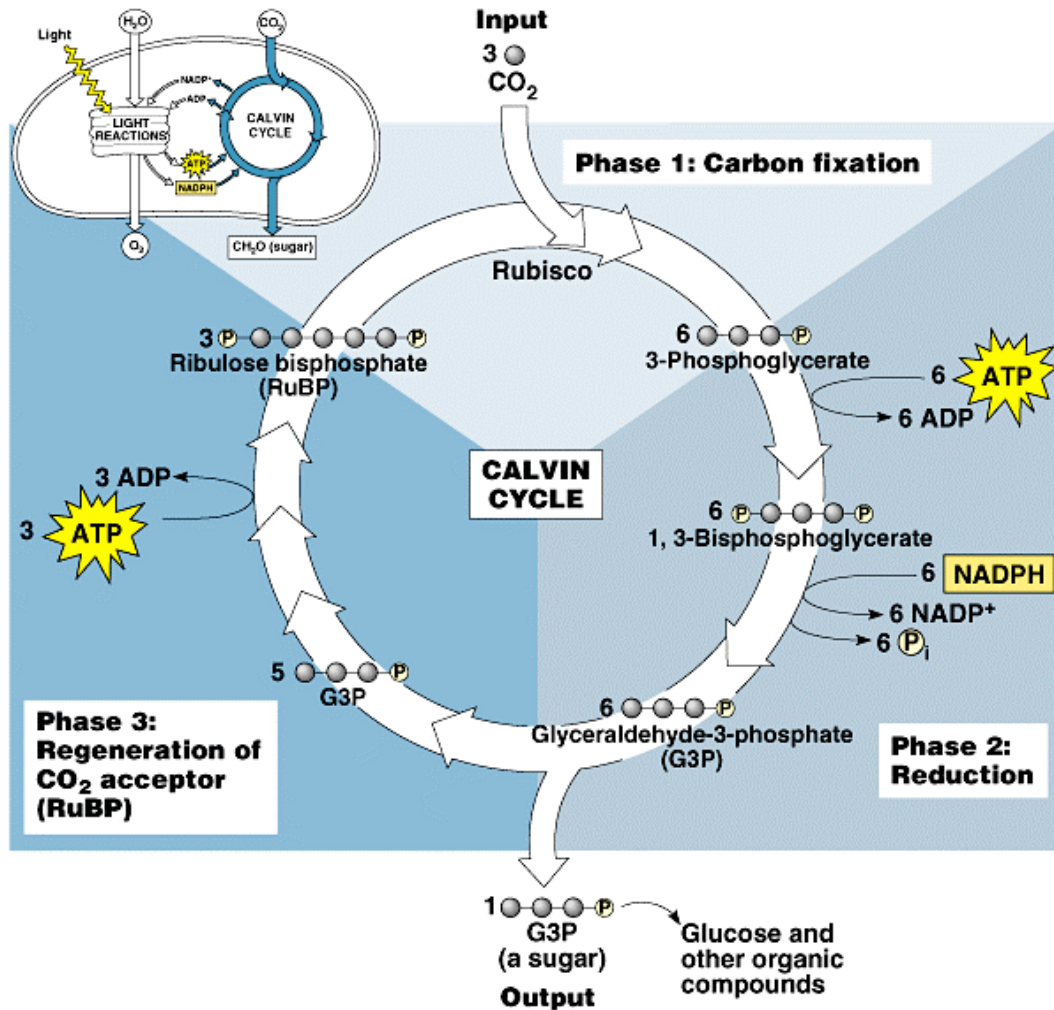
This is the second step in the mechanism of photosynthesis. The chemical processes of photosynthesis occurring independent of light is called *dark reaction*. It takes place in the stroma of chloroplast. The dark reaction is purely enzymatic and it is slower than the light reaction. The dark reactions occur also in the presence of light. In dark reaction, the sugars are synthesized from CO₂. The energy poor CO₂ is fixed to energy rich carbohydrates using the energy rich compound, ATP and the assimilatory power, NADPH₂ of light reaction. The process is called carbon fixation or carbon assimilation. Since Blackman demonstrated the existence of dark reaction, the reaction is also called as *Blackman's reaction*. In dark reaction two types of cyclic reactions occur

1. Calvin cycle or C₃ cycle
2. Hatch and Slack pathway or C₄ cycle

Calvin cycle or C₃ cycle

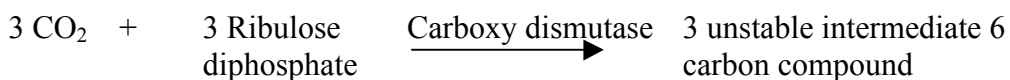
It is a cyclic reaction occurring in the dark phase of photosynthesis. In this reaction, CO₂ is converted into sugars and hence it is a process of carbon fixation. The Calvin cycle was first observed by Melvin Calvin in chlorella, unicellular green algae. Calvin was awarded Nobel Prize for this work in 1961. Since the first stable compound in Calvin cycle is a 3 carbon compound (3 phosphoglyceric acid), the cycle is also called as C₃ cycle. The reactions of Calvin's cycle occur in three phases.

1. Carboxylative phase
2. Reductive phase
3. Regenerative phase

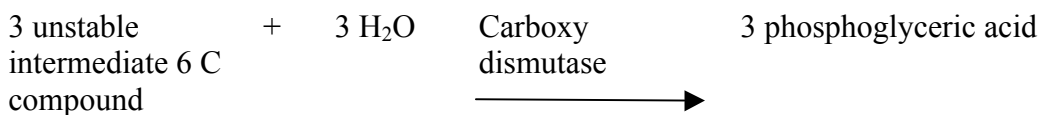


1. Carboxylative phase

Three molecules of CO_2 are accepted by 3 molecules of 5C compound viz., ribulose diphosphate to form three molecules of an unstable intermediate 6C compound. This reaction is catalyzed by the enzyme, carboxy dismutase



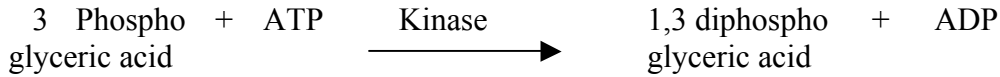
The three molecules of the unstable 6 carbon compound are converted by the addition of 3 molecules of water into six molecules of 3 phosphoglyceric acid. This reaction is also catalyzed by the enzyme carboxy mutase.



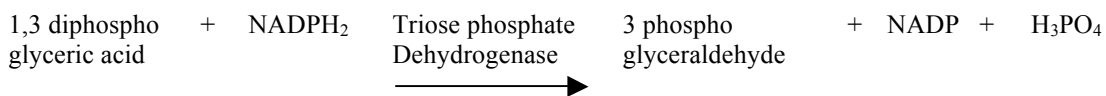
3 phosphoglyceric acid (PGA) is the first stable product of dark reaction of photosynthesis and since it is a 3 carbon compound, this cycle is known as C3 cycle.

2. Reductive phase

Six molecules of 3PGA are phosphorylated by 6 molecules of ATP (produced in the light reaction) to yield 6 molecules of 1-3 diphospho glyceric acid and 6 molecules of ADP. This reaction is catalyzed by the enzyme, Kinase



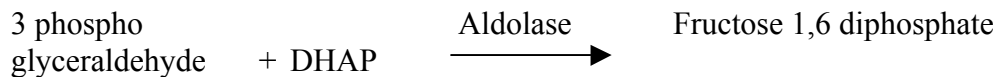
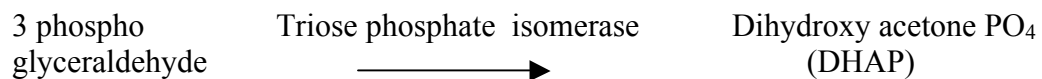
Six molecules of 1, 3 diphosphoglyceric acid are reduced with the use of 6 molecules of NADPH₂ (produced in light reaction) to form 6 molecules of 3 phospho glyceraldehyde. This reaction is catalysed by the enzyme, triose phosphate dehydrogenase.



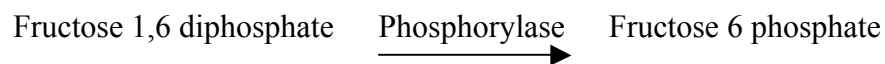
3. Regenerative phase

In the regenerative phase, the ribose diphosphate is regenerated. The regenerative phase is called as *pentose phosphate pathway* or *hexose monophosphate shunt*. It involves the following steps.

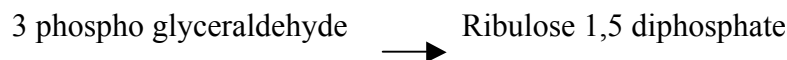
1. Some of the molecules of 3 phospho glyceraldehyde into dihydroxy acetone phosphate. Both 3 phospho glyceraldehyde and dihydroxy acetone phosphate then unite in the presence of the enzyme, aldolase to form fructose, 1-6 diphosphate.



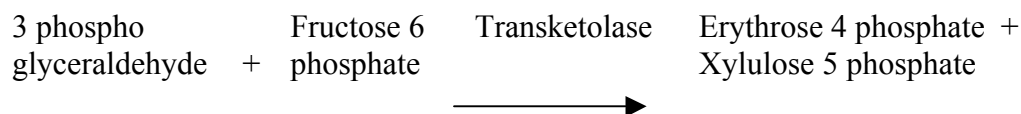
2. Fructose 6 phosphate is converted into fructose 6 phosphate in the presence of phosphorylase



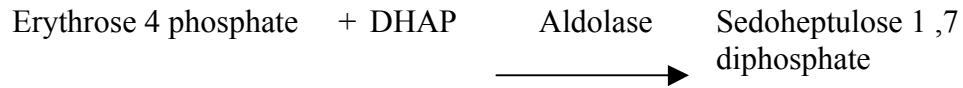
3. Some of the molecules of 3 phospho glyceraldehyde instead of forming hexose sugars are diverted to regenerate ribulose 1-5 diphosphate



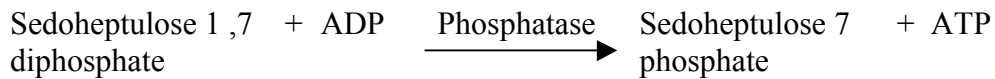
4. 3 phospho glyceraldehyde reacts with fructose 6 phosphate in the presence of enzyme transketolase to form erythrose 4 phosphate (4C sugar) and xylulose 5 phosphate (5C sugar)



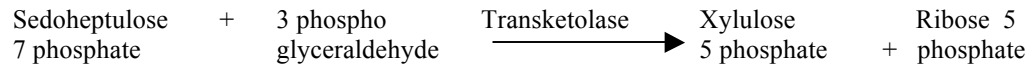
5. Erythrose 4 phosphate combines with dihydroxy acetone phosphate in the presence of the enzyme aldolase to form sedoheptulose 1,7 diphosphate(7C sugar)



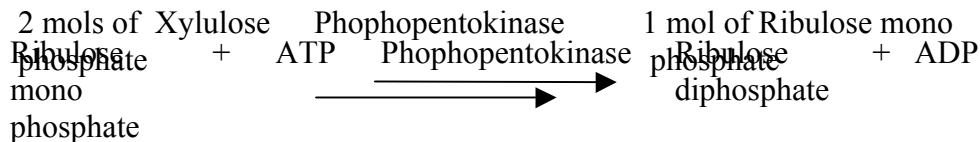
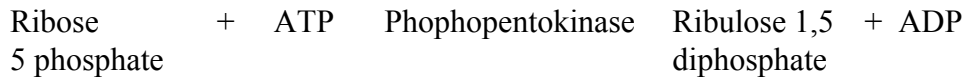
6. Sedoheptulose 1, 7 diphosphate loses one phosphate group in the presence of the enzyme phosphatase to form sedoheptulose 7 phosphate.



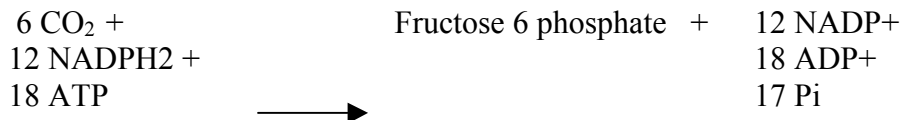
7. Sedoheptulose phosphate reacts with 3 phospho glyceraldehyde in the presence of transketolase to form xylulose 5 phosphate and ribose 5 phosphate (both % c sugars)

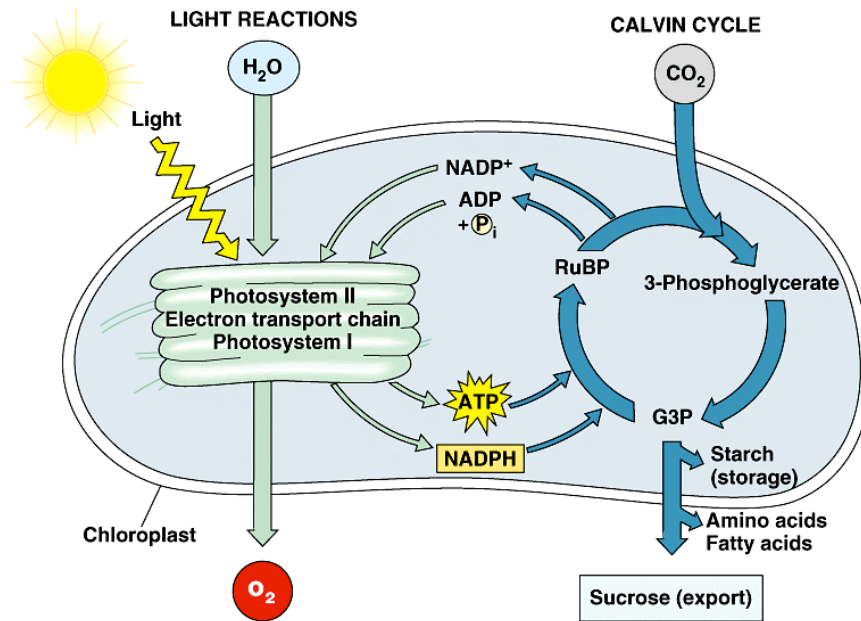


8. Ribose 5 phosphate is converted into ribulose 1, 5 diphosphate in the presence of enzyme, phosphopentose kinase and ATP. Two molecules of xylulose phosphate are also converted into one molecule of ribulose monophosphate. The ribulose monophosphate is phosphorylated by ATP to form ribulose diphosphate and ADP, thus completing Calvin cycle.



In the dark reaction, CO₂ is fixed to carbohydrates and the CO₂ acceptor ribulose diphosphate is regenerated. In Calvin cycle, 12 NADPH₂ and 18 ATPs are required to fix 6 CO₂ molecules into one hexose sugar molecule (fructose 6 phosphate).





Schematic diagram of light reaction and Calvin cycle

C4 cycle or Hatch and Slack pathway

It is the alternate pathway of C3 cycle to fix CO_2 . In this cycle, the first formed stable compound is a 4 carbon compound viz., oxaloacetic acid. Hence it is called C4 cycle. The path way is also called as Hatch and Slack as they worked out the pathway in 1966 and it is also called as C4 dicarboxylic acid pathway. This pathway is commonly seen in many grasses, sugar cane, maize, sorghum and amaranthus.

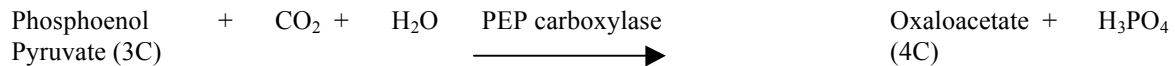
The C4 plants show a different type of leaf anatomy. The chloroplasts are dimorphic in nature. In the leaves of these plants, the vascular bundles are surrounded by bundle sheath of larger parenchymatous cells. These bundle sheath cells have chloroplasts. These chloroplasts of bundle sheath are larger, lack grana and contain starch grains. The chloroplasts in mesophyll cells are smaller and always contain grana. This peculiar anatomy of leaves of C4 plants is called Kranz anatomy. The bundle sheath cells are bigger and look like a ring or wreath. Kranz in German means wreath and hence it is called Kranz anatomy. The C4 cycle involves two carboxylation reactions, one taking place in chloroplasts of mesophyll cells and another in chloroplasts of bundle sheath cells. There are four steps in Hatch and Slack cycle:

1. Carboxylation
2. Breakdown

- 3. Splitting
- 4. Phosphorylation

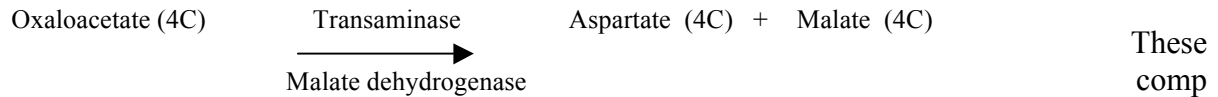
1. Carboxylation

It takes place in the chloroplasts of mesophyll cells. Phosphoenolpyruvate, a 3 carbon compound picks up CO₂ and changes into 4 carbon oxaloacetate in the presence of water. This reaction is catalysed by the enzyme, phosphoenol pyruvate carboxylase.



2. Breakdown

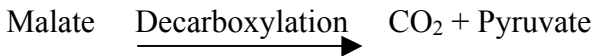
Oxaloacetate breaks down readily into 4 carbon malate and aspartate in the presence of the enzyme, transaminase and malate dehydrogenase.



ounds diffuse from the mesophyll cells into sheath cells.

3. Splitting

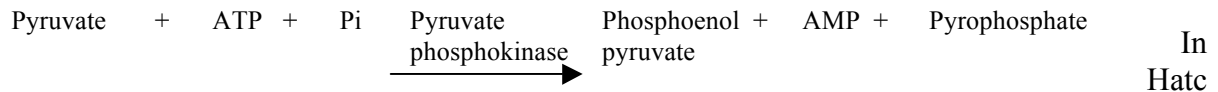
In the sheath cells, malate and aspartate split enzymatically to yield free CO₂ and 3 carbon pyruvate. The CO₂ is used in Calvin's cycle in the sheath cell.



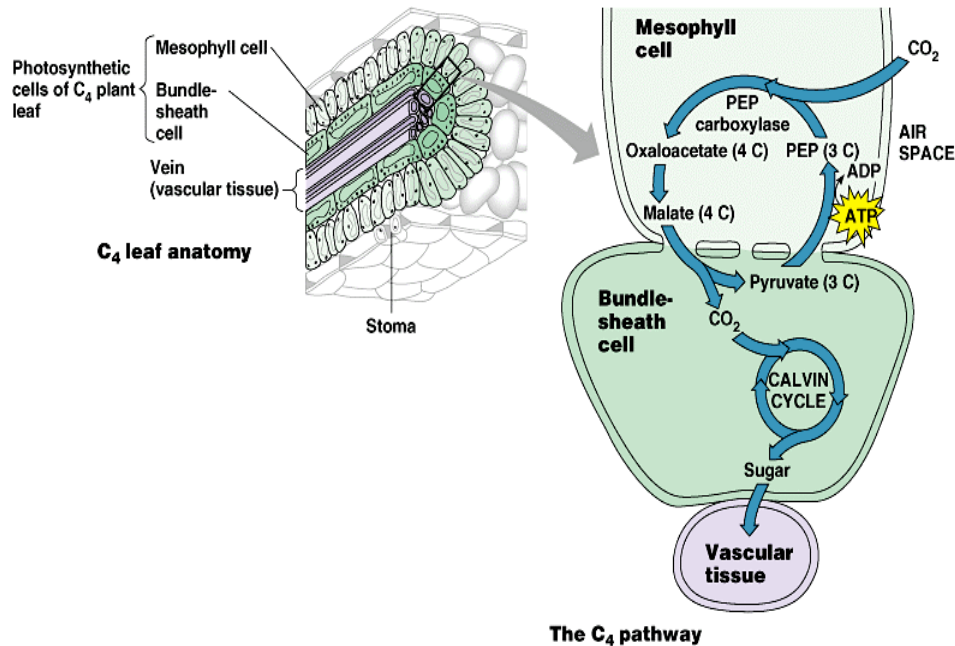
The second Carboxylation occurs in the chloroplast of bundle sheath cells. The CO₂ is accepted by 5 carbon compound ribulose diphosphate in the presence of the enzyme, carboxy dismutase and ultimately yields 3 phosphoglyceric acid. Some of the 3 phosphoglyceric acid is utilized in the formation of sugars and the rest regenerate ribulose diphosphate.

4. Phosphorylation

The pyruvate molecule is transferred to chloroplasts of mesophyll cells where, it is phosphorylated to regenerate phosphoenol pyruvate in the presence of ATP. This reaction is catalysed by pyruvate phosphokinase and the phosphoenol pyruvate is regenerated.



h and Slack pathway, the C₃ and C₄ cycles of carboxylation are linked and this is due to the Kranz anatomy of the leaves. The C₄ plants are more efficient in photosynthesis than the C₃ plants. The enzyme, phosphoenol pyruvate carboxylase of the C₄ cycle is found to have more affinity for CO₂ than the ribulose diphosphate carboxylase of the C₃ cycle in fixing the molecular CO₂ in organic compound during Carboxylation.



Crassulacean Acid Metabolism (CAM) cycle or the dark fixation of CO₂ in succulents

CAM is a cyclic reaction occurring in the dark phase of photosynthesis in the plants of Crassulaceae. It is a CO₂ fixation process wherein, the first product is malic acid. It is the third alternate pathway of Calvin cycle, occurring in mesophyll cells. The plants exhibiting CAM cycle are called CAM plants. Most of the CAM plants are succulents e.g., Bryophyllum, Kalanchoe, Crassula, Sedium, Kleinia etc. It is also seen in certain plants of Cactus e.g. Opuntia, Orchid and Pine apple families.

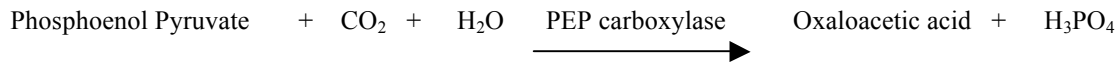
CAM plants are usually succulents and they grow under extremely xeric conditions. In these plants, the leaves are succulent or fleshy. The mesophyll cells have larger number of chloroplasts and the vascular bundles are not surrounded by well defined bundle sheath cells. In these plants, the stomata remain open during night and closed during day time. The CAM plants are adapted to photosynthesis and survival under adverse xeric conditions. CAM plants are not as efficient as C₄ plants in photosynthesis. But they are better suited to conditions of extreme desiccation.

CAM involves two steps:

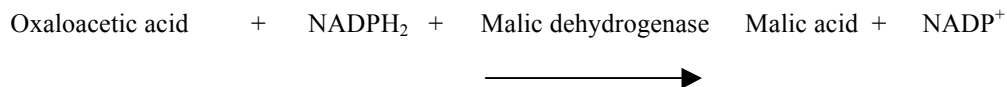
1. Acidification
2. Deacidification

Acidification

In darkness, the stored carbohydrates are converted into phosphoenol pyruvic acid by the process of Glycolysis. The stomata in CAM plants are open in dark and they allow free diffusion of CO₂ from the atmosphere into the leaf. Now, the phosphoenolpyruvic acid is carboxylated by the enzyme phosphoenol pyruvic acid carboxylase and is converted into oxaloacetic acid.



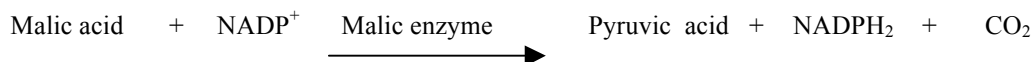
The oxaloacetic acid is then reduced to malic acid in the presence of the enzyme malic dehydrogenase. The reaction requires NADPH₂ produced in Glycolysis.



The malic acid produced in dark is stored in the vacuole. The malic acid increases the acidity of the tissues.

Deacidification

During day time, when the stomata are closed, the malic acid is decarboxylated to produce pyruvic acid and evolve carbon dioxide in the presence of the malic enzyme. When the malic acid is removed, the acidity decreases the cells. This is called deacidification. One molecule of NADP⁺ is reduced in this reaction.

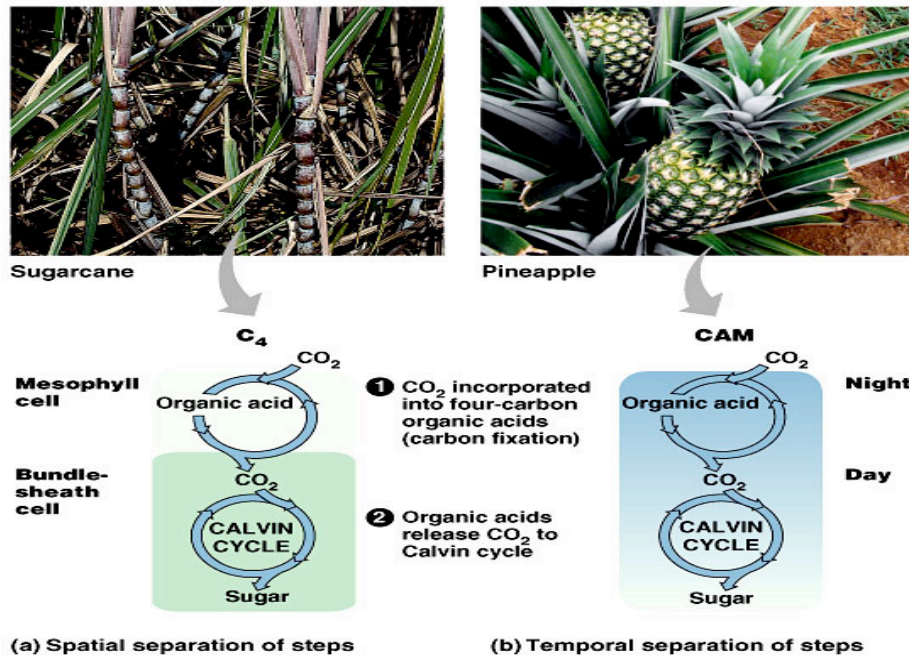


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The pyruvic acid may be oxidized to CO₂ by the pathway of Krebs's cycle or it may be reconverted to phosphoenol pyruvic acid and synthesize sugar by C₃ cycle. The CO₂ released by deacidification of malic acid is accepted by ribulose diphosphate and is fixed to carbohydrate by C₃ cycle.

CAM is a most significant pathway in succulent plants. The stomata are closed during day time to avoid transpiration loss of water. As the stomata are closed, CO₂ cannot enter into the leaves from the atmosphere. However, they can carry out photosynthesis during the day time with the help of CO₂ released from organic acids. During night time, organic acids are synthesized in plenty with the help of CO₂ released in respiration and the CO₂ entering from

the atmosphere through the open stomata. Thus, the CO_2 in dark acts as survival value to these plants.



Comparison of the plants of C₃ and C₄ cycle

	C ₃ Plant	C ₄ Plant
1.	Only C ₃ cycle is found	Both C ₄ and C ₃ cycles are found.
2.	The efficiency of CO ₂ absorption at low concentration is far less and hence, they are less efficient.	The efficiency of CO ₂ absorption at low concentration is quite high and hence, they are more efficient plants.
3.	The CO ₂ acceptor is Ribulose-1, 5-diphosphate.	The CO ₂ acceptor is phospho enol pyruvate.
4.	The first stable product is phospho glyceric acid (PGA).	Oxaloacetate (OAA) is the first stable product.
5.	Plants show one type of chloroplast (monomorphic type).	Plants show dimorphic type of chloroplast. The chloroplast of parenchymatous bundle sheath is different from that of mesophyll cells (dimorphic type). The chloroplasts in bundle sheath cell are centripetally

		arranged and lack grana. Leaves show <i>Kranz type</i> of anatomy.
6.	In each chloroplast, two pigment systems (Photosystem I and II) are present.	In the chloroplasts of bundle sheath cells, the photosystem II is absent. Therefore, these are dependent on mesophyll chloroplasts for the supply of NADPH + H ⁺ .
7.	The Calvin cycle enzymes are present in mesophyll chloroplast. Thus, the Calvin cycle occurs.	Calvin cycle enzymes are absent in mesophyll chloroplasts. The cycle occurs only in the chloroplasts of bundle sheath cells.
8.	The CO ₂ compensation point is 50-150 ppm CO ₂ .	The CO ₂ compensation point is 0-10 ppm CO ₂ .
9.	Photorespiration is present and easily detectable.	Photorespiration is present only to a slight degree or absent.
10.	The CO ₂ concentration inside leaf remains high (about 200 ppm).	The CO ₂ concentration inside the leaf remains low (about 100 ppm).
11.	The ¹³ C/ ¹² C ratio in C-containing compounds remains relatively low (both ¹³ CO ₂ and ¹² CO ₂ are present in air).	The ratio is relatively high, <i>i.e.</i> C ₄ plants are more enriched with ¹³ C than C ₃ plants.
12.	Net rate of photosynthesis in full sunlight (10,000 – 12,000 ft. c.) is 15-25 mg. of CO ₂ per dm ² of leaf area per hour.	It is 40-80 mg. of CO ₂ per dm ² of leaf area per hour. That is, photosynthetic rate is quite high. The plants are efficient.
13.	The light saturation intensity reaches in the range of 1000-4000 ft. c.	It is difficult to reach saturation even in full sunlight.
14.	Bundle sheath cells are unspecialized.	The bundle sheath cells are highly developed with unusual construction of organelles.
15.	The optimum temperature for the process is 10-25°C.	In these plants, it is 30-45°C and hence, they are warm climate plants. At this temperature, the rate of photosynthesis is double than that is in C ₃ plants.

16.	18 ATPs are required to synthesize one glucose molecule.	30 ATPs are required to synthesize one glucose molecule.
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Factors affecting photosynthesis

I. External factors

1. Light

It is the most important factor of photosynthesis. Any kind of artificial light such as electric light can induce photosynthesis. Out of the total solar energy, only 1-2 % is used for photosynthesis and the rest is used for other metabolic activities. The effect of light on photosynthesis can be studied under three categories.

a. Light intensity

Wolkoff (1966) found that the rate of photosynthesis is directly proportional to light intensity. But the extremely high light intensities do not favor for higher photosynthetic rates. The high light intensity which fails to accelerate photosynthesis is called light saturation intensity. Of the light falling on a leaf, about 80 per cent is absorbed, 10 per cent is reflected and 10 % is transmitted. The rate of photosynthesis is greater in intense light than in diffused light. The plants are grouped into two types on the basis of light requirement.

i. Heliophytes (Sun plants)

ii. Sciophytes (Shade plants)

At a specific light intensity, the amount of CO₂ used in photosynthesis and the amount of CO₂ released in respiration are volumetrically equal. This specific light intensity is known as *light compensation point*.

At very high light intensity, beyond a certain point, the photosynthetic cells exhibit *photo oxidation*. This phenomenon is called *solarisation* and a result of this, inactivation of chlorophyll molecules, bleaching of chlorophyll molecules and even inactivation of some enzymes take place resulting in the destruction of whole photosynthetic apparatus. In general, low light intensity favours stomatal closure and in turn reduced rate of photosynthesis.

b. Light quality (wavelength)

Photosynthesis occurs only in the visible part of the light spectrum i.e., between 400 and 700 nm. The maximum rate of photosynthesis occurs at red light followed by blue light.

The green light has minimum effect and photosynthesis cannot take place either in the infrared or in the ultraviolet light.

c. Light duration

In general tropical plants get 10-12 hours of light per day and this longer period of light favours photosynthesis.

2. Carbon dioxide

CO₂ is one of the raw materials required for photosynthesis. If the CO₂ concentration is increased at optimum temperature and light intensity, the rate of photosynthesis increases. But, it is also reported that very high concentration of CO₂ is toxic to plants inhibiting photosynthesis.

3. Temperature

The rate of photosynthesis increases by increase in temperature up to 40 °C and after this, there is reduction in photosynthesis. High temperature results in the denaturation of enzymes and thus, the dark reaction is affected. The temperature requirement for optimum photosynthesis varies with the plant species. For example, photosynthesis stops in many plants at 0 °C but in some conifers, it can occur even at -35 °C. Similarly photosynthesis stops beyond 40-50 °C in certain plants; but certain bacteria and blue green algae can perform photosynthesis even at 70 °C.

4. Water

Water has indirect effect on the rate of photosynthesis although it is one of the raw materials for the process. The amount of water utilized in photosynthesis is quite small and even less than 1 per cent of the water absorbed by a plant. Water rarely acts as a limiting factor for photosynthesis. During water scarcity, the cells become flaccid and the rate of photosynthesis might go down.

5. Oxygen

Oxygen is a byproduct of photosynthesis and an increase in the O₂ concentration in many plants results in a decrease in the rate of photosynthesis. The phenomenon of inhibition of photosynthesis by O₂ was first discovered by Warburg (1920) in green alga *Chlorella* and this effect is known as Warburg's effect. This is commonly observed in C₃ plants.

In plants, there is a close relationship between Warburg's effect and photorespiration. The substrate of photorespiration is glycolate and it is synthesized from some intermediates of Calvin's cycle. In plants that show Warburg's effect, increased O₂ concentration result in diversion of these intermediates of Calvin cycle into the synthesis of glycolate, thereby showing higher rate of photorespiration and lower photosynthetic productivity.

6. Mineral elements

The elements like Mg, Fe, Cu, Cl, Mn, P etc are involved in the key reactions of photosynthesis and hence, the deficiency of any of these nutrients caused reduction in photosynthesis.

7. Chlorophyll content

It is very much essential to trap the light energy. In 1929, Emerson found direct relationship between the chlorophyll content and rate of photosynthesis. In general, the chlorophyll sufficient plants are green in colour showing efficient photosynthesis. The chlorotic leaves due to irregular synthesis of chlorophyll or breakdown of chlorophyll pigment exhibit inefficient photosynthesis.

8. Leaf

The leaf characters such as leaf size, chlorophyll content, number of stomata. Leaf orientation and leaf age are some of the factors that are responsible for photosynthesis. The maximum photosynthetic activity is usually seen in the physiologically functional and full size leaves (usually third/fourth leaf from the tip of the shoot system).

9. Carbohydrates

If the accumulated carbohydrates are not translocated, the photosynthetic rate is reduced and respiration is increased. Sugar is converted into starch and gets accumulated in the chloroplasts. This reduces the effective surface in the chloroplast and the rate of photosynthesis is decreased.

10. Phytohormones

Treharne (1970) reported first that photosynthesis may be regulated by plant hormone system. He found that gibberellic acid and cytokinin increase the carboxylating activity and photosynthetic rates. Meidner (1967) also reported that kinetin @ 3µm causes 12 per cent increase in photosynthesis within one hour of the treatment.

PHOTORESPIRATION

The excessive respiration that takes place in green cells in the presence of light is called as photorespiration. Decker (1955) discovered the process and it is also called as C₂ cycle as the 2 carbon compound glycolic acid acts as the substrate in photorespiration. In general, respiration takes place under both light and dark conditions. However in some plants, the respiration is more in light than in dark. It is 3-5 times higher than the rate of respiration in dark. Photorespiration is carried out only in the presence of light. But the normal respiration is not light dependent and it is called dark respiration.

In photorespiration, temperature and oxygen concentration play an important role. Photorespiration is very high when the temperature is between 25 and 30 °C. The rate of photorespiration increases with the increase in the concentration of oxygen. Three cell organelles namely chloroplast, peroxisome and mitochondria are involved in the photorespiration. This kind of respiration is seen in plants like cotton, pulses, capsicum, peas, tomato, petunia soybean, wheat, oats, paddy, chlorella etc and it is absent in grasses.

Mechanism

1. In the presence of excess oxygen and low CO₂, ribulose 1,5 diphosphate produced in the chloroplast during photosynthesis is split into 2 phospho glycolic acid and 3 phospho glyceric acid by the enzyme, ribulose 1,5 diphosphate oxygenase
2. The 3 phospho glyceric acid enters the Calvin cycle.
3. In the next step, phosphate group is removed from 2 phosphoglycolic acid to produce glycolic acid by the enzyme, phosphatase.
4. Glycolic acid then it come out of chloroplast and enter the peroxisome. Here, it combines with oxygen to form glyoxylic acid and hydrogen peroxide. This reaction is catalyzed by the enzyme, glycolic acid oxidase. Hydrogen peroxide is toxic and it is broken down into water and oxygen by the enzyme, Catalase. Photorespiration is an oxidation process. In this process, glycolic acid is converted into carbohydrate and CO₂ is released as the by product. As glycolic acid is oxidized in photorespiration, it is also called as glycolate metabolism.
5. The glyoxylic acid converted into glycine by the addition of one amino group with the help of the enzyme, amino transferase.

2. The process causes oxidation of glycolic acid which arises as an unwanted byproduct of photosynthesis. The glycolic acid after oxidation is converted into carbohydrate but the remainder is converted into CO₂.
3. Photorespiration uses energy in the form of ATP and reduced nucleotides, but normal respiration yields ATP and reduced nucleotides.
4. It is believed that photorespiration was common in earlier days when CO₂ content was too low to allow higher rates.