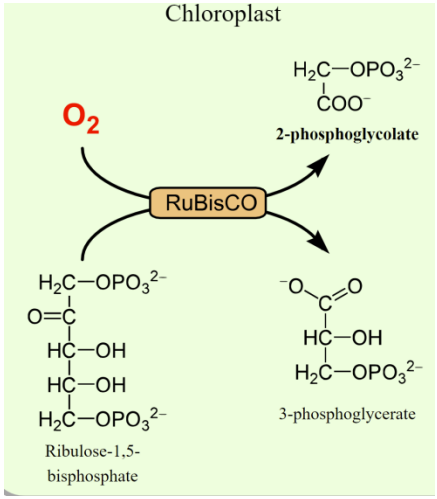
C4 and CAM

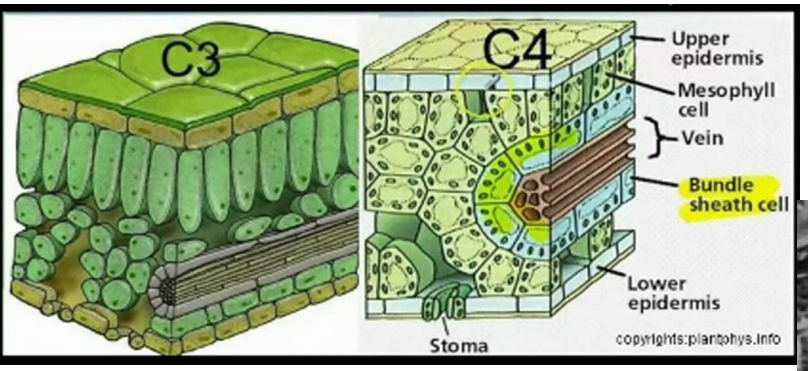
CO_2-Concentrating Mechanisms

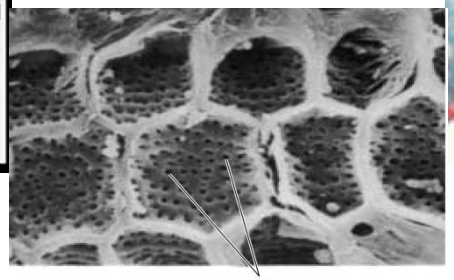


- Rubisco can add oxygen to RuBP:
  - 3-phosphoglycerate (3-PGA) and
  - 2-phosphoglycolate.
- Phosphoglycolate in higher concentrations it is toxic for the plant.
- It has to be processed in a metabolic pathway: ***photorespiration***.
- Photorespiration
  - Is energy demanding,
  - Leads to a net loss of CO_2.
- The efficiency of photosynthesis can be decreased by 40% (high temperatures and dryness).
- Many plants
  - Either do not photorespire at all,
  - Or they do so to only a limited extent.
- These plants have
  - Normal RUBISCOs, and
  - Their lack of photorespiration: They concentrate CO_2 in the RUBISCO environment and
  - Thereby suppress the oxygenation reaction.
- There are mechanisms for concentrating CO_2 at the site of carboxylation:
  1. C4 photosynthetic carbon fixation (C4)
  2. Crassulacean acid metabolism (CAM)
- They involve “add-ons” to the Calvin cycle.
  - Plants with **C4 metabolism** are often found in **hot** environments;
  - **CAM** plants are typical of **desert** environments.

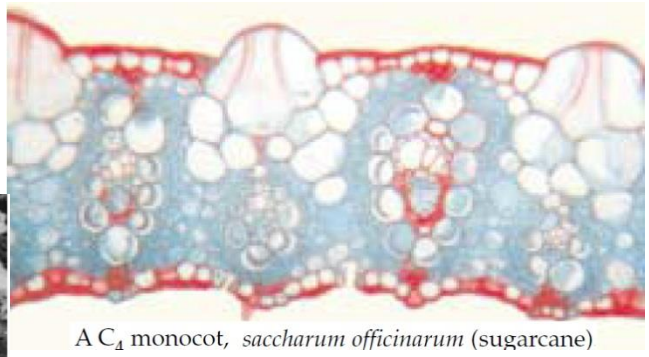
THE C_4 CARBON CYCLE

- There are differences in leaf anatomy
  - Plants: Photosynthesize solely via the Calvin photosynthetic cycle (C3 plants) and
  - Plants with C4 carbon cycle (called C4 plants)
- C_3 leaf (cross section): Mesophyll is the one major cell type with chloroplasts,.
- A typical C_4 leaf has two distinct chloroplast-containing cell types:
  - Mesophyll and
  - Bundle sheath (or Kranz, German for "wreath") cells
- But operation of the C4 cycle requires the cooperative effort of both cell types.
- No mesophyll cell of a C4 plant is more than two or three cells away from the nearest bundle sheath cell.
- Networks of plasmodesmata connects mesophyll and bundle sheath cells: flow of metabolites.





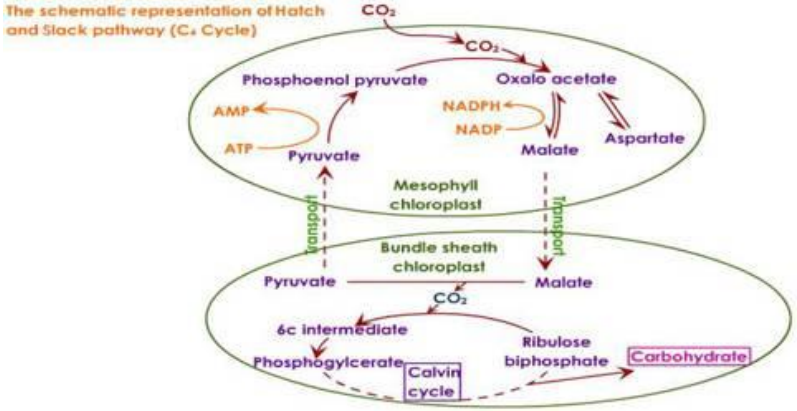
Scanning electon micrograph of *Triodia irritans* (C4 plant) showing the plasmodesmata pits in the bundle sheath heath cell walls



A C_4 monocot, *saccharum officinarum* (sugarcane)

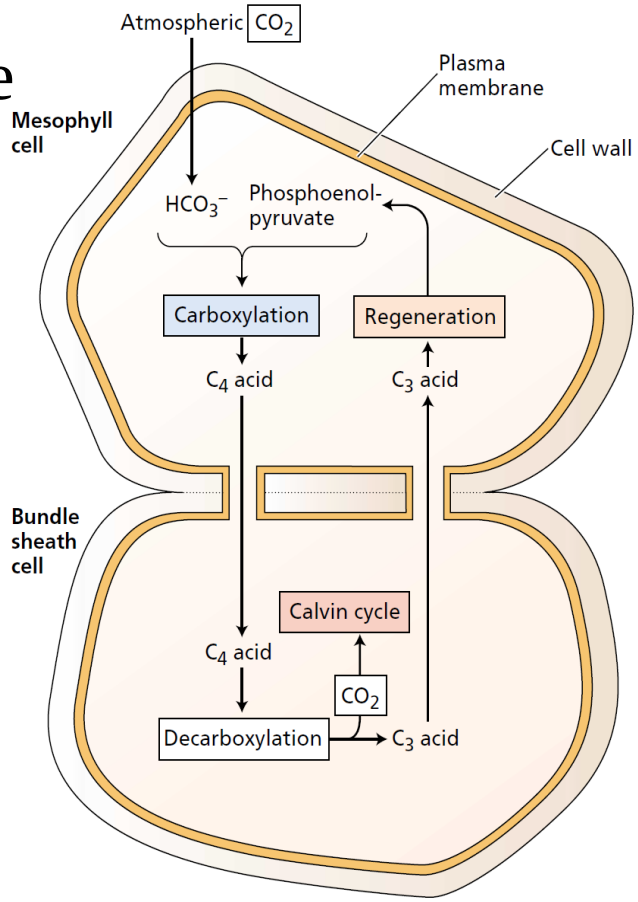
Malate and Aspartate Are Carboxylation Products

- When leaves were exposed for a few seconds to $^{14}CO_2$ in the light
  - 70 to 80% of the label was found in the C_4 acids malate and aspartate.
  - a pattern different from the leaves that photosynthesize solely via the Calvin cycle.
- ***M. D. Hatch*** and ***C. R. Slack*** elucidated the C_4 photosynthetic carbon cycle.
  - The C_4 acids malate and aspartate are the first stable, detectable intermediates of photosynthesis in leaves of sugarcane
  - The primary carboxylation in these leaves is catalyzed by ***PEP (phosphoenylpyruvate) carboxylase*** (not by RUBISCO).
- The CO_2 concentration mechanism
  - a division of labor between the mesophyll and the bundle sheath cells.



The C_4 Cycle Concentrates CO_2 in Bundle Sheath Cells

- The basic C_4 cycle consists of four stages:
  - ***a.*** ***Fixation:*** Carboxylation of PEP in the mesophyll cells to form a C_4 acid.
  - ***b.*** ***Transport:*** of the C_4 acids to the bundle sheath cells
  - ***c.*** ***Decarboxylation:*** Within the bundle sheath cells and generation of CO_2, which is then reduced to carbohydrate via the Calvin cycle
  - ***d.*** ***Transport:*** C3 acid (decarboxylated from C4 acid), back to the mesophyll cell and regeneration of the CO_2-acceptor PEP.

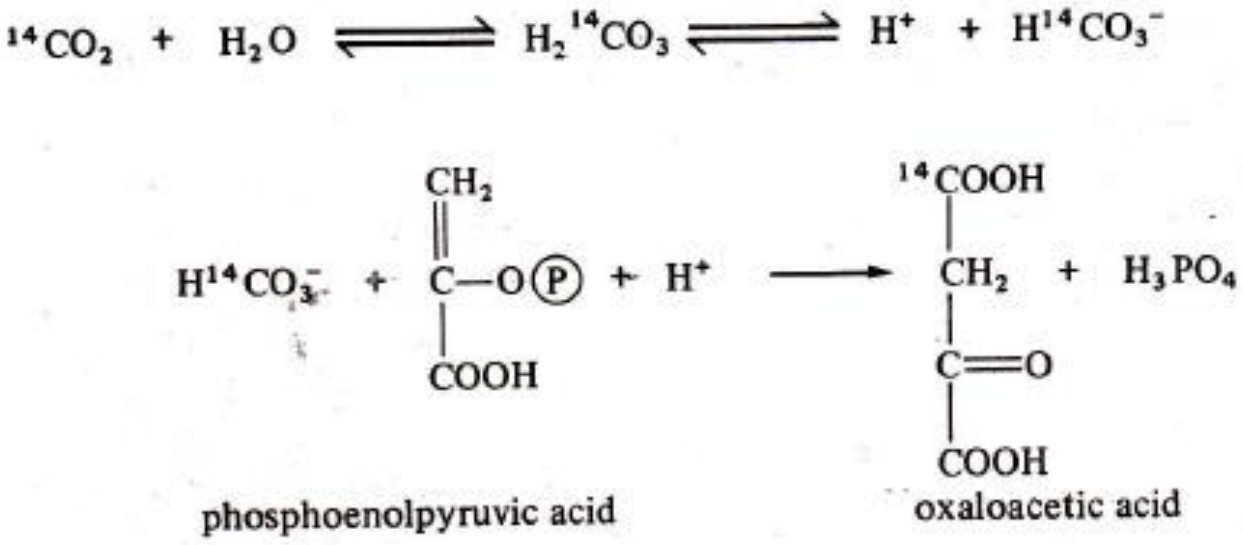


CO_2 Concentration….

- Rubisco operate under high CO_2 concentrations in the bundle sheath cells (more efficiently than in C_3 plants).
- C_4 plants need less of this enzyme which leads to a better nitrogen-use efficiency of C_4 compared to C_3 plants, since the rate of photosynthesis per unit nitrogen in the leaf is increased.
- C_4 plants exhibit better water-use efficiency.
- They can acquire enough CO_2 even when keeping their stomata more closed.
- Water loss by transpiration is reduced.

Reactions of the C_4 photosynthetic carbon cycle

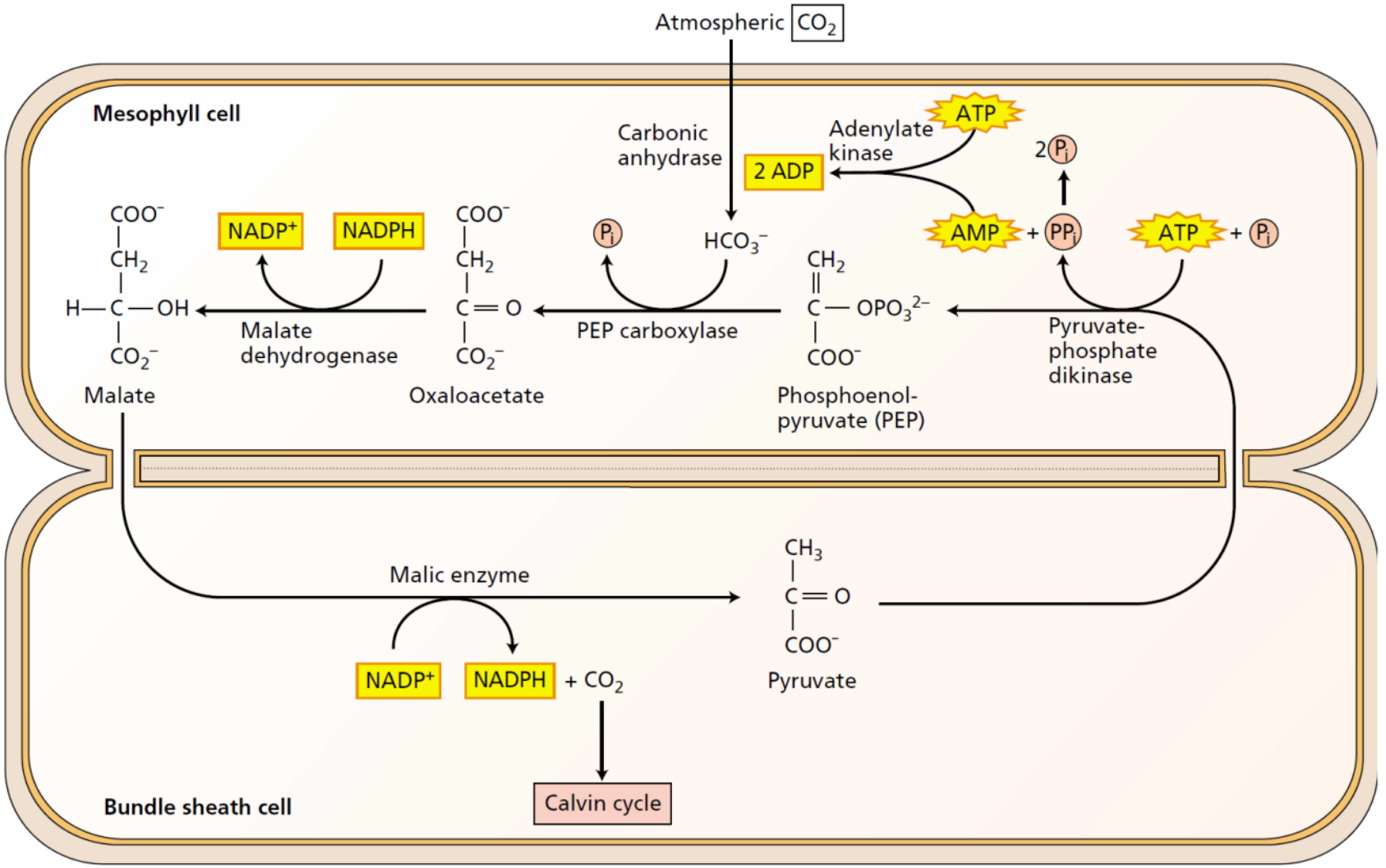
- Air passes through the open stomata of the leaf into the extensive intercellular spaces and bathes the mesophyll cells.
- CO_2 then passes into the cytoplasm of these cells where it dissolves and ionizes by carbonic anhydrase.
- The resulting HCO_3^-, is then used by phosphoenolpyruvate carboxylase (PEPC) to carboxylate PEP with the formation of OAA.
- HCO_3-, rather than CO_2, is the substrate of PEP carboxylase.
- The resulting oxaloacetate is composed of four carbon atoms, (the basis for the name of this metabolic pathway).



Fate of OAA

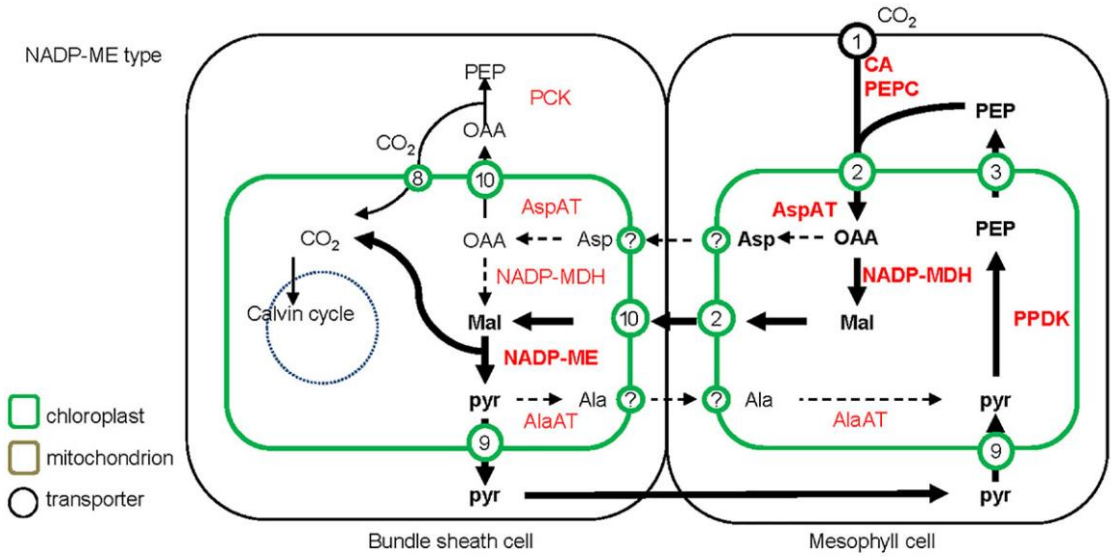
- Oxaloacetate is rapidly converted to the more stable C4 acids malate or Asp that diffuse to the bundle sheath cells.
- Here, CO_2 is released by one of three different decarboxylating enzymes.
- These define the 3 basic biochemical subtypes of C_4 photosynthesis:
  - NADP-dependent malic enzyme (NADP-ME),
  - NAD-dependent ME (NAD-ME), and
  - PEP carboxykinase (PEPCK).
- The released CO_2 is refixed by Rubisco, which exclusively operates in the bundle sheath cells in C_4 plants.
- The three-carbon compound resulting from CO_2 release diffuses back to the mesophyll cells.
- In the mesophyll cells, the primary CO_2 acceptor PEP is regenerated by pyruvate orthophosphate dikinase by the consumption of two molecules of ATP, at the end.

The C4 photosynthetic pathway



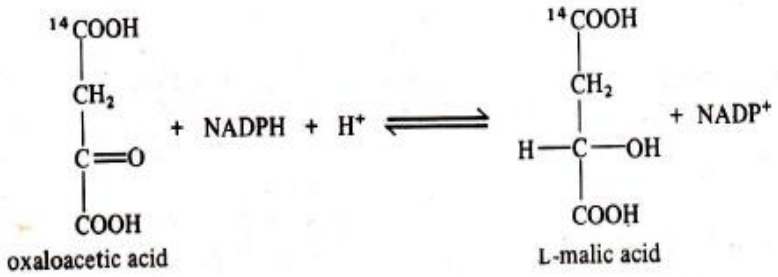
NADP-ME subtype of C4 photosynthesis

- Maize (*Zea mays*), sugarcane (*Saccharum spp.*), and sorghum (*Sorghum bicolor*) etc.
- Malate is the dominant transport metabolite while Asp can be used in parallel.
- The synthesis of malate occurs in the mesophyll chloroplasts, the decarboxylation by NADP-ME in the bundle sheath chloroplasts.
- The two other biochemical subtypes differ from the NADP-ME type by
  - the transport metabolites used and
  - the subcellular localization of the decarboxylation reaction.

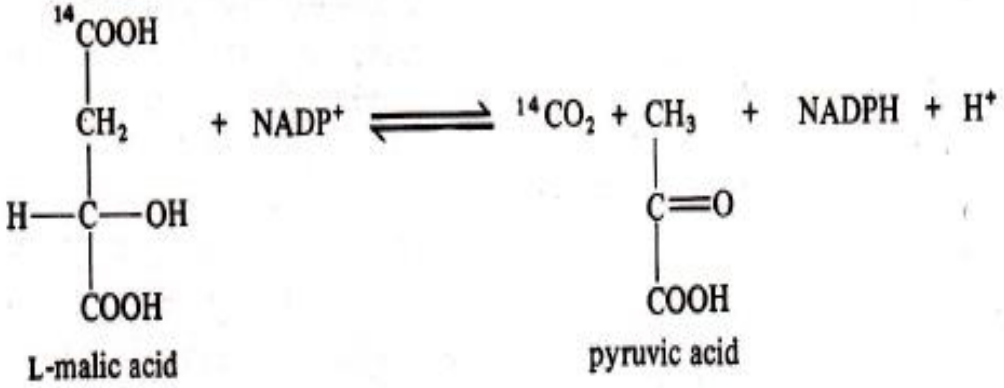


Details of the NADP-ME reactions

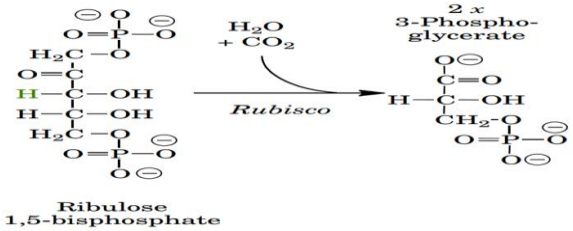
- The OAA passes into the mesophyll chloroplasts where NADP malate dehydrogenase catalyzes its reduction to malic acid using NADPH generated by the light phase of photosynthesis



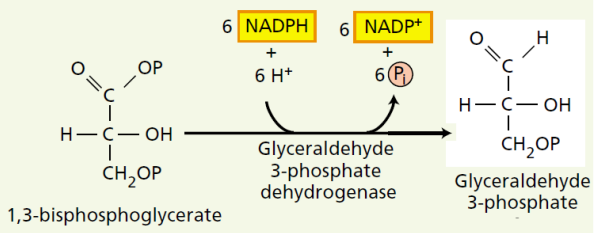
- NADP-malate dehydrogenase is activated in the presence of light.
- The malic acid then passes from the mesophyll chloroplasts into the chloroplasts of the bundle sheath cells by the plasmodesmata which connect the two types of cell.
- It is then decarboxylated by the NADP-specific malic enzymes to yield CO_2 and pyruvic acid.



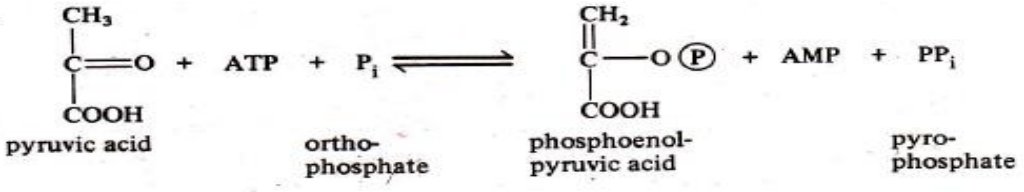
- The CO_2 becomes the substrate for RuDP carboxylase and is incorporated into 3-PGA which is then converted into F-6-P by the operation of the Calvin cycle.



- The NADPH produced is re-oxidized by using it as the reductant in the conversion of 1,3-diPGA into 3-PGAlD.

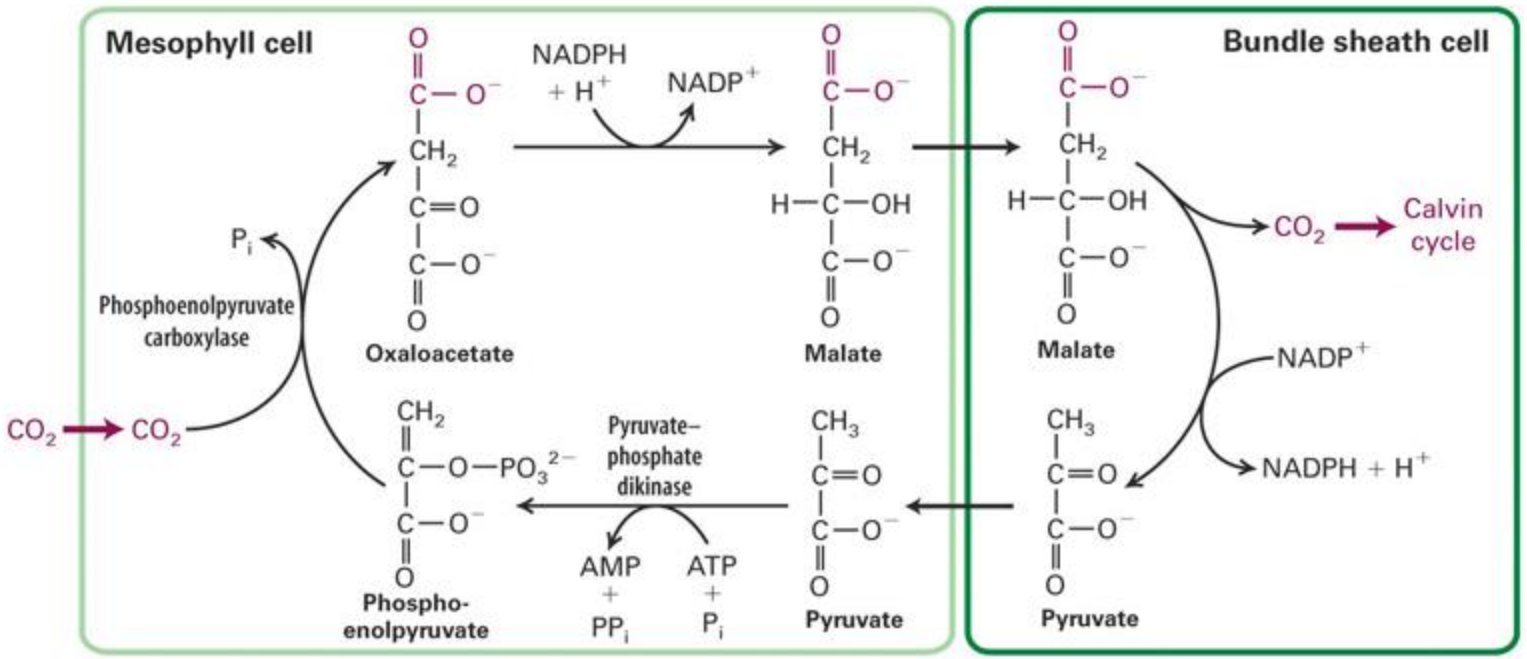


- The pyruvic acid produced passes back from the bundle-sheath chloroplasts into the mesophyll chloroplasts by the plasmodesmata.
- It is then converted into PEP by the enzyme pyruvate orthophosphate dikinase.



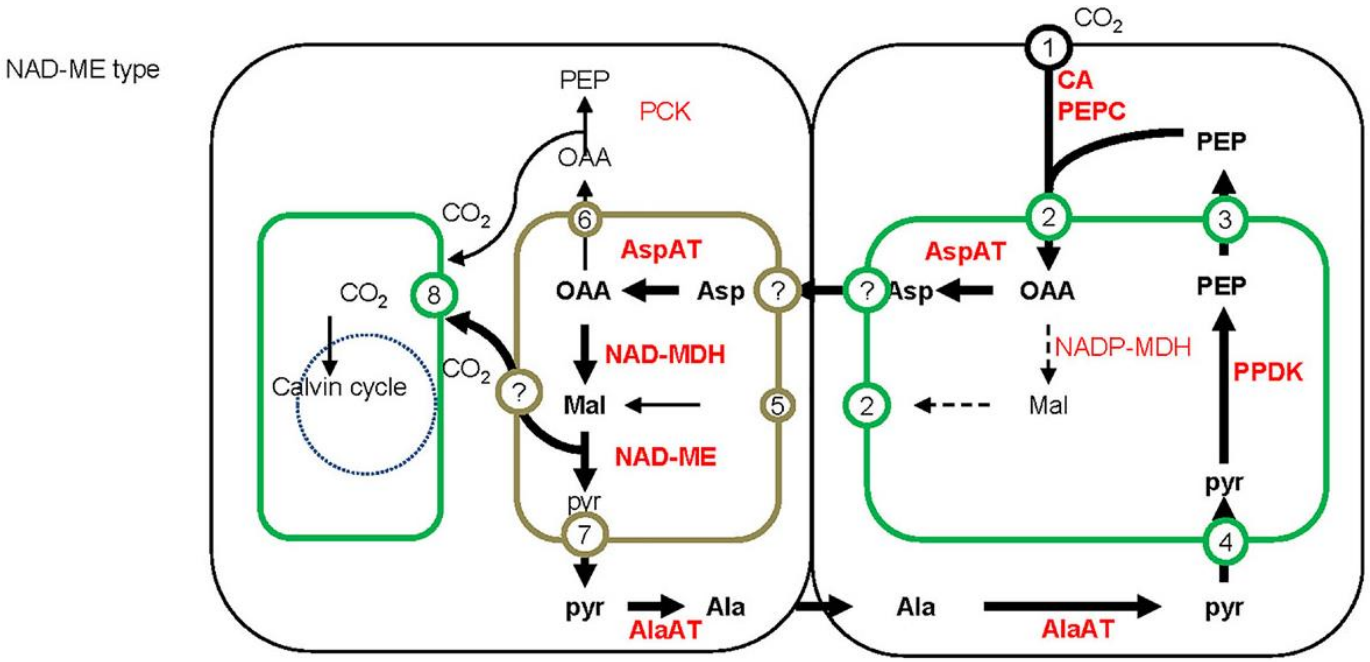
- The PEP diffuses from the chloroplast into the cytoplasm of the mesophyll cell and becomes the substrate for PEP carboxylase; the cycle of C4 photosynthesis is thus complete.

Chemical Reaction: NADP-ME



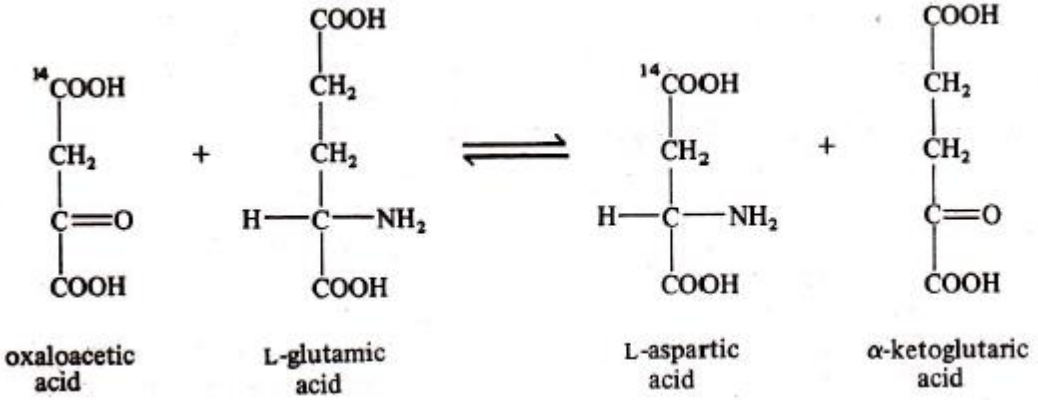
NAD-ME subtype of C4 photosynthesis

- Switch grass (*Panicum virgatum* L.), pearl millet [*Pennisetum glaucum* (L.) R. Br], and amaranth (Amaranthaceae) etc.
- In NAD-ME plants **Asp** is synthesized in the mesophyll cytosol.
- It is used as transport metabolite.
- After deamination and reduction, the resulting malate is decarboxylated by NAD-ME in the **bundlesheath mitochondria**.

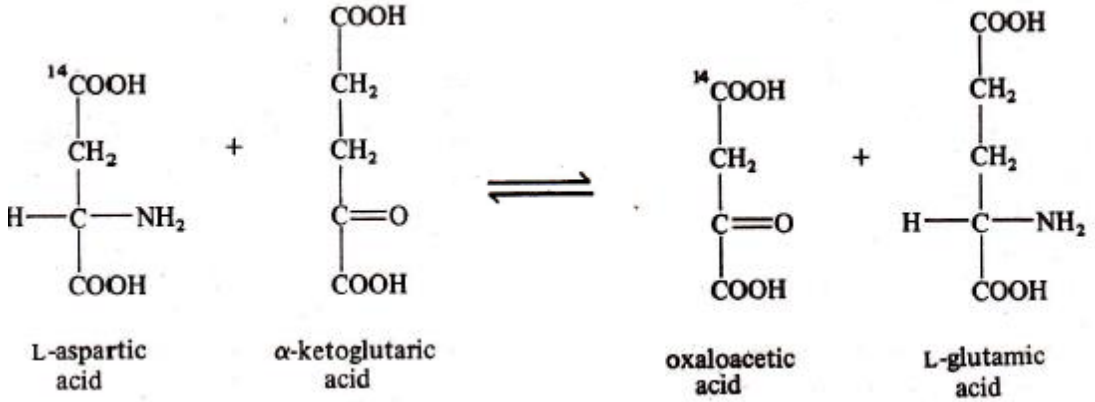


Details of the NAD-ME reactions

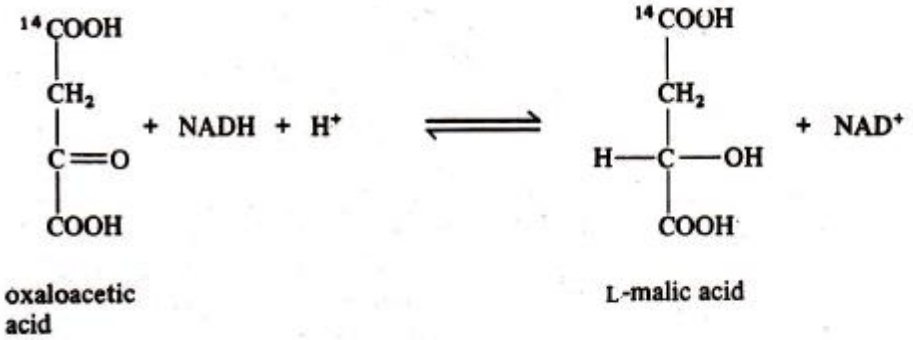
- OAA produced, is trans aminated by the cytoplasmic L-aspartate aminotransferase utilizing L-glutamic acid as the amino-donor forming L-aspartic acid and α-ketoglutaric acid.



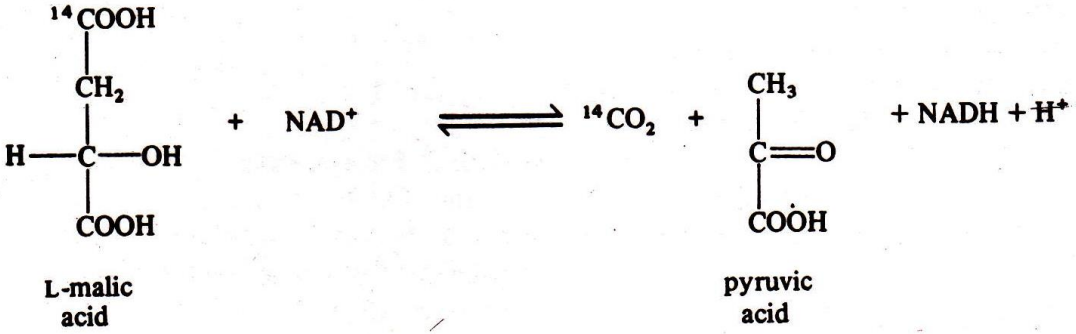
- The resulting aspartic acid then passes from the cytoplasm of the mesophyll cell to the mitochondria of the bundle-sheath cells via the plasmodesmata.
- There OAA is re-formed by reversal of the transamination reaction.



- The OAA is then reduced by the mitochondrial NAD specific malate dehydrogenase to L -malic acid

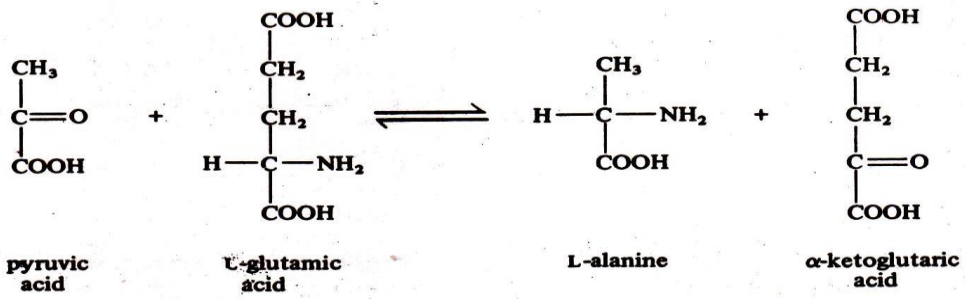


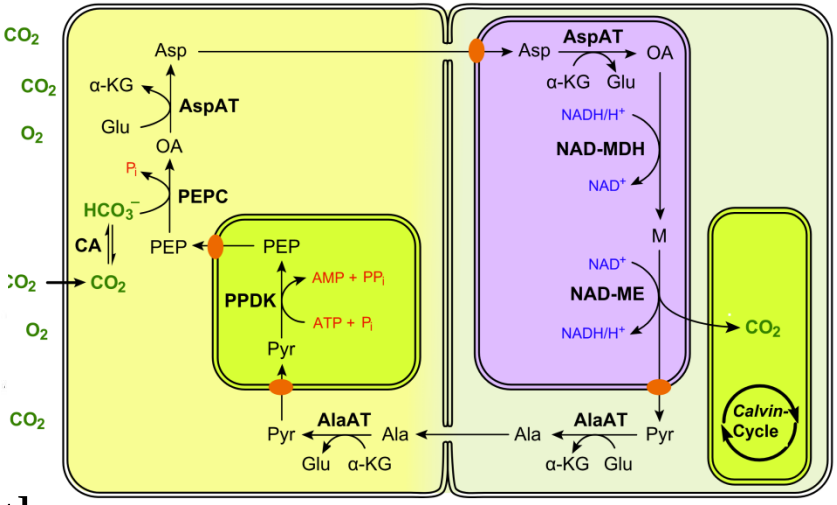
- The malic acid is decarboxylated by the mitochondrial NAD-malic enzyme to yield pyruvic acid and CO_2.



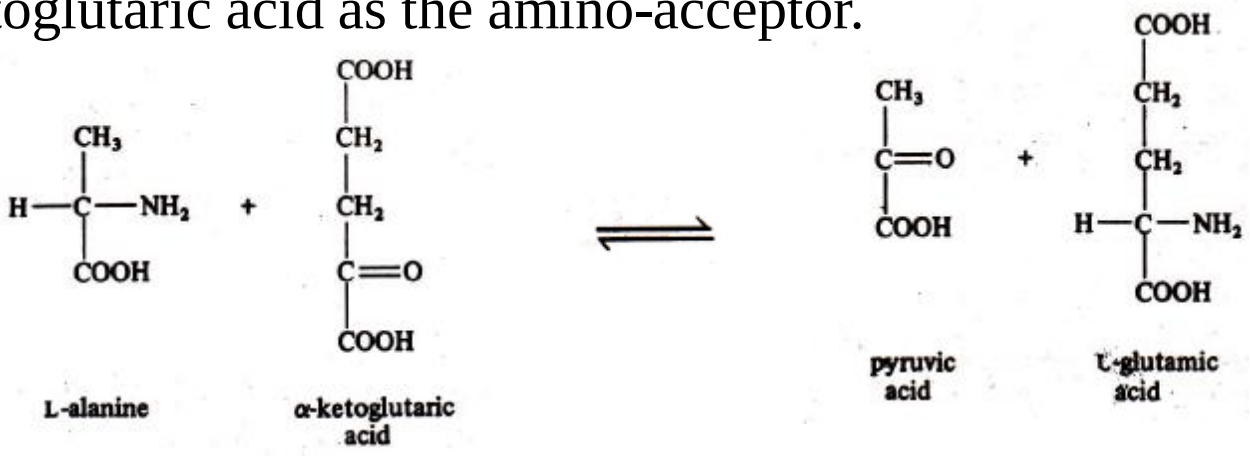
- The CO_2 diffuses from the **mitochondria** to the **chloroplasts** where it is incorporated into carbohydrate by the **Calvin cycle**.
- The pyruvic acid diffuses into the cytoplasm where it is trans-aminated by L-alanine aminotransferase utilizing L-glutamic acid as the amino donor.

- L-alanine and α-ketoglutaric acid are produced. The two transamination reactions usually balance one another.

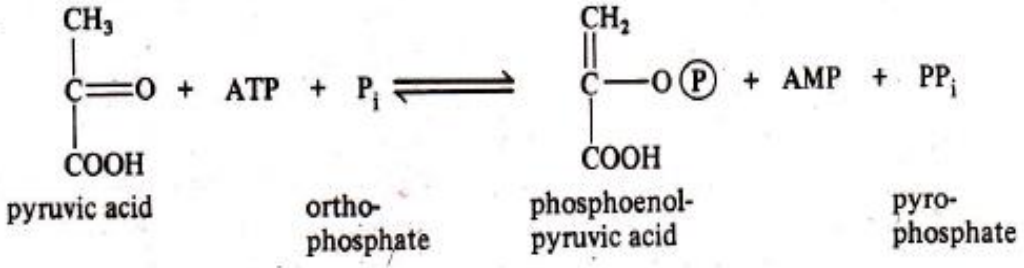


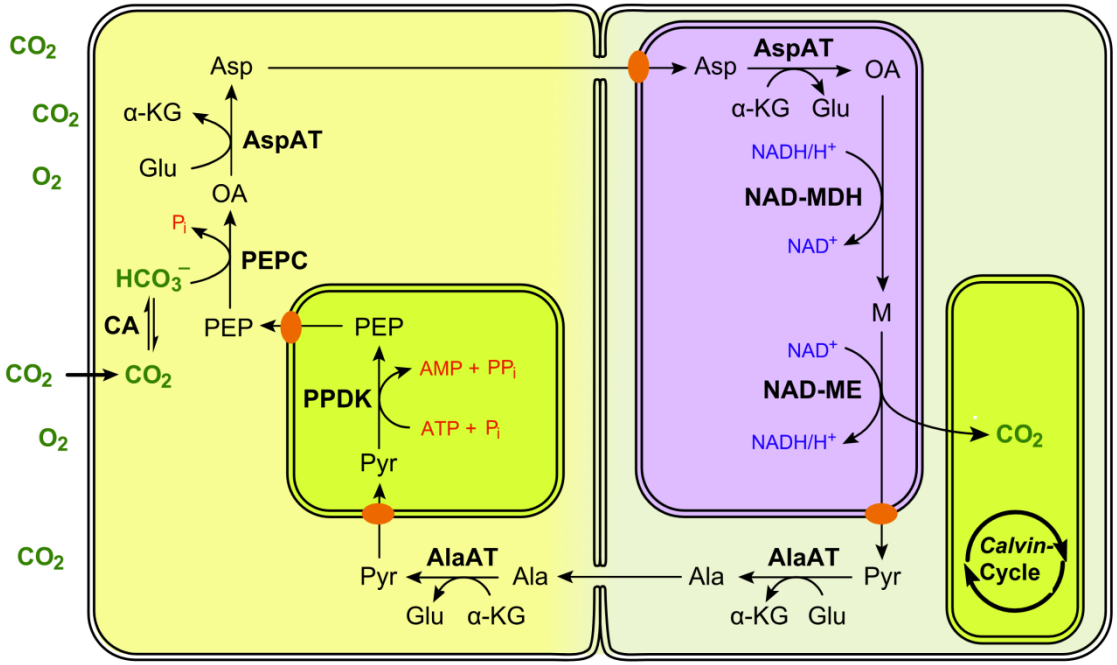


- The alanine passes from the cytoplasm of the bundle sheath cell to that of the mesophyll cell via the plasmodesmata.
- It is then converted into pyruvic acid by L-alanine aminotransferase utilizing the α-ketoglutaric acid as the amino-acceptor.



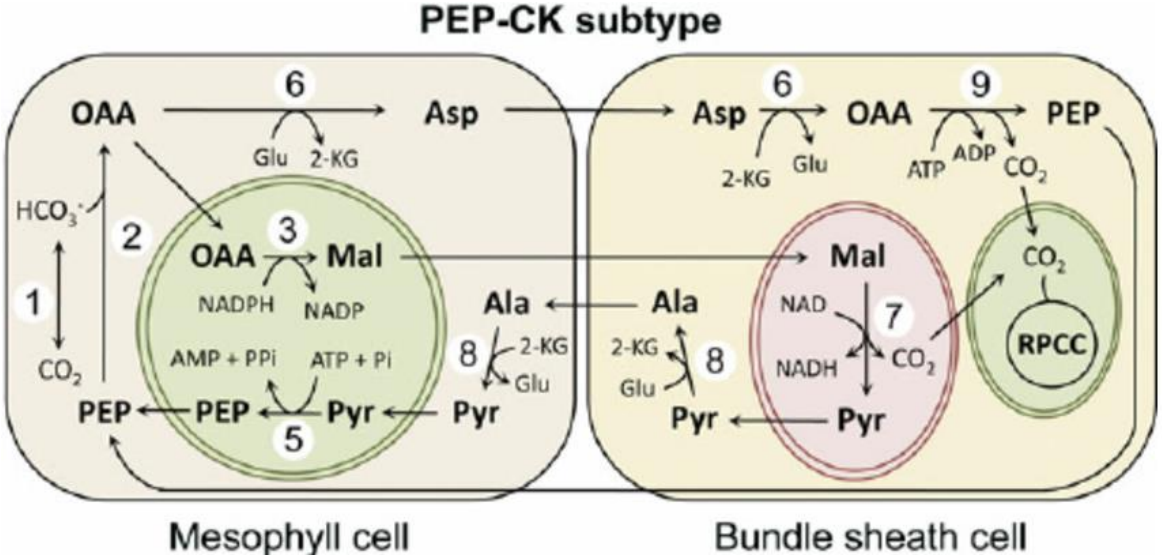
- The pyruvic acid then passes into the **mesophyll chloroplasts** for conversion into PEP by the mechanism as before, thus completing the cycle of C4 photosynthesis.





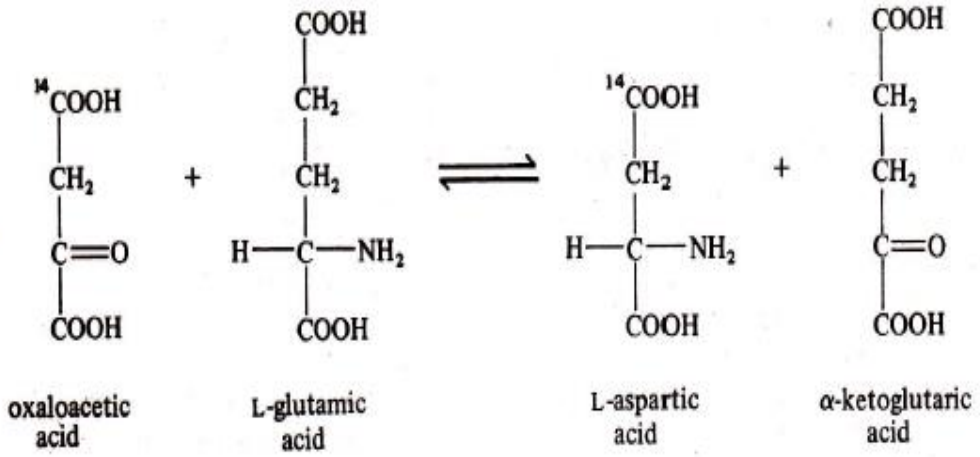
PEPCK type subtype of C4 photosynthesis

- Example: *Panicum maximum*, *Chloris gayana*, *Sporobolus fimbriatui*
- Plants of the PEPCK type use Asp as well as malate as transport metabolites.
- Asp is synthesized in the cytosol of mesophyll cells and decarboxylated in the cytosol of bundle sheath cells by the combined action of Asp amino transferase and PEPCK (RPCC, reductive photosynthetic carbon cycle).
- Malate is synthesized in the mesophyll chloroplasts but decarboxylated by NAD-ME in the mitochondria of bundle sheath cells.
- This reaction produces NADH that is used in the mitochondria to produce the ATP needed to drive the PEPCK reaction (Hatch, 1987).
- The three-carbon decarboxylation product, pyruvate, is transported back to the mesophyll cells in the form of Ala to maintain the ammonia balance between the two cell types



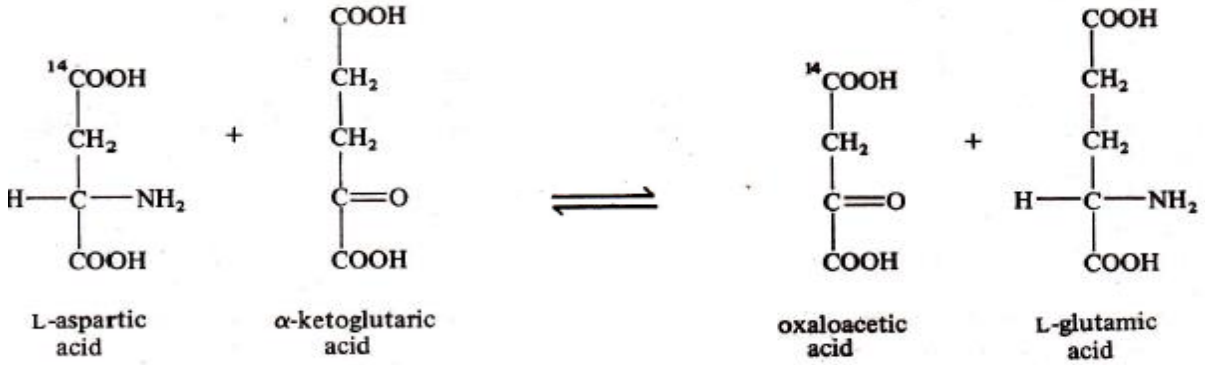
Details of the PEP Carboxykinase reactions

- The reaction sequence is very similar to that of the species utilizing the NAD-malic enzyme .
- The OAA produced is trans-aminated by the cytoplasmic L-aspartate aminotransferase utilizing L-glutamic acid as the amino-donor forming L-aspartic acid and α-ketoglutaric acid.

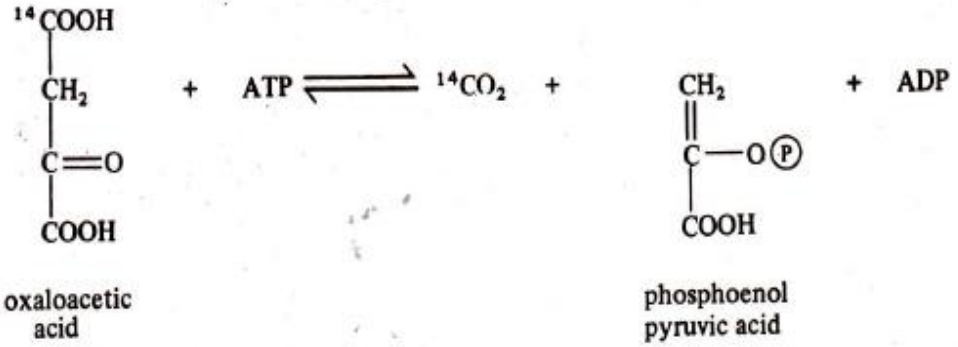


- The resulting aspartic acid then passes from the cytoplasm of the mesophyll cell to the **cytoplasm** of the **bundle-sheath cells** via the plasmodesmata.

- There OAA is re-formed by reversal of the transamination reaction.



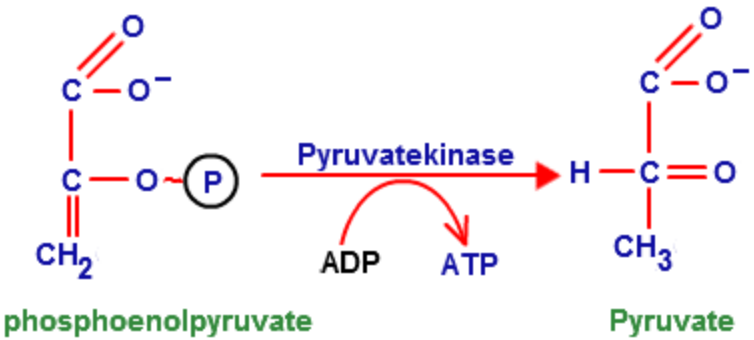
- The OAA produced by transamination of the L-aspartic acid imported from the mesophyll cells is decarboxylated by the enzyme PEP carboxykinase to yield CO_2 and PEP



- The CO_2 produced is utilized as the substrate for the Calvin cycle in the chloroplasts of the bundle-sheath cells.
- PEP, is converted into pyruvate which is then transaminated to yield L-alanine and translocated to the mesophyll cells.
- Alanine from the bundle-sheath cells is converted into PEP in the mesophyll cells (like the NAD-malic enzyme-utilizing species).

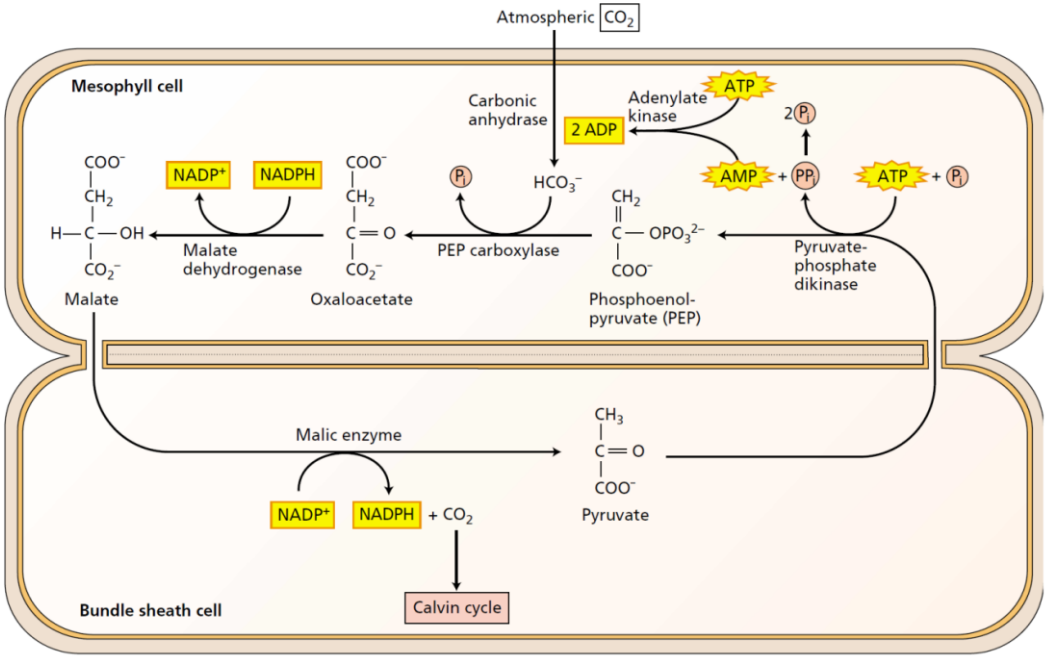
Stichoiometry of the C4 mode of Photosynthesis

- The operation of the Calvin cycle in the bundle-sheath cell is identical with that in the chloroplasts of C3 plants.
- Thus the stoichiometry is the same and 3ATP and 2NADPH are required for the fixation of each CO_2.
- In the C4 mode an additional 2ATP is required for the conversion of pynrvic acid into PEP in the mesophyll cells.
- In the PEP-carboxykinase species it is assumed that the ATP required by the decarboxylation reaction in the bundle-sheath cells is regenerated when PEP is converted into pyruvate (of glycolysis).
- There is, no extra requirement for NADPH.
- That this is so needs a little explanation in the case of the NADP-malic enzyme species.



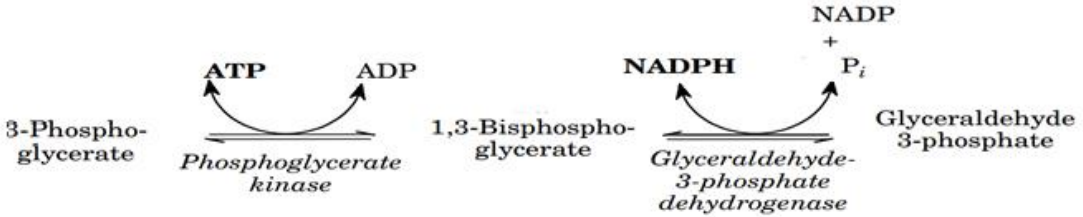
Stoichiometry of the NADP-ME type

- One molecule of photogenerated NADPH is utilized per CO_2 fixed during the reduction of **OAA to malic** acid in the mesophyll chloroplast.
- During the decarboxylation of that same malic acid in the bundle-sheath chloroplast one molecule of NADPH is generated per CO_2 produced.
- This NADPH is then utilized along with one photogenerated NADPH, to provide the 2NADPH required to fix one CO_2 in the Calvin cycle.
- So the C4 mode of photosynthesis requires 5ATP and 2NADPH for the fixation of each CO_2 (of 3ATP and 2NADPH per CO_2 in C3 plants).
- Thus the C4 mode of photosynthesis is less efficient than the C3 mode. This conclusion, does not accurately reflect the overall situation in the two types of plant because no account has been taken of the effect of photorespiration.
- The photorespiration rate in C4 plants is very much less than in C3 plants. Thus the net numbers of molecules of ATP and NADPH required to fix one molecule of CO_2 (i.e. the resultant of photosynthesis minus photorespiration) are considerably lower in C4 plants than in C3 plants.
- In the overall sense, therefore, C4 plants are more efficient assimilatory organism than C3 plants.

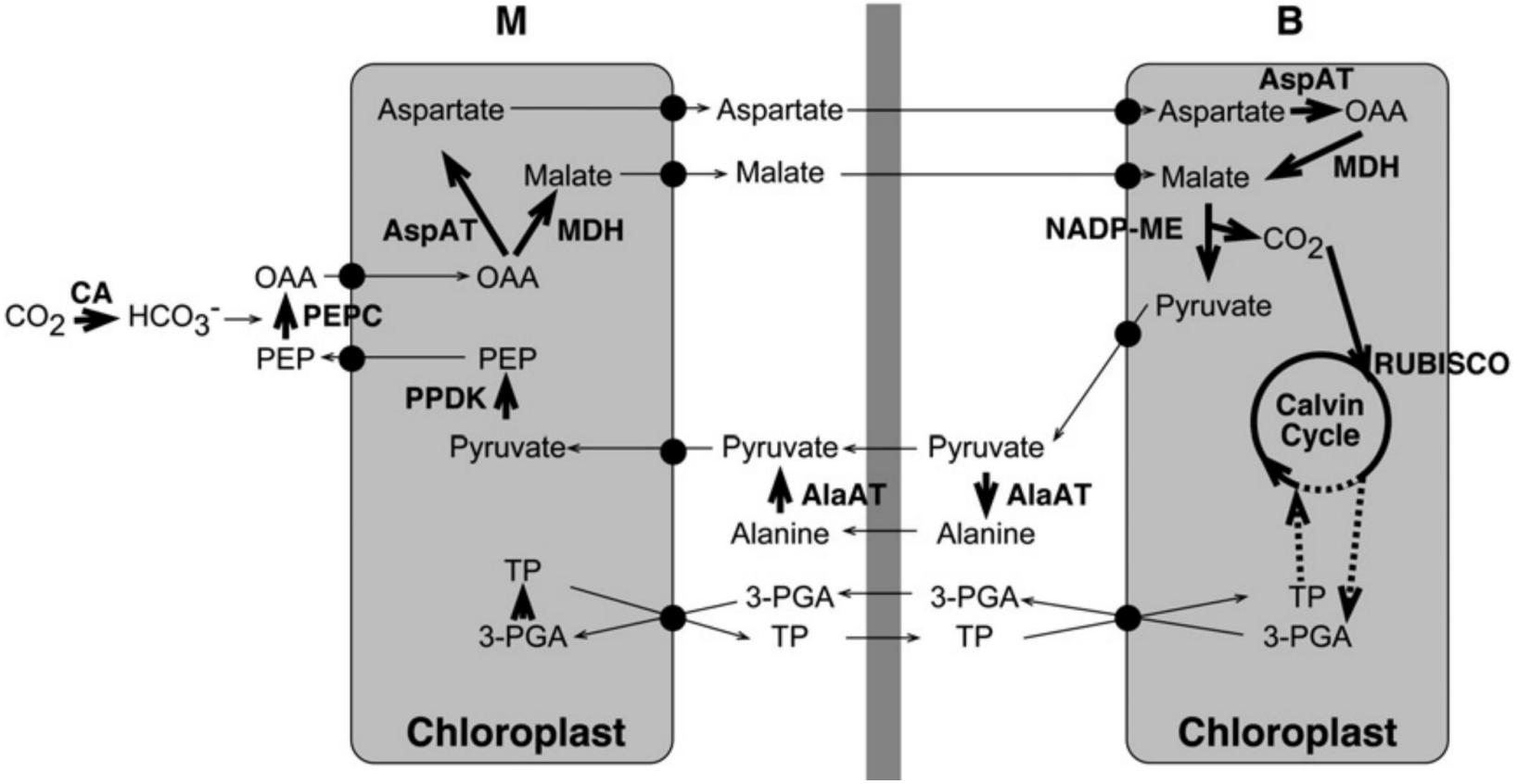


Bundle sheath cells: characteristic leaf anatomy

- Enlarged and a higher organelle content of these cells in most C4 species.
- A close contact between mesophyll and bundle sheath cells.
- They are tightly interconnected to each other by plasmodesmata.
- The bundle sheath cells enclose the vascular bundles and are themselves surrounded by the mesophyll cells.
- The internal anatomy of a C4 leaf is often composed of a repeating pattern of vein-bundle sheath-mesophyll-mesophyll-bundle sheath-vein.
- Because of its ***wreath-like structure*** this type of leaf anatomy was termed ***Kranz anatomy*** by the German botanist G. Haberlandt (1904).
- Kranz anatomy is found in nearly all monocotyledonous and dicotyledonous lineages that use the two-cell mode of C4 photosynthesis.
- C4 species of the NADP-ME subtype are depleted in PSII in their bundle sheath cells to lower oxygen production in these cells.
- The production of reduction equivalents in the bundle sheath cells is reduced and the reduction phase of the Calvin-Benson cycle (the conversion of **3-phosphoglycerate to triose phosphate**) has been at least partially shifted to the mesophyll cells.



NADP-ME type of C_4 photosynthesis



Photosynthetic efficiency of C3 and C4 plants and crop productivity

- The peculiar reactions of C4 photosynthesis (synthesis of a C4 compound by carboxylation of PEP), its translocation from mesophyll to bundle-sheath cell and its subsequent decarboxylation to regenerate CO_2, seem pointless and wasteful of 2ATP per CO_2 fixed.
- They have a purpose:
  - C4 photosynthesis serve to concentrate CO_2, in the bundle-sheath chloroplasts.
  - High rate of CO_2 fixation is observed in C4 plants.
  - C4 plants have a higher stomatal resistance to gaseous diffusion than C3 plants.
  - This causes a much greater CO_2 concentration gradient, during steady-state photosynthesis, between the atmosphere and the mesophyll cell surface in C4 plants than that in C3 plants.
  - The CO_2 concentration in the mesophyll cell is high enough in C_3 plants to allow RuBP carboxylase to operate satisfactorily but would be far too low to allow this in C4 plants.
  - The CO_2 concentration in C4 plants (though low) is high enough for the satisfactory operation of ***PEP carboxylase***.
  - Hence PEP carboxylase is used to raise the low concentration of CO_2 in the mesophyll cell to a concentration in the bundle-sheath cell high enough to allow RUDP carboxylase to function satisfactorily.

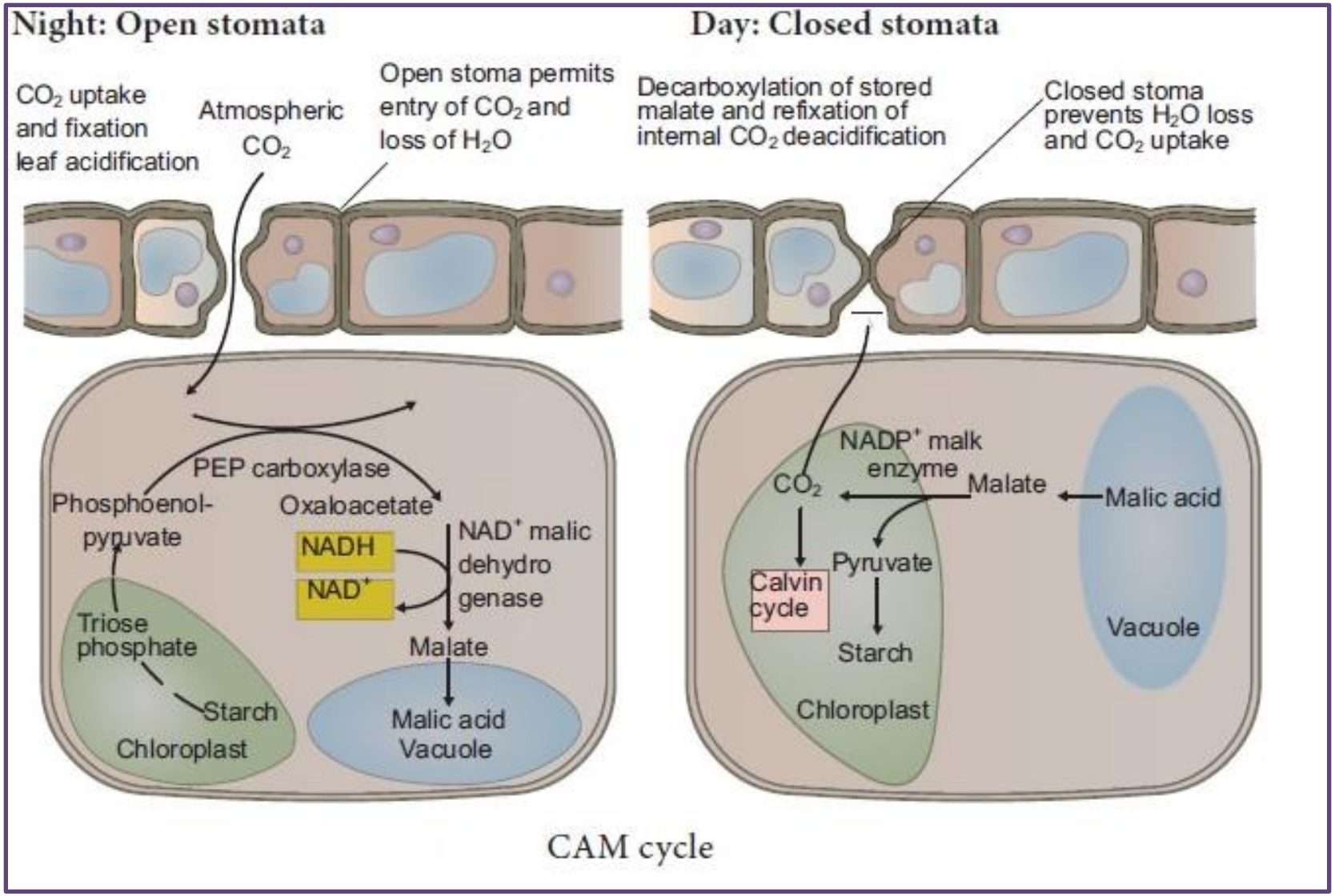
- A higher stomatal resistance reduces the **loss of water** from the plant through transpiration.
- This saving in water allows the C4 plant to grow in **hotter**, **drier** environments than C3 plants, but its penalty is that CO_2 uptake is more difficult.
- This difficulty in CO_2 uptake, in turn, leads to the requirement for a CO_2 -concentrating mechanism.
- From this line of reasoning C4 photosynthesis, is an evolutionary adaptation to hotter, drier conditions.
- Another advantage to the C4 plant of concentrating CO_2 in the bundle-sheath cells is
  - it depresses photorespiration,
  - reduces the considerable loss (20-40%) of photosynthetically fixed carbon seen in C3 plants.
- RuBP carboxylase also has RuBP oxygenase activity; that catalyses the-oxygen-dependent cleavage of RuBP into 3-PGA and phosphoglycollic acid
- Since CO_2 and O_2 are competitive substrates for RuBP carboxylase, a higher CO_2:O_2 concentration resulting from CO_2 concentration within the bundle-sheath chloroplast will favour the carboxylase activity and depress the oxygenase activity, thereby decreasing photorespiration.

Crassulacean Acid Metabolism (CAM)

- Succulents plants exhibit Crassulacean Acid Metabolism.
- Many belong to the dicotyledonous family Crassulaceae,
  - e.g. *Kalanchoe* spp.,
  - *Sedum* spp.
- Other dicotyledonous families
  - Aizoaceae,
  - Asclepiadaceae,
  - Cactaceae,
  - Calyophyllaceae,
  - Chenopodiaceae,
  - Compositae,
  - Convolvulaceae,
  - Euphorbiaceae,
  - Plantaginaceae etc.
- To the monocotyledonous families,
  - Agavaceae,
  - Bromeliaceae,
  - Liliaceae and
  - Orchidaceae

Characteristics of CAM Plants

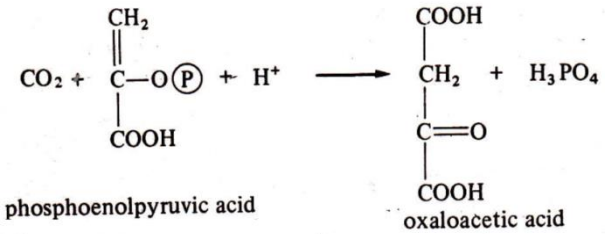
- Normally:
  - their stomata are open during the night (i.e. in the dark) and
  - closed during the day (reverse of those of non-CAM plants).
- At Night:
  - CO_2 is fixed during the hours of darkness in chloroplast-containing cells of photosynthetic leaf or stem tissue and
  - Considerable quantities of free L-malic acid are synthesized.
  - This malic acid is stored in the large vacuoles which are characteristic of the cells of CAM plants.
- During the ensuing hours of daylight:
  - the malic acid is decarboxylated and
  - the resulting CO_2 converts into sucrose and storage glucan (e.g. starch) by light-driven, C_3 photosynthesis.
- During the next period of darkness
  - some of the storage glucan present in the cell is catabolized to provide an acceptor molecule for the dark CO_2-fixation reaction.
- Thus, CAM tissues show a ***diurnal cycle***:
  - The level of malate rises and the level of storage glucan falls during the night and
  - The converse occurs during the day.


Night: Open stomata
Day: Closed stomata
CO_2 uptake and fixation leaf acidification
Atmospheric CO_2
Open stoma permits entry of CO_2 and loss of H_2O
Decarboxylation of stored malate and refixation of internal CO_2 deacidification
Closed stoma prevents H_2O loss and CO_2 uptake
PEP carboxylase
Phosphoenol-pyruvate
Oxaloacetate
NADH
NAD^+
NAD^+ malic dehydro genase
Triose phosphate
Starch
Chloroplast
Malate
Malic acid
Vacuole
$NADP^+$ malk enzyme
CO_2
Malate
Malic acid
Pyruvate
Calvin cycle
Starch
Chloroplast
Vacuole

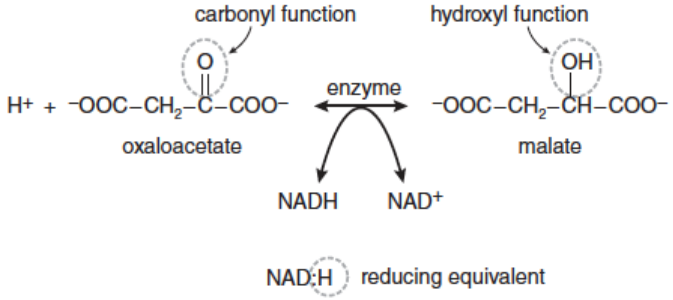
CAM cycle

Biochemistry of CAM Plants

- During the night CO_2 enters the tissue through the open stomata.
- Once within the cells of the tissue the CO_2 reacts with phosphoenolpyruvate (PEP) under the catalytic influence of ***PEP carboxylase*** to form oxaloacetic acid (OAA).

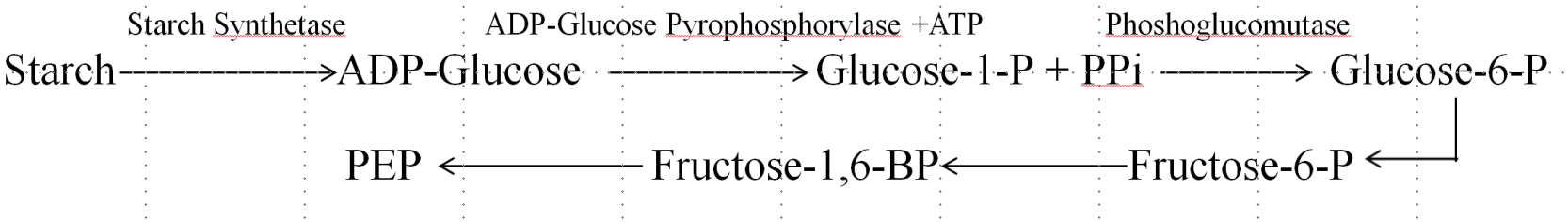


- The enzyme is allosterically **inhibited** by **malate:** regulation of CAM.
- **Malate dehydrogenase** then catalyses the reduction of OAA by NADH



- Malic acid is actively transported across the tonoplast membrane into the vacuole.
- This keeps the cytoplasmic malic acid concentration below the $K_{i\ (malate)}$ of PEP carboxylase.
- The production of malic acid at the expense of CO_2 and PEP proceeds throughout the night but its rate slackens off as dawn approaches.

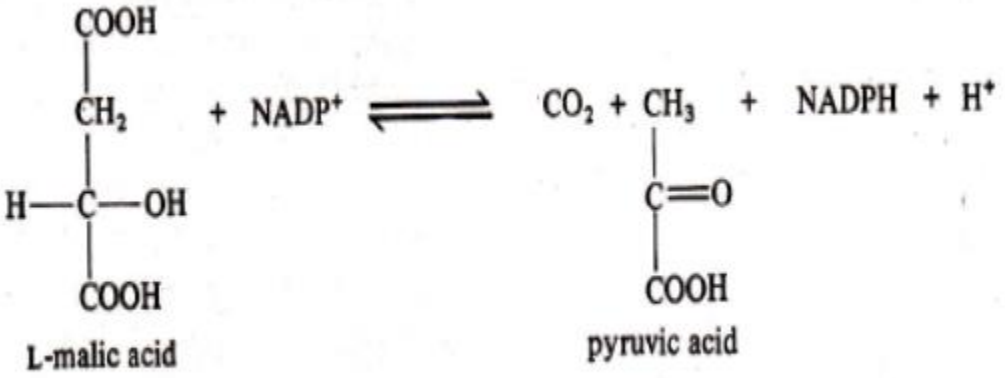
- The source of PEP is the storage glucan produced during the previous light period.



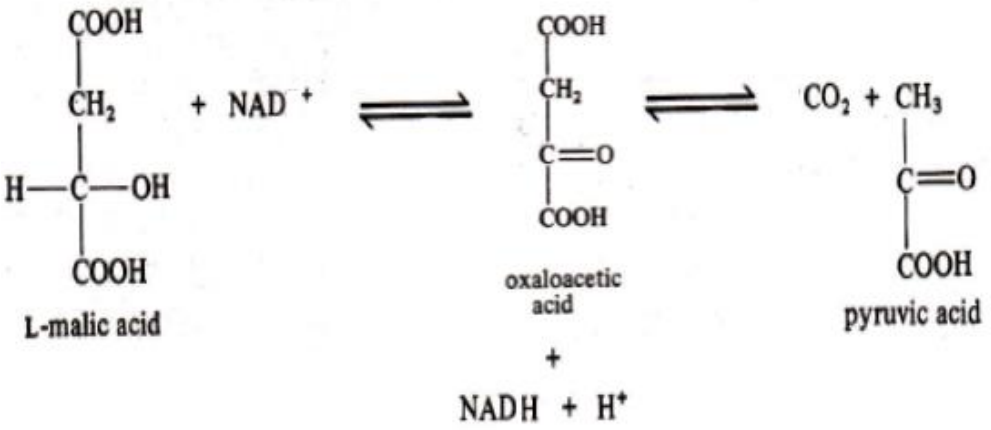
- During the following day malic acid passes from the vacuole back into the cytoplasm and is decarboxylated to provide CO_2 for light-driven C_3 photosynthesis.

Malic Acid $\longrightarrow$ CO_2 and Pyruvic Acid

- Two **different** decarboxylation mechanisms are used in CAM although they never occur together in the same plant species.
  - Members of the Crassulaceae, Cactaceae and Agavaceae decarboxylate malic acid directly utilizing the ***NADP-malic enzymes*** which produces CO_2 and pyruvate.



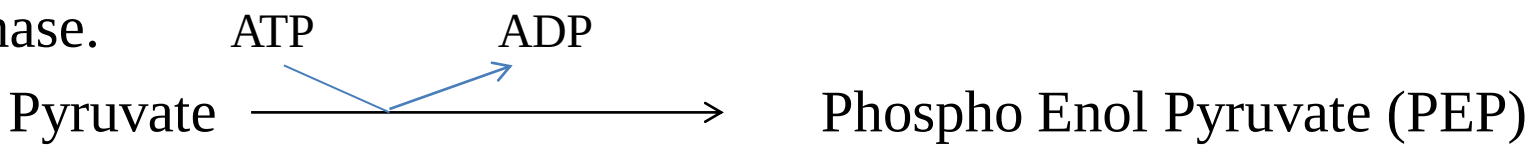
- Members of the Liliaceae, Bromeliaceae and Asclepiadaceae do not possess the NADP-malic enzyme but instead have a very active ***PEP carboxykinase***.
  - In these families malic acid is firstly oxidized to OAA by a malate dehydrogenase.
  - The OAA is then converted into CO_2 and PEP-with the utilization of ATP by PEP carboxykinase.



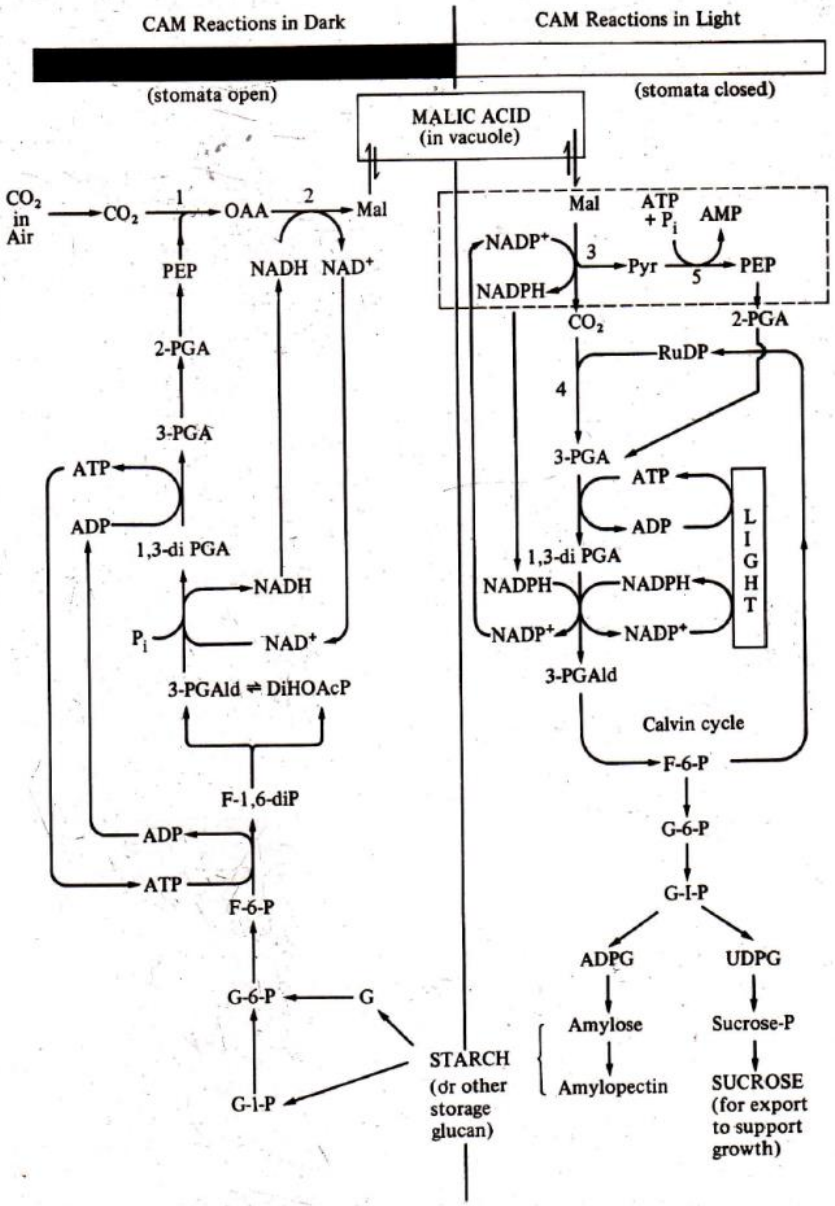
- In the family Euphorbiaceae some species use the PEP carboxykinase is the decarboxylating mechanism whilst others use the NADP-malic enzymes.

- The CO_2 produced by malic acid decarboxylation is fixed into 3-phosphoglycerate (3-PGA) by RuBP carboxylase (Calvin cycle): enzymes located in the chloroplast.

- ***Fructose 6-phosphate*** is used for the synthesis of storage glucan, e.g. starch.
- The starch is stored in the chloroplast during the day and then utilized as a source of PEP for the dark carboxylation reaction during the following night.
- ***DHAP***
  - is tapped off the Calvin cycle and
  - It passes from the chloroplast to the cytoplasm, where it is converted, via F-6-P, into sucrose which is then exported from the cell and utilized for growth of the plant.
- The pyruvate produced are utilized for carbohydrate synthesis during the day.
- The pyruvate is firstly converted into PEP by pyruvate orthophosphate dikinase.



- This enzyme is not not found in those CAM plants which utilize PEP carboxykinase, since PEP is produced directly.
- PEP in both types of CAM plant is then converted via' reversed glycolysis into 3-PGA which is then utilized in the Calvin cycle.



Pathways of crassulacean acid metabolism during darkness and daylight. (1 = PEP carboxylase; 2 = malate dehydrogenase; 3 = NADP malic enzyme; 4 = RuDP carboxylase; 5 = pyruvate, orthophosphate dikinase, activated by light—see Section D.2(iv). Reactions within dashed rectangle occur as such in CAM plants of Crassulaceae, Cactaceae and Agavaceae but not in other families, see text.)

Regulation of CAM

- There are apparently two types of CAM regulation which may be termed
  - Short-term regulation: it refers to the regulation of CAM during the daily cycle.
  - Long term regulation: It refers to regulation over periods longer than a day (e.g. a week, several weeks or a season).

Short-term regulation of CAM

- Towards the end of the day: PEP carboxylase begins to generate malic acid by the dark fixation of CO_2.
- At this time the vacuolar concentration of malic acid is low (depleted during the light period).
- So, malic acid can be readily actively transported into the vacuole.
- This has two effects:
  1. It keeps the concentration of malic acid in the cytoplasm low thus preventing the allosteric inhibition of PEP-carboxylase and
  2. It increases the concentration of malic acid in the vacuole: It causes the osmotically driven uptake of water into the vacuole.
- This in turn increases the turgor pressure.
- When a critical turgor pressure is reached:
  - The net influx of malate into the vacuole is switched to net efflux.
  - The malic acid so released into the cytoplasm and
  - Inhibits PEP-Carboxylase
  - Switching off dark fixation of CO_2 into malic acid.
- The malic acid now in the cytoplasm is decarboxylated and the resulting CO_2 (and 3C compound) utilized in C3 photosynthesis.
- This process will eventually use up the malic acid produced by dark CO_2 fixation and so lower the cytoplastnic malic acid concentration to below the $K_{i\ (malate)}$ of PEP carboxylase.
- This allows PEP carboxylasels to function once again and the cycle to repeat itself.

Long-term regulation of CAM

- The most important factor in the long-term regulation of CAM is the *availability of water*.
- The cacti which can survive and grow in some of the most arid desert regions in the world.
- During the daytime when it is extremely hot
  - The cacti close their stomata.
  - This virtually prevents any gaseous exchange between the plant and its environment.
  - Thus evaporative water loss and CO_2 influx are almost zero.
  - CO_2 is being provided for C3 photosynthesis by malic acid decarboxylation.
- During the night, when evaporative demand is much lower,
  - The stomata may or may not open.
- Whether they open depends upon the availability of water.
- If there has been recent rainfall the stomata open and CO_2 can be taken in and malic acid formed.
  - During the daylight this malic acid is used for C3 photosynthesis.
  - There is a net production of carbohydrate and
  - Growth of the cactus can take place.

- If, there has been no rainfall for some time:
  - The stomata remain tightly shut since even the lower evaporative water loss of the night could not be made up by root absorption.
  - No CO_2 uptake during the night and
  - Therefore no net carbohydrate production during the following day.
  - Under these conditions there is no growth;
  - The cactus can achieve is to survive.
  - This it can do for periods of months and years.
  - During the day malic acid is decarboxylated to provide CO_2 for C3 photosynthesis until it is depleted.
  - During the night the starch formed during the preceding day is converted into CO_2 and PEP by the normal respiratory processes of glycolysis and operation of the TCA cycle.
  - The CO_2 and PEP are then converted back into malate.
  - The diurnal fluctuation of malic acid under these conditions is very much lower than that under conditions of water availability.
  - This recycling of CO_2 through the CAM and the other pathways is frequently referred to as '***idling***' and persists throughout the period of drought.

- With the coming of the first rainfall the cactus:
  - Takes up water,
  - Its tissues rehydrate and
  - The stomata recommence their night-time opening thus allowing the net intake of CO_2 and
  - The transition from idling to productive CAM.
- Some CAM plants grow in regions where water availability is less of a problem (tropical regions).
  - These plants appear to shift from CAM to C3 photosynthesis during periods of water abundance and
  - Then back again to CAM as the water supply diminishes and
  - Is characterized by the reversal of the stomatal opening and shutting pattern, i.e. from night opening and day.
- It appears that CAM is an adaptation which allows plants to survive and grow in extremely arid environments.
- There would therefore appear to be two types of CAM plants:
  - Obligate or constitute CAM plants (e.g. the cacti) and
  - Facultative or inducible CAM plants (e.g. *Mesembryanthemum crystallinium*).

Comparisons of C_3,C_4 and CAM plants

- C_3, C_4 and CAM plants all use the Calvin cycle to make sugars from CO_2. These pathways for fixing CO_2 have different advantages and disadvantages and make plants suited for different habitats. The C_3 mechanism works well in cool environments, while C_4 and CAM plants are adapted to hot, dry areas.
- Both the C_4 and CAM pathways have evolved independently over time and they give plant species a significant evolutionary advantage, in hot climates.

Type	Separation of initial CO_2 fixation and Calvin cycle	Stomata open	Best adapted to
C_3	No separation	Day	Cool, wet environments
C_4	Between mesophyll and bundle-sheath cells (in space)	Day	Hot, sunny environments
CAM	Between night and day (in time)	Night	Very hot, dry environments

The Effect of Temperature on the Rate of Photosynthesis

- The rate of photosynthesis is critically dependent upon **temperature, pH and intensity of light**. The photosynthetic rate is usually measured **indirectly** by detecting the amount of carbon dioxide released by plants.
- Optimum photosynthetic rates lead to the removal of greater amounts of carbon dioxide from the local atmosphere, producing greater amounts of glucose. Since glucose levels within plants are difficult to measure, scientists utilize the amount of carbon dioxide assimilation or its release as a means to measure photosynthetic rates.
- **Low Temperature**
  - Enzymes are protein molecules to carry out biochemical reactions. The proteins are folded into a very particular shape, and this allows them to bind efficiently to the molecules of interest.
  - At low temperatures, between 32 and 50 degrees Fahrenheit (**0 and 10 degrees Celsius**) the enzymes that carry out photosynthesis do **not work efficiently** and this decreases the photosynthetic rate.
  - This leads to a decrease in glucose production and will result in stunted growth.
- **Medium Temperatures**
  - At medium temperatures, between 50 and 68 degrees Fahrenheit (**10 and 20 degrees Celsius**) the photosynthetic enzymes work at their **optimum** levels, so photosynthesis rates gauge high.
- **High Temperatures**
  - At temperatures above 68 degrees Fahrenheit (**20 degrees Celsius**) the rate of photosynthesis decreases because the enzymes do not work as efficiently at this temperature. This is despite the increase of carbon dioxide diffusion into leaves.
  - At a temperature above 104 degrees Fahrenheit (**40 degrees Celsius**) the enzymes that carry out photosynthesis lose their shape and functionality, and the photosynthetic rate declines rapidly.

Adaptive features:

- Across section of a typical C3 leaf reveals one major cell type that has chloroplasts, the mesophyll. In contrast, a typical C4 leaf has two distinct chloroplast-containing cell types: mesophyll and bundle sheath (or Kranz, German for “wreath”)
- There is considerable anatomic variation in the arrangement of the bundle sheath cells with respect to the mesophyll and vascular tissue.
- Operation of the C4 cycle requires the cooperative effort of both cell types.
- No mesophyll cell of a C4 plant is more than two or three cells away from the nearest bundle sheath cell.
- An extensive network of plasmodesmata connects mesophyll and bundle sheath cells, thus providing a pathway for the flow of metabolites between the cell types.
- Their vascular bundles are surrounded by two rings of cells; the inner ring, called bundle sheath cells, contains starch-rich chloroplasts lacking grana, which differ from those in mesophyll cells present as the outer ring.
- The chloroplasts are called dimorphic.
- The primary function of kranz anatomy is to provide a site in which CO2 can be concentrated around RuBisCO, thereby avoiding photorespiration.
- In order to maintain a significantly higher CO2 concentration in the bundle sheath compared to the mesophyll, the boundary layer of the kranz has a low conductance to CO2, a property that may be enhanced by the presence of suberin.

Summary of notable characteristics, advantages and features of Kranz type pathways in comparison to the C3 pathway

Recap	C_3	Kranz C_4
Rubisco localisation	Leaf mesophyll cell chloroplasts	Leaf bundle sheath cell chloroplasts
PEPcasea	Present but does not have photosynthetic function	Initial fixation of atmospheric CO_2. Located in mesophyll cell cytoplasm
Leaf anatomy	No Kranz anatomy	Kranz anatomy, dimorphic cells and chloroplasts
Initialcarbon molecule fixed from atmospheric CO_2 has :	3 Carbon 3-PGA	4 Carbon acid
Photorespiration	Present, high in high temperature arid environments	Greatly reduced or absent
Mesophyll cells	Primary leaf photosynthetic cells	Outer layer of photosynthetic cells that surround leaf bundle sheath cells. Chloroplasts present, pump C_4 acids to bundle sheath.
Bundle sheath cells	Present, but few chloroplasts, minimal photosynthetic function	Usually larger than M cells, more abundant and larger chloroplasts. Decarboxylate C_4 acids and refix CO_2
Calvin–Benson cycle located	Leaf mesophyll cells	Bundle sheath cells
Energy cost	Lower	Higher, by 2 ATPs consumed for every atmospheric CO_2 molecule fixed
Nitrogen use	Less efficient than C_4	More efficient than C_3
Hot aride Environments	Less adaptive thanC_4	More adaptive than C_3
CO_2 compensation	Lower than C_4	Higher than C_3
C-12 and C-13 discrimination	More discrimination against C-13 due to initial fixation by Rubisco	Less discrimination against C-13, due to initial fixation by PEPcase

Thank you