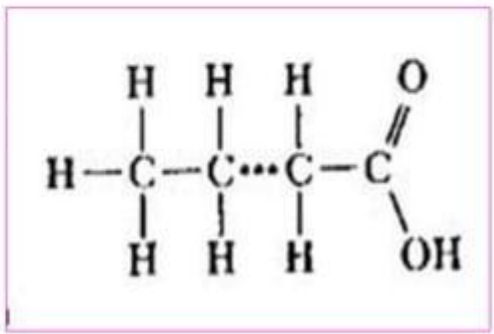
5.1. Synthesis and breakdown of triglycerides, ß-oxidation, glyoxalate cycle, gluconeogenesis and its role in mobilization of the lipids during seed germinations, α- oxidation.

Introduction

- Building block of lipids
- C,H,O Element present
- High amount energy other then carbohydrates and proteins
- **Fatty acid** is a carboxylic acid with a long chain hydrocarbon side groups.



- Fatty acid are bind or attach with different group of biomolecule & make complex molecule such as Glycolipids, Phospholipids, Sterols.
- Simple form is Triglycerols.
- Chemically diverse group.
- Common feature is insolubility in water

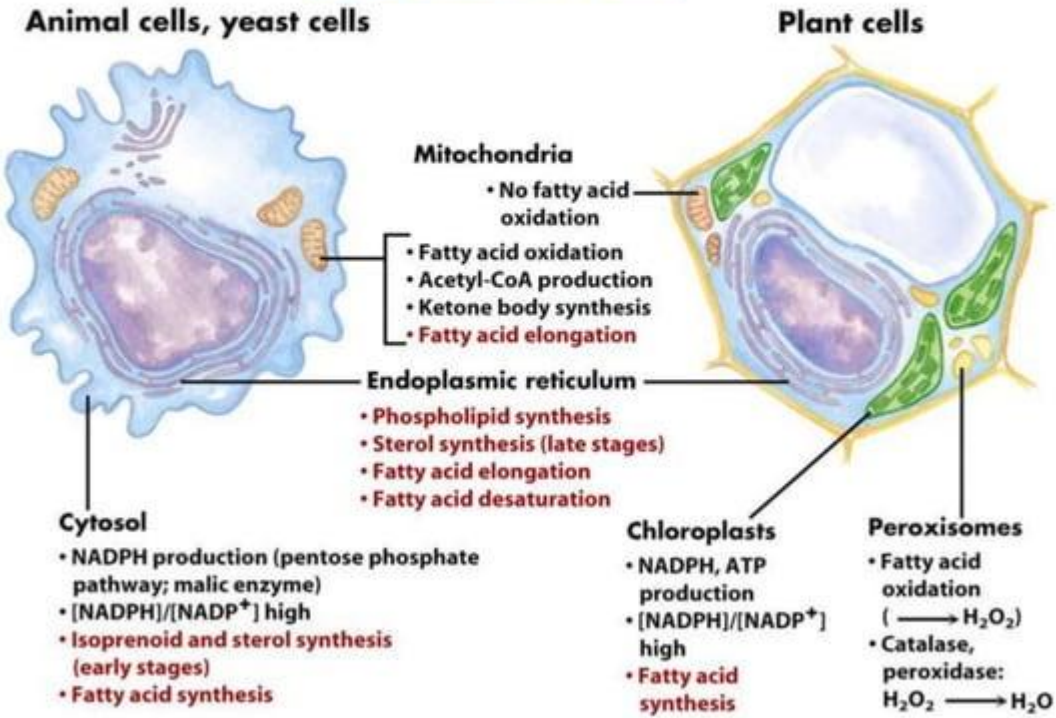
Types

- Based on Carbon bond-
  1) saturated
  2) unsaturated

- Based on Requirement-
  1) Essential
  2) Non essential

- Length of chain-
  1) SCFA
  2) MCFA
  3) LCFA
  4) VLCFA

Localization



Biosynthesis

- In 1945 David Rittenberg and Konrad Bloch demonstrated through isotopic labelling techniques

- In 1950 Sahil Wakil discovered a requirement for bio carbonate in fatty acid biosynthesis and malonyl-CoA was shown to be an intermediate

- In vertebrates its happen into cytosol but in plant and bacteria its occur into chloroplast.

- The pathway of biosynthesis is not exactly reverse as β oxidation.

- Fatty acid breakdown and biosynthesis occur into different compartments of cells, catalysed by different pathways & catalysed by different enzymes

Enzymes & co factors

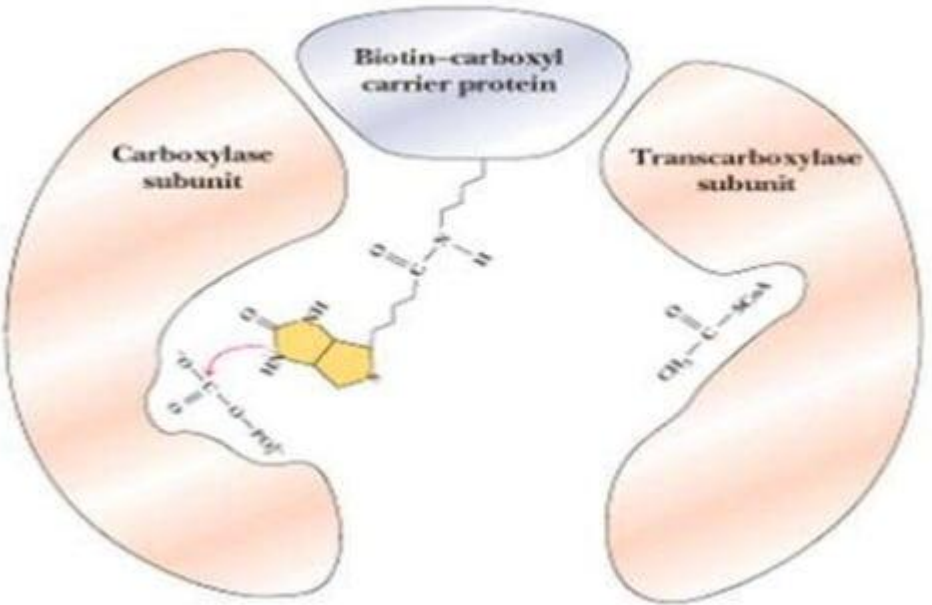
Two main enzyms-

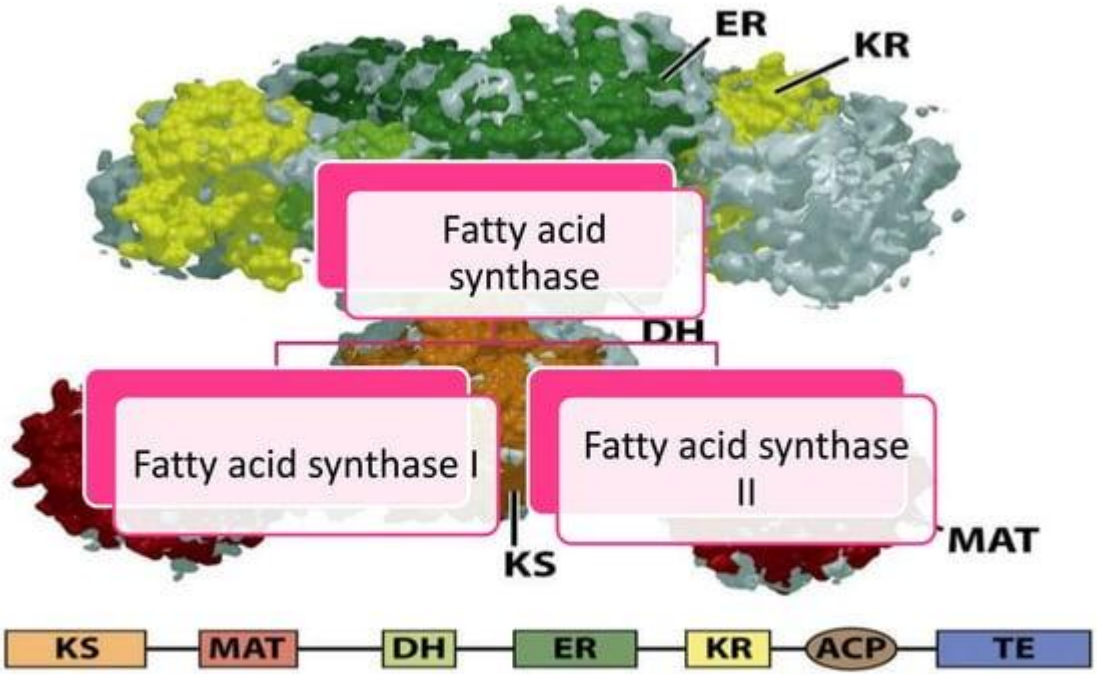
- ✓ 1)acetyl CoA carboxylase
- ✓ 2)fatty acid synthase

Co factors-

- ✓ 1)Biotin
- ✓ 2)NADH
- ✓ 3)Mg†

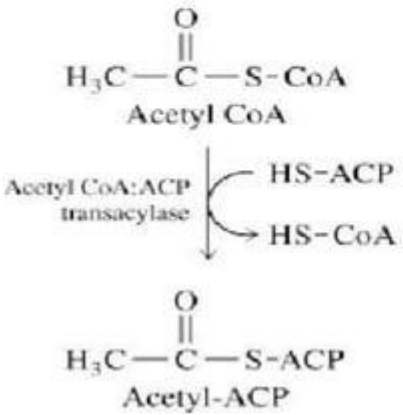
Acetyl CoA carboxylase




ER
KR
Fatty acid synthase
DH
Fatty acid synthase I
Fatty acid synthase II
KS
MAT
KS
MAT
DH
ER
KR
ACP
TE

Activation

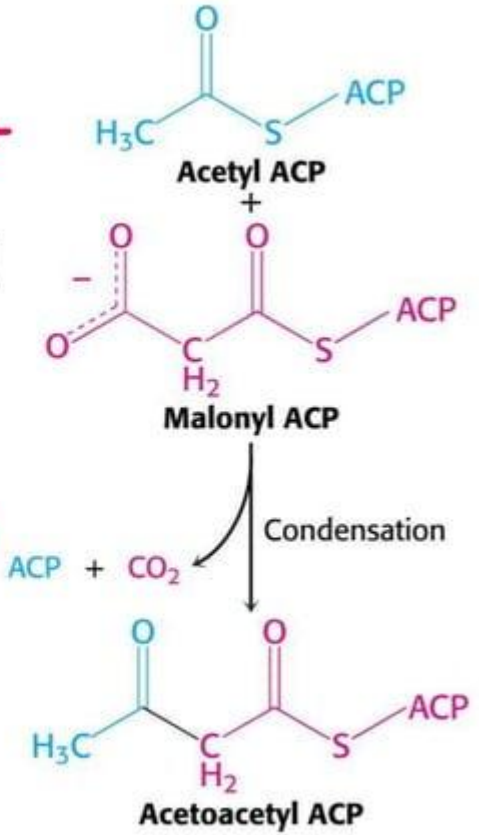
- ✓ Fatty acid synthesis starts with the formation of acetyl ACP and malonyl ACP.
- ✓ ***Acetyl transacylase*** and ***malonyl transacylase*** catalyze these reactions.
- ✓ **Acetyl CoA + ACP ⇔ acetyl ACP + CoA**
  Malonyl CoA + ACP ⇔ malonyl ACP + CoA



Condensation reaction-

Acetyl ACP and malonyl ACP react to form acetoacetyl ACP.

Enzyme - ***acyl-malonyl ACP condensing enzyme.***

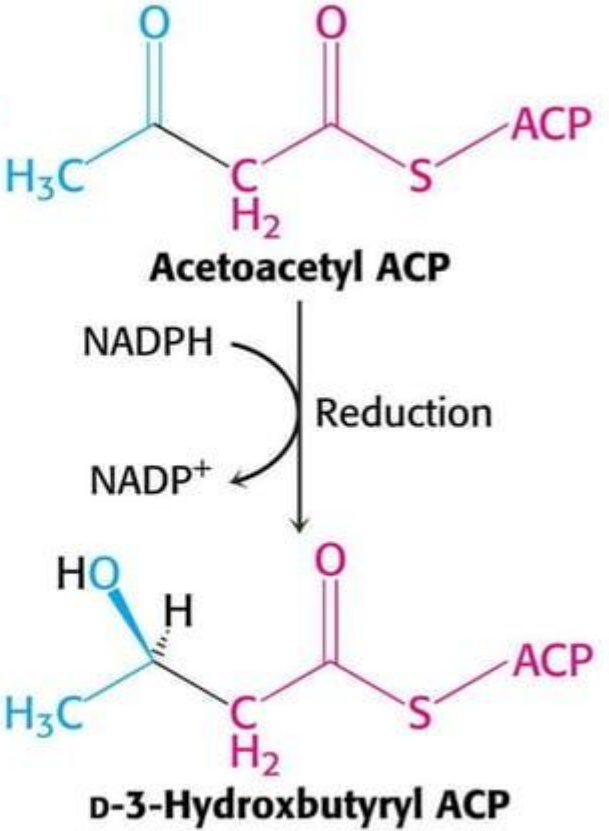


Reduction Reaction-

Acetoacetyl ACP is reduced to D-3-hydroxybutyryl ACP.

NADPH is the reducing agent

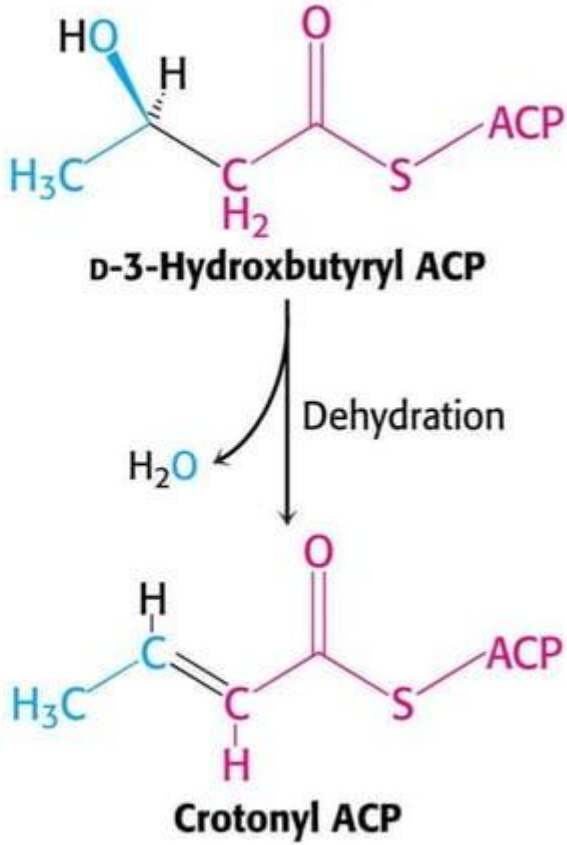
Enzyme: *β-ketoacyl ACP reductase*



Dehydration Reaction-

D-3-hydroxybutyryl ACP is *dehydrated* to form crotonyl ACP (trans-Δ^2-enoyl ACP).

Enzyme:
3-hydroxyacyl ACP dehydratase



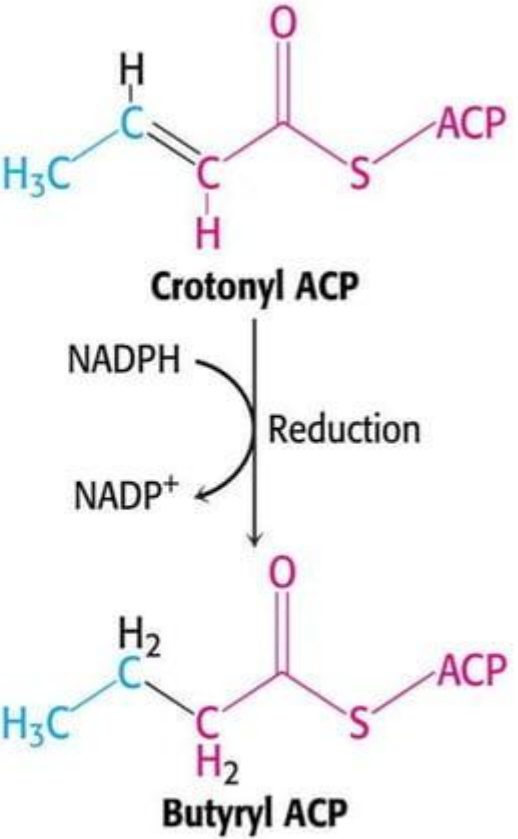
Reduction Reaction-

The final step in the cycle reduces crotonyl ACP to **butyryl ACP.**

NADPH is reductant.

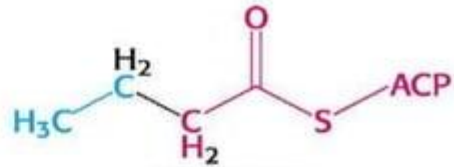
Enzyme - ***enoyl ACP reductase.***

This is the end of first elongation cycle (first round).

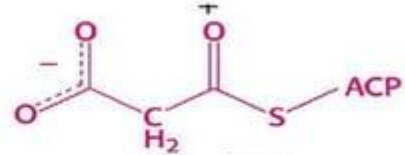


In the second round butyryl ACP condenses with malonyl ACP to form a C_6-β-ketoacyl ACP.

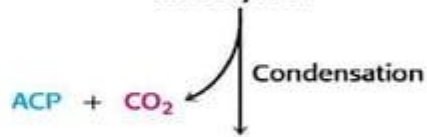
Reduction, dehydration, and a second reduction convert the C_6-β-ketoacyl ACP into a C_6-acyl ACP, which is ready for a third round of elongation.

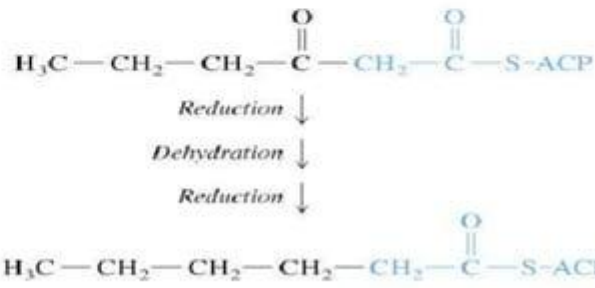


Butyryl ACP



Malonyl ACP

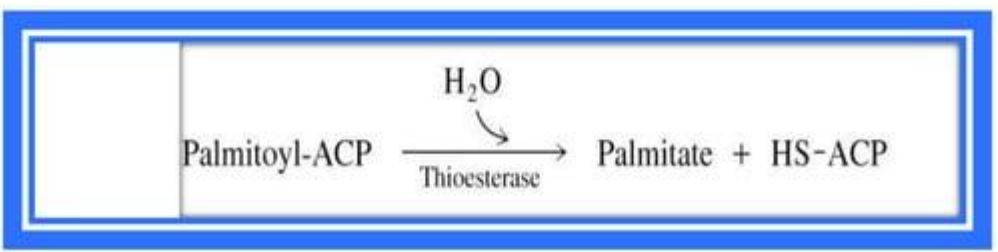




Termination

- Rounds of synthesis continue until a C_{16} palmitoyl group is formed

- Palmitoyl-*ACP* is hydrolyzed by a ***thioesterase***



Net Production

- **Net reaction-**

$$8\,\text{acetyl-CoA} + 14\,\text{NADPH} + 7\,\text{ATP} \rightarrow \text{palmitate} + 14\,\text{NADP}^+ + 8\,\text{CoA} + 7\,\text{ADP} + 7\,\text{P}_i$$

- **Over all Net Reaction-**

$$\text{acetyl-CoA} + 7\,\text{malonyl-CoA} + 14\,\text{NADPH} \rightarrow \text{palmitate} + 7\,\text{CO}_2 + 14\,\text{NADP}^+ + 8\,\text{CoA}$$

Regulation

Acetyl CoA carboxylase- rate limiting step

In vertebrate

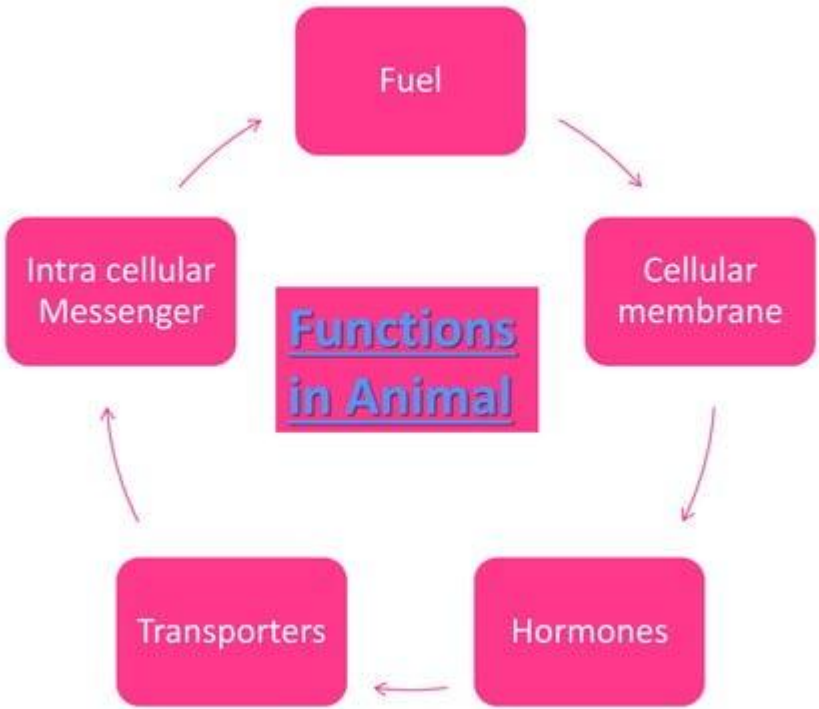
- ✓ Feedback inhibitor- palmitate
- ✓ Allosteric activator- citrate
- ✓ Covalent modification- glucagon & epinephrine

In plants

- ✓ Not citrate
- ✓ Not Hormonal
- ✓ pH
- ✓ [Mg+]
- ✓ At gene level

Difference

Difference	Biosynthesis	oxidation
Location	Chloroplast	Peroxisome
Carrier	Acyl carrier protien	CoA
Isomer	D	L
Activation of Co_2	Require	Not
Carbon chain	Add 2 Carbon	Remove 2 Carbon
NADH	Require	Generate
Enzyme	Multi complex	Independent


Fuel
Cellular membrane
Hormones
Transporters
Intra cellular Messenger
Functions in Animal

Functions in Plants

- Photosyntthesis- eg.Chlorophyll
- Electrone transport chain- eg.ubiquinone
- Photoprotection- eg.Caretenoids
- Coloration- eg.Pigments
- Cellular membrane- eg.Galactolipid
- Transporters
- Communication

Summery

- Fatty acid biosynthesis in plant occure into chloroplast.
- Not reverse as Oxidation process.
- Four steps- condensation, reduction, dehydration and reduction.
- Regulation is different then animals.
- Important role as a structural and biological functions in plants

Breakdown (or Degradation) of Fats in Plants

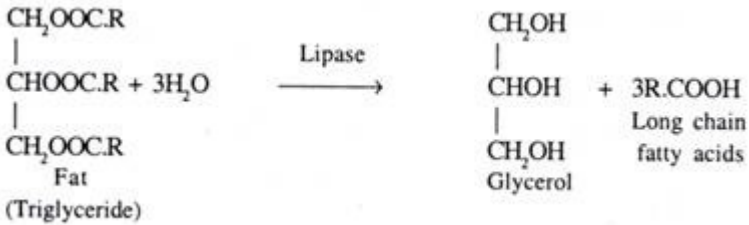
Like many other metabolites fats also exist in dynamic state in plants i.e., at one time they are being synthesized and at other times broken down to meet specific requirement of the cells.

Active breakdown of fats (which are insoluble) takes place:

(1) During the germination of fatty seeds so that the decomposition products may enter into glycolysis and Krebs' cycle to release energy and also to synthesise soluble sucrose through glyoxylic acid cycle which is then trans located to the growing regions of the young germinating seedling till it develops green leaves to manufacture its own food.

(2) In plants, when carbohydrates reserve declines, the fats (also the proteins), may form the respiratory substrates which are broken down and oxidised to release energy.

The fats are first hydrolysed in the presence of the enzymes lipases to yield fatty acids and glycerol.



The above hydrolysis takes place in 3 steps. The first two steps are reversible in which diglyceride and monoglyceride are produced as intermediates. The third step is irreversible which completes the process of hydrolysis. The hydrolysis of fats (triglycerides) is accelerated by the presence of Ca++ ions.

(i) Triglyceride $\rightleftharpoons$ Diglyceride + Fatty acid

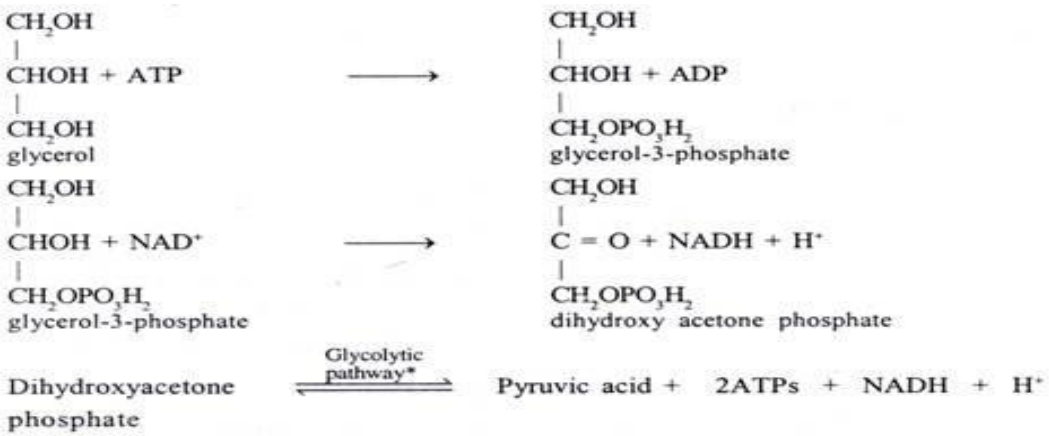
(ii) Diglyceride $\rightleftharpoons$ Monoglyceride + Fatty acid

(iii) Monoglyceride → Glycerol + Fatty acid

The first two steps occur in cytosol while the third step occurs both in cytosol and glyoxysomes.

Oxidation of Glycerol:

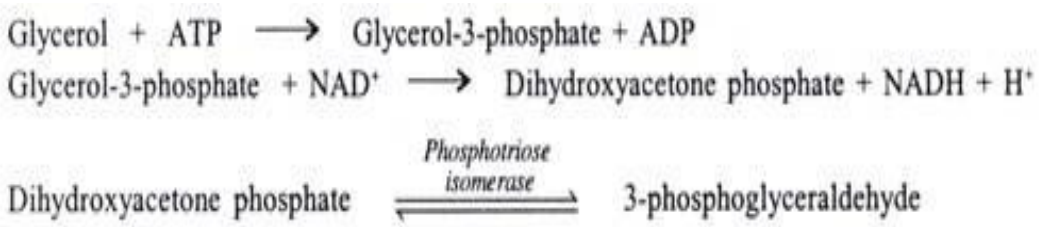
The glycerol may react with ATP under the catalytic influence of glycerol kinase to form glycerol-3-phosphate which is then oxidised in the presence of glycerol-3-phosphate dehydrogenase and NAD+ to produce dihydroxyacetone phosphate and enters into glycolysis.

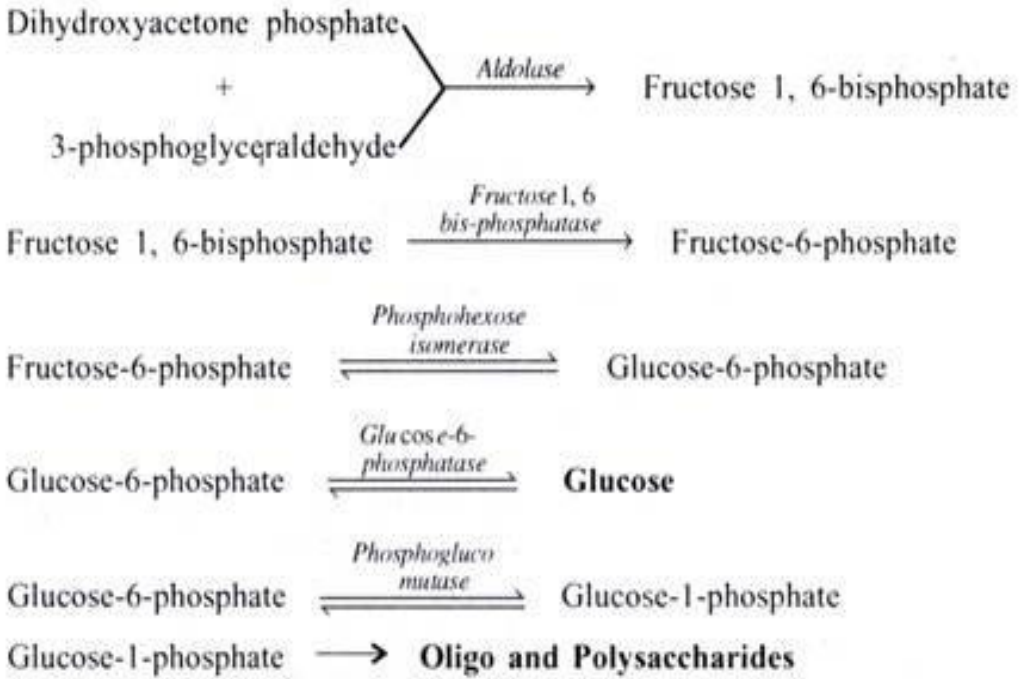


This conversion of glycerol into pyruvic acid which takes place in cytoplasm yields 2ATPs by substrate level phosphorylation and 2 NADH which on re-oxidation by terminal electron transport chain via the external NADH dehydrogenase (located on the outer surface of the inner mitochondrial membrane in plants) further generate 4 ATP molecules (2 mol./NADH oxidised).

If the pyruvic acid also undergoes complete oxidation into CO_2 and H_2O in Krebs' cycle (or TCA cycle) it will produce another 15 ATP molecules. Thus a total of 2 + 4 + 15 = 21 ATPs are produced per glycerol molecule oxidised. However, there is consumption of 1 ATP molecule in the glycerol kinase catalysed reaction. Therefore, the net gain is 21 – 1 = 20 ATPs per glycerol molecule oxidised.

The glycerol produced after the hydrolysis of triglycerides in spherosomes or of monoglycerides in glyoxysomes diffuses out into cytosol and may also be utilised in the synthesis of glucose and other carbohydrates first by converting into dihydroxyacetone phosphate and then by reverse reactions of glycolysis (although with slight modification) as follows:




Dihydroxyacetone phosphate
+
3-phosphoglyceraldehyde
Aldolase
Fructose 1, 6-bisphosphate
Fructose 1, 6-bisphosphate
Fructose 1, 6 bis-phosphatase
Fructose-6-phosphate
Fructose-6-phosphate
Phosphohexose isomerase
Glucose-6-phosphate
Glucose-6-phosphate
Glucose-6-phosphatase
Glucose
Glucose-6-phosphate
Phosphogluco mutase
Glucose-1-phosphate
Glucose-1-phosphate
Oligo and Polysaccharides

Fatty acid β-oxidation is a multistep process by which fatty acids are broken down by various tissues to produce energy. Fatty acids primarily enter a cell via fatty acid protein transporters on the cell surface.

- Fatty acid transporters include fatty acid translocase (FAT/CD36), tissue specific fatty acid transport proteins (FATP), and plasma membrane bound fatty acid binding protein (FABPpm).
- Once inside the cell, a CoA group is added to the fatty acid by fatty acyl-CoA synthase (FACS), forming long-chain acyl-CoA. Carnitine palmitoyltransferase 1 (CPT1) conversion of the long-chain acyl-CoA to long-chain acylcarnitine allows the fatty acid moiety to be transported across the inner mitochondrial membrane via carnitine translocase (CAT), which exchanges long-chain acylcarnitines for carnitine.

•An inner mitochondrial membrane CPT2 then converts the long-chain acylcarnitine back to long-chain acyl-CoA. The long-chain acyl-CoA enters the fatty acid β-oxidation pathway, which results in the production of one acetyl-CoA from each cycle of fatty acid β-oxidation.

•This acetyl-CoA then enters the mitochondrial tricarboxylic acid (TCA) cycle. The NADH and FADH2 produced by both fatty acid β-oxidation and the TCA cycle are used by the electron transport chain to produce ATP. An overview of fatty acid oxidation is provided in Figure 1.

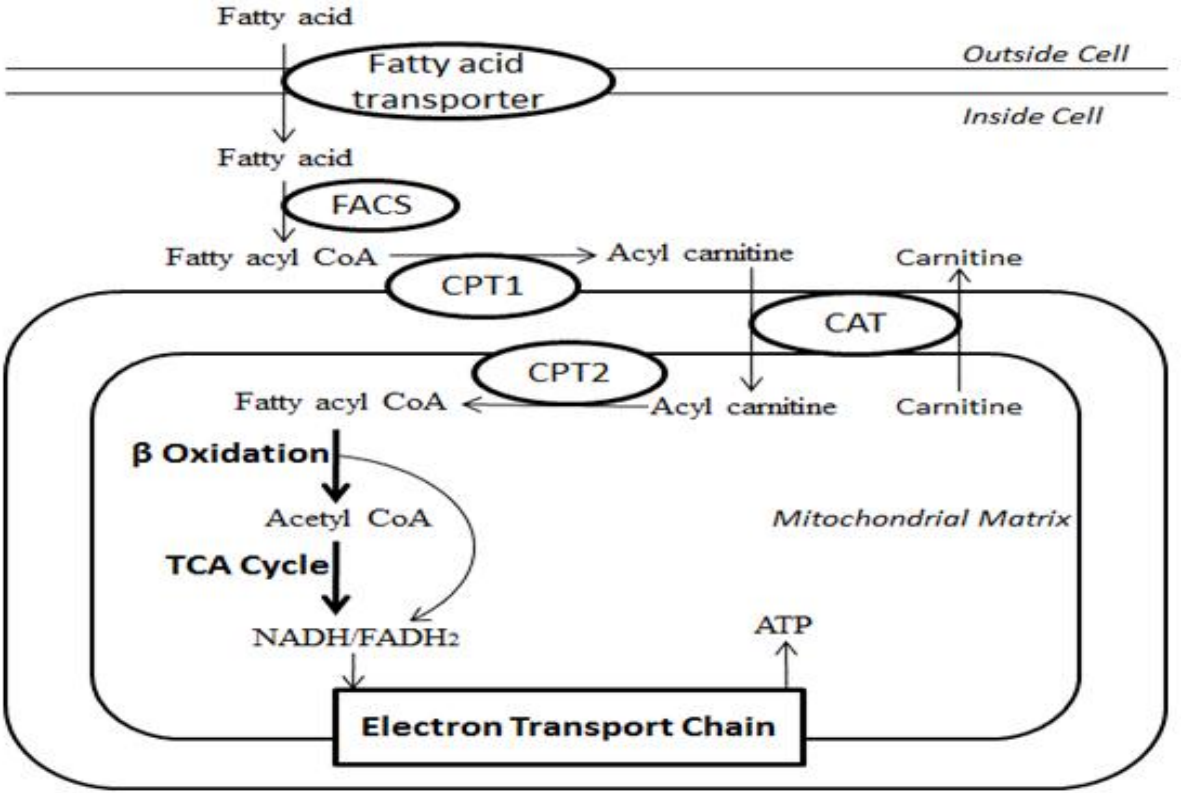


Figure 1. Fatty Acid Oxidation Overview

•The four steps are dehydrogenation, hydration, oxidation, and thiolysis. Dehydrogenation is catalyzed by Acyl-CoA-dehydrogenase and coverts FAD to FADH2 to form a double bond between C2 and C3. Hydration results in a hydroxyl group on C3 as a result of the double bond being attacked by a water molecule.

α- oxidation

•Alpha oxidation of fatty acids occurs in the peroxisome as well; this metabolic pathway exists to degrade by-products of chlorophyll, a component of green vegetables in the diet. Phytanic acid is the primary molecule that requires the enzymes dedicated to alpha-oxidation.

•It is a minor oxidation pathway that occurs in peroxisomes. The chain is broken between C1 and C2 and releases CO_2 per cycle. Omega oxidation: It is another minor oxidation pathway that occurs in the endoplasmic reticulum.

Types of Oxidation in Fatty Acids

Beta oxidation: It is the major mechanism of oxidation of fatty acids that occurs in mitochondria and peroxisomes. It releases acetyl CoA by breaking the carbon chain between C2 and C3.

Alpha oxidation: It is a minor oxidation pathway that occurs in peroxisomes. The chain is broken between C1 and C2 and releases CO2 per cycle.

Omega oxidation: It is another minor oxidation pathway that occurs in the endoplasmic reticulum. The action site for this type of reaction is the methyl end of the molecule.

Steps of Alpha Oxidation

The phytanic acid, a branched-chain fatty acid that is obtained in humans by the fat of ruminant animals, dairy products and plant materials, is the primary target for alpha oxidation. Plant materials contain chlorophyll that releases phytanic acid in humans. The steps of alpha oxidation of phytanic acid are as follows:

- Phytanic acid first attaches with CoA to form phytanoyl-CoA. The phytanoyl-CoA is oxidised to 2-hydroxy phytanoyl-CoA in the presence of phytanoyl-CoA dioxygenase using Fe^{2+} and O_2.
- 2-hydroxy phytanoyl-CoA is cleaved to form pristanal and formyl-CoA in the presence of 2-hydroxyphytanoyl-CoA lyase. Pristanal undergoes oxidation to form pristanic acid in the presence of aldehyde dehydrogenase. The pristanic acid can then undergo beta oxidation.

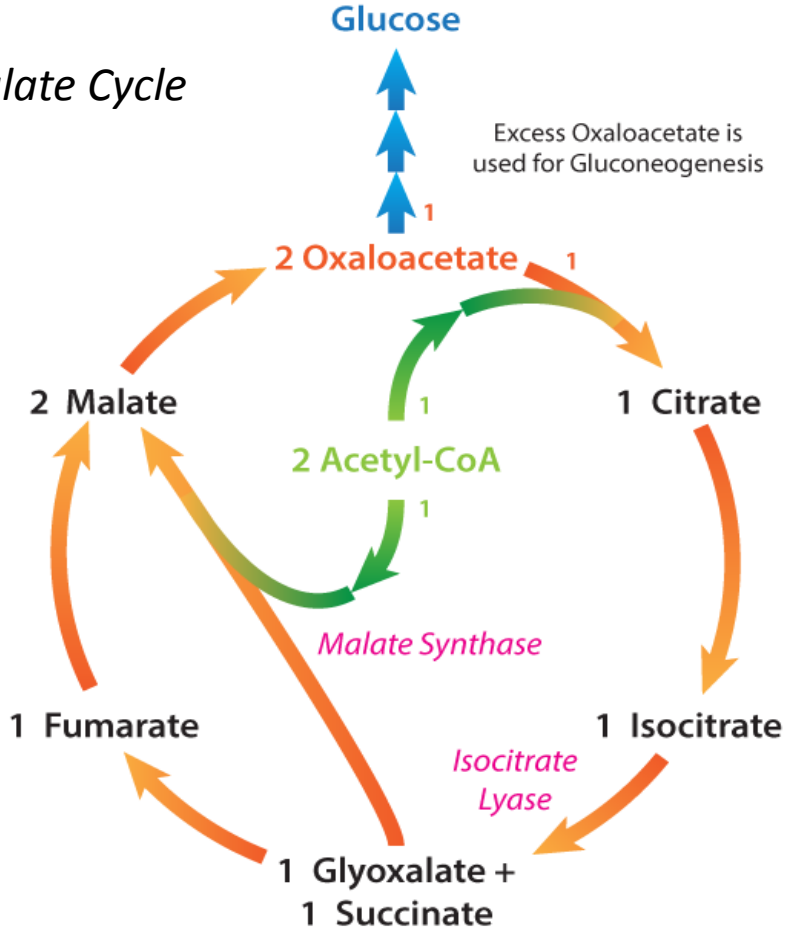
Significance of Alpha Oxidation

- Alpha oxidation yields simpler forms of fatty acids that can later undergo beta oxidation. For example – beta oxidation cannot occur in phytanic acids because of the presence of a β-methyl group.
- Alpha oxidation removes the methyl group by decarboxylation, and hence beta oxidation can proceed.
- It produces intermediate hydroxy fatty acids such as cerebronic acid that can be used to synthesise cerebrosides and sulfatides.
- The decarboxylation of fatty acids produces odd chain fatty acids that can be used in the synthesis of sphingolipids.

The glyoxylate cycle is a special variant of the tricarboxylic cycle (TCA) that allows utilization of two carbons compounds in the absence of glucose. The glyoxylate cycle is generally not present in human and animal tissue, and can only be found in plants, bacteria, fungi and protists.

A pathway related to the Citric Acid Cycle (CAC) is the glyoxylate pathway. This pathway, which overlaps all of the non-decarboxylation reactions of the CAC does not operate in animals, because they lack two enzymes necessary for the pathway – isocitrate lyase and malate synthase. Isocitrate lyase catalyzes the conversion of isocitrate into succinate and glyoxylate. Because of this, all six carbons of the CAC survive and do not end up as carbon dioxide.

The Glyoxalate Cycle



•Succinate continues through the remaining reactions of the CAC to produce oxaloacetate. Glyoxylate combines with another acetyl-CoA (one acetyl-CoA was used to start the cycle) to create malate (catalyzed by malate synthase). Malate can, in turn, be oxidized to oxaloacetate.

•It is at this point that the pathway's contrast with the CAC is apparent. After one turn of the CAC, a single oxaloacetate is produced and it balances the single one used in the first reaction of the cycle.

•Thus, in the CAC, no net production of oxaloacetate is realized. By contrast, at the end of a turn of the glyoxylate cycle, two oxaloacetates are produced, starting with one. The extra oxaloacetate can then be used to make other molecules, including glucose in gluconeogenesis.

- Because animals do not run the glyoxylate cycle, they cannot produce glucose from acetyl-CoA in net amounts, but plants and bacteria can. As a result, these organisms can turn acetyl-CoA from fat into glucose, while animals can't.
- Bypassing the decarboxylations (and substrate level phosphorylation) has its costs, however. Each turn of the glyoxylate cycle produces one FADH and one NADH instead of the three NADHs, one FADH2, and one GTP made in each turn of the CAC.

Gluconeogenesis and its role in mobilization of the lipids during seed germinations:

- In plants, the mobolisation of oil (triacylglycerol) is best understood in relation to seed germination. As soon as a seed germinates, it enters a phase of rapid growth and development culminating in the formation of a photosynthetically active seedling.
- The energy and building blocks that are necessary to support this growth period are supplied by the metabolism of storage reserve compounds that were laid down during seed development, and are derived from the mother plant. The form of these reserves varies, but oil, carbohydrate and protein are usually predominant.
- The tissue where the reserves are stored also differs considerably with species, depending on the anatomy and physiology of the seed.

- Regardless of these variations, efficient breakdown and utilisation of stored reserves is considered to be extremely important for the rapid transition from seed to seedling. The speed of this transition and the ability to complete it in adverse environmental conditions is crucial for a species' reproductive success (evolutionary fitness).

- Triacylglycerol is believed to be the predominant store of carbon in the majority of seed species and can account for more than 60% of the weight of the seed.
- Triacylglycerol consists of three fatty acids esterified to a glycerol backbone. It is an extremely compact energy store. The complete oxidation of triacylglycerol yields more than twice the energy of protein or carbohydrate.

•It is most commonly located in the cotyledon or endosperm tissues of the seed, and is found in the cell cytosol in ~0.5 to 1 μm diameter lipid droplets surrounded by a phospholipid monolayer and structural proteins (oleosins), which prevent coalescence.

• The mobilisation of triacylglycerol in seed tissues requires a complex metabolic programme that is activated following germination and enables the net conversion of oil to sugars (Fig. 1). This capacity is not present in mammals, which lack a key linking pathway called the glyoxylate cycle.

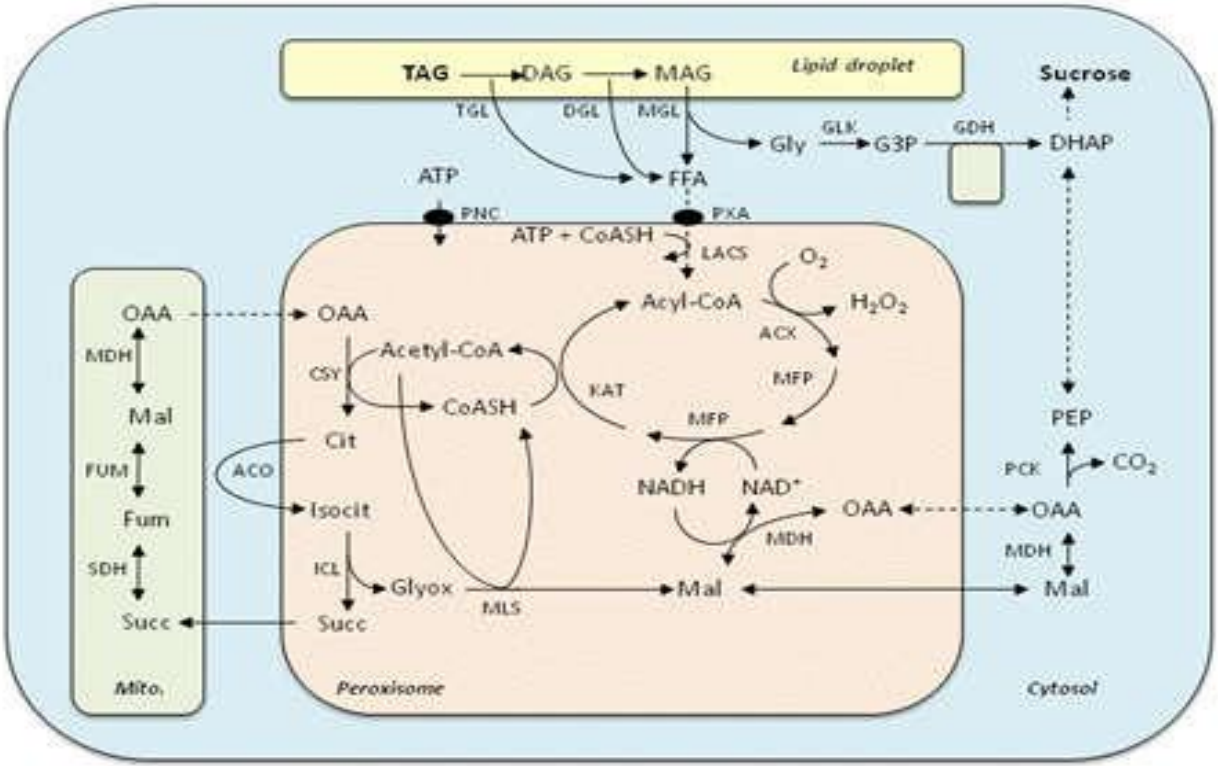


Figure 1. Pathways involved in the conversion of oil to sucrose.

Figure 1. Pathways involved in the conversion of oil to sucrose. TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; FFA, free fatty acid; Gly, glycerol; G3P, glycerol-3-phosphate; DHAP, dihydroxyacetone phosphate; PEP, phosphoenolpyruvate; Glyox, glyoxylate; Succ, succinate; Mal, malate; OAA, oxaloacetate; Cit, citrate; Isocit, isocitrate; Fum, fumarate; ATP, adenosine triphosphate; CoASH, Coenzyme A; TGL, TAG lipase; DGL, DAG lipase; MGL, MAG lipase; GLK, glycerol kinase; GDH, FADglycerol-3-phosphate dehydrogenase; PXA, ATP-binding cassette transporter; PNC, ATP transporter; LACS, long-chain acyl-CoAsynthetase; MDH, malatedehydrogenase; PCK, PEP carboxykinase; ACX, acyl-CoAoxidase; MLS, malatesynthase; MFP, multifunctional protein, ICL, isocitratelyase; SDH, succinatedehydrogenase; ACO, acontiase; FUM, fumarase; KAT, ketoacyl-CoAthiolase; CYS, citrate synthase. Dashed lines denote multistep processes.

• Much of our understanding of the biochemistry of oil mobilisation in plants comes from ground breaking work using the castor bean (Ricinus communis) as an experimental system. More recently the model oilseed plant Arabidopsis thaliana has provided a convenient genetic model to decipher the underlying molecular mechanisms.

Gluconeogenesis:

- Organic acids generated by the glyoxylate cycle are metabolised to sugars via gluconeogenesis. Sugars are then available to be transported throughout the seedling, supporting growth and development.
- A key step in this process is the conversion of oxaloacetate to phosphoenolpyruvate (PEP) catalysed by phosphoenolpyruvatecarboxykinase. It is noteworthy that ATP is consumed by this step and one quarter of the carbon is lost as CO_2.
- Glycolytic enzymes then allow phosphoenolpyruvate to be further metabolised to sugar phosphates, which provide substrates for sucrose synthesis.

Regulation:

•Upon seed germination, a coordinate induction of many of the genes involved in storage oil mobilisation occurs, and this induction generally reflects the levels of protein and enzyme activity.

•However, the regulation of the process is relatively poorly understood. The phyto-hormones gibberellins (GAs) and abscisic acid (ABA) are well known to have a positive and negative effect on seed germination, respectively.

•This antagonistic action also extends to the control of oil breakdown. Oil metabolism is also governed by metabolic status and high levels of sugar repress mobilisation.

•In Arabidopsis the APETALA2 domain–containing transcription factor ABSCISIC ACID INSENSITIVE4 (ABI4) is required for both ABA and sugar repression of oil mobilisation in the embryo.

Physiological Significance :

- The physiological importance of oil mobilisation for seedling establishment is likely to vary depending on the species and environment.
- In Arabidopsis triacylglycerol makes up 35-40% of the seed's weight and is therefore the main store of carbon.
- A lipase double mutant (sdp1 sdp1L) that retains less than ~5% of wild type TAG hydrolytic capacity exhibits only a slight reduction in the rate of seed germination but subsequent seedling growth is strongly retarded.