KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University Established Under Section 3 of UGC Act 1956)
Coimbatore – 641 021.

SYLLABUS

STAFF NAME: Dr.P.Anusooriya
SUBJECT NAME: Plant Biochemistry

SUB.CODE: 17BCU503A

SEMESTER: V CLASS: III B.Sc (BC)

SCOPE

To provide sufficient knowledge about plant cell and its organelles basically, plant growth substances, plant nutrition and senescence.

OBJECTIVES

Understanding of the plant cell organelles and their functions. To gain a wide knowledge about plant growth substances, plant nutrition and photo morphogenesis.

Unit 1

Plant cell structure and Photosynthesis

Structure of Plasma membrane, Vacuole and tonoplast membrane, cell wall, plastids and peroxisomes. Photosynthesis - Structure of PSI and PSII complexes, Light reaction, Cyclic and non cyclic photophosphorylation.

Unit 2

Carbon assimilation and Plant Respiration

Carbon assimilation - Calvin cycle and regulation; C4 cycle and Crassulacean acid metabolism (CAM). Respiration - Overview of glycolysis, Alternative reactions of glycolysis, Regulation of plant glycolysis, Translocation of metabolites across mitochondrial membrane, TCA cycle, Alternative NAD(P)H oxidative pathways; Cyanide resistant respiration and Photorespiration.

Unit 3

Nitrogen metabolism

Biological Nitrogen fixation by free living and in symbiotic association, structure and function of enzyme Nitrogenase. Nitrate assimilation: Nitrate and Nitrite reductase. Primary and secondary ammonia assimilation in plants; ammonia assimilation by Glutamine synthetase- glutamine oxoglutarate amino transferase (GS-GOGAT) pathway. Seed storage proteins in legumes and cereals.

Unit 4

Regulation of plant growth and Plant tissue culture

Introduction to plant hormones and their effect on plant growth and development, Regulation of plant morphogenetic processes by light. Plant tissue culture - Cell and tissue culture techniques, types of cultures: organ and explants culture, callus culture, cell suspension culture and protoplast culture. Plant regeneration pathways: organogenesis and somatic embryogenesis. Applications of cell and tissue culture and somoclonal variation.

Unit 5

Plant Secondary metabolites

Representatives alkaloid group and their amino acid precursors, function of alkaloids, Examples of major phenolic groups; simple phenylpropanoids, Coumarins, Benzoic acid derivatives, flavonoids, tannins and lignin, biological role of plant phenolics, Classification of terpenoids and representative examples from each class, biological functions of terpenoids.

REFERENCES

Bowsher, C., Steer, M., Tobin, A., (2008). Plant Biochemistry. Garland science ISBN 978-0-8153-4121-5.

Biochemistry and molecular Biology of plant-Buchanan. (2005) 1st edition. Publisher: I K International. ISBN-10: 8188237116, ISBN-13: 978-8188237111.

Dey, P.M., and Harborne, J.B., (1997). Plant Biochemistry. Academic Press ISBN-10:0122146743, ISBN-13:978-0122146749.

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LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

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Sl. No	LECTURE DURATION	TOPICS	BOOK REFERENCE	PAGE NO	WEB REFERENCE
Unit-1					
1	1	Structure of plant cell-cell wall, vacuoles, plastids	T1 T2	03-11, 36-42 06-10	
2	1	Mitochondria, peroxisomes and golgi complex			
3	1	Overview of photosynthesis	T1 T2	139-142 229-235	
4	1	Photosynthetic apparatus,	T1	142-148	
5	1	Reaction center	T2	222-229	
6	1	Photosystems I and II	T1 T2	148-151 244-247	
7	1	Mechanism of photosynthesis-cyclic	T1	156-159	
8	1	Non cyclic photophosphorylation	T1 T2	153-156 248-250	
9	1	Evidences in support of light and dark reactions	T1	160-163	
10	1	Revision and Possible QP discussion			
Total: 10 hours					
Unit-2					
1	1	Photorespiration and water consumption	T1 T2	174-177 271-277	
2	1	Co_2 assimilation by C_3 and C_4 plants, CAM	T2	247-248	

		plants	T1 T2	160-163 255-260	
3	1	Nitrogen assimilation; reduction of nitrate	T1	233-236	
4	1	Nitrogen fixation in symbiotic plants	T1 T2	237-244 363-372	
5	1	Nitrogen fixation in non-symbiotic plants	T1 T2	237-244 363-372	
6	1	Nitrogen cycle	T1	233-236	
7	1	Sulphate metabolism in leaf			
8	1	Sulfite reduction and sulphur cycle	T3	278-280	
9	1	Glutathione synthesis	T3	278-280	
10	1	Carbon and phosphorus cycle	T2	280-283	
11	1	Revision and Possible QP discussion			
Total: 11 hours					
Unit-3					
1	1	Biosynthesis of fatty acids in plastids, Synthesis of waxes	T4	343-346 349 805-814	
2	1	Synthesis of triacylglycerols	T2	820-824	
3	1	Glycolipids	T2	256	
4	1	Synthesis of chlorophyll	T1 T2	139-142 236-238	
5	1	Carotenoid formation	T1 T2	141 236-238	
6	1	Secondary oxidative mechanisms-β-oxidation, ψ-oxidation	T4	652-654	
7	1	Glyoxylate pathway	T4	663-664	
8	1	Revision and Possible QP discussion			
Total: 8 hours					
Unit-4					
1	1	Plant growth substances; chemistry	T1 T2	334-336 446-447	
2	1	Biosynthesis, mode of action and physiological role of auxins, gibberellins	T1 T2	338-351 447-469	
3	1	Cytokinins, abscisic acid and ethylene	T1 T2	351-356 469-479	

4	1	Phytochromes: molecule, biological display	T2 T1	319-321 498-502	
5	1	Functions as light sensor			
6	1	Senescene: Biochemical changes	T2	521-524	
7	1	Senescence: regulation	T5	625-632	
8	1	Revision and Possible QP discussion			
Total: 8 hours					
Unit-5					
1	1	Plant secondary metabolites: Alkaloids			Article 1
2	1	Flavonoids, Terpenoids			Article 1
3	1	Phenols-occurrence, distribution and functions			Article 1
4	1	Production of secondary metabolites in plants			Article 2
5	1	Stages of secondary metabolite production			Article 2
6	1	PTC Totipotency, meristematic			W1
7	1	Somatic embryogenesis			Article 3
8	1	Metabolic engineering for increased production of secondary metabolites			Article 1
9	1	Revision and Possible QP discussion			
PREVIOUS YEAR END SEMESTER EXAMINATION QUESTION PAPER DISCUSSION					
1	1	Previous year ESE question paper discussion			
2	1	Previous year ESE question paper discussion			
Total: 11 hours					
Grand Total: 48 hours					

REFERENCE

T1 : SK Verma (1999). A text book of plant physiology and Biochemistry; 3rd edition. S. Chand and Company Ltd, New Delhi.

T2 : SN Pandey and BK Sinha (2006). Plant Physiology, 4th edition; UBS Publications and Distributors Pvt Ltd., New Delhi.

T3 : G Ray Noggle and George J Fritz (1999). Introductory Plant Physiology; 2nd edition Pentice-Hall of India Pvt Ltd, New Delhi.

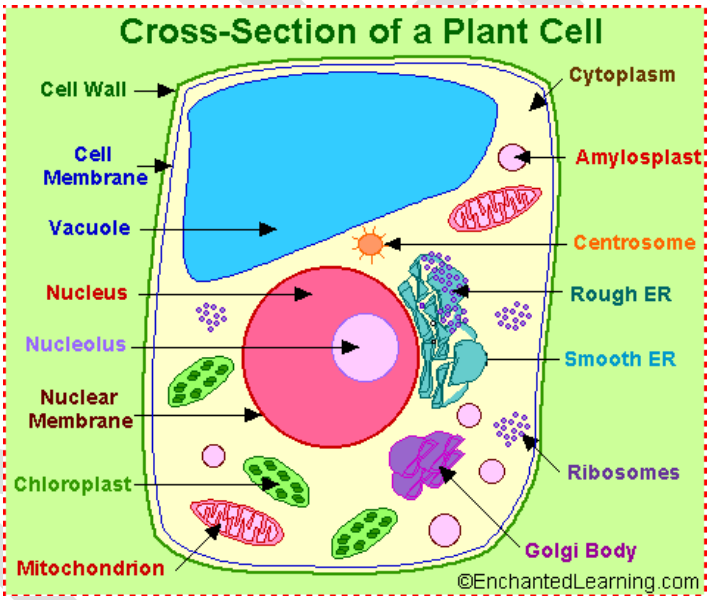
UNIT-I

Plant cell: Structure of plant cell-cell wall, vacuoles, plastids, mitochondria, peroxisomes and Golgi complex. Overview of photosynthesis: photosynthetic apparatus, reaction center, photosystems I and II, mechanism of photosynthesis-cyclic and non-cyclic photophosphorylation; evidences in support of light and dark reactions.

Plant cell

Plant cells are eukaryotic cells that differ in several key respects from the cells of other eukaryotic organisms. Their distinctive features include:

Structure of plant cell



A large central vacuole, a water-filled volume enclosed by a membrane known as the tonoplast maintains the cell's turgor, controls movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.

A cell wall composed of cellulose and hemicelluloses, pectin and in many cases lignin, are secreted by the protoplast on the outside of the cell membrane. This contrasts with the cell walls of fungi (which are made of chitin), and of bacteria, which are made of peptidoglycan.

Plastids, notably the chloroplasts which contain chlorophyll and the biochemical systems for light harvesting and photosynthesis, but also amyloplasts specialized for starch storage, elaioplasts specialized for fat storage and chromoplasts specialized for synthesis and storage of pigments. As in mitochondria, which have a genome encoding 37 genes plastids have their own genomes of about 100-120 unique genes. Unlike animal cells, plant cells are stationary.

Parts of a Plant Cell Structure

Plant cells are classified into three viz. parenchyma cells, collenchyma cells and sclerenchyma cells based on the structure and function. Now let us see the different parts of a plant cell.

Cell Wall

Cell wall is the outermost rigid layer composed of cellulose, hemicellulose, pectin and sometimes lignin. The function of cell wall is protection, structural support and also it helps in filtering mechanism.

Cell Membrane

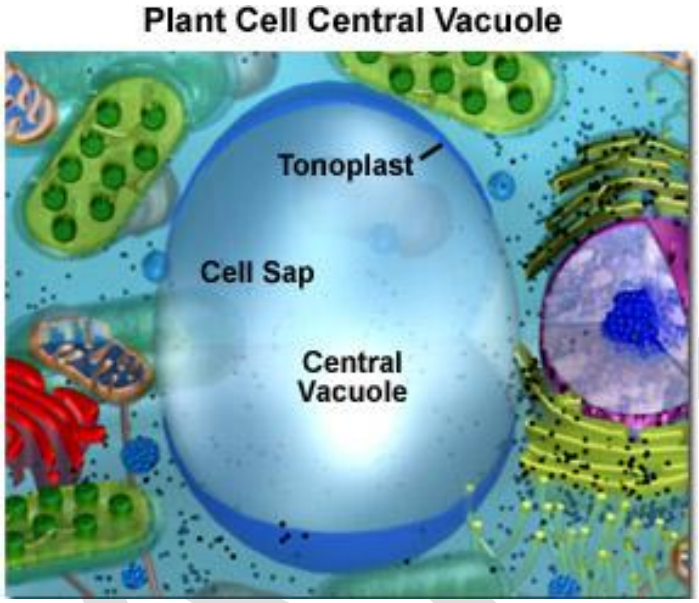
Cell membrane also called as plasma membrane is present inside the cell wall and surrounds the cytoplasm. It connects the intracellular components with the extracellular environment and helps in protection and transportation.

Plasmodesmata

Plasmodesmatas are small openings, which connect plant cells with each other enabling transport and communication between them.

Vacuole

Vacuoles are large membrane bound compartments, which stores compounds and provides storage, excretory and secretory functions. The membrane surrounding vacuole is called tonoplast.



Cytoplasm

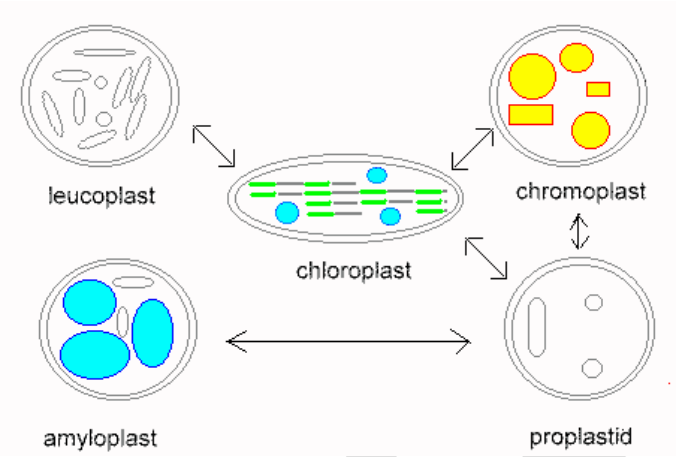
Cytoplasm is filled up by cytosol, which is a gelatinous, semitransparent fluid.

Nucleus

Nucleus is a specialized organelle, which contains the plant's hereditary material i.e. DNA (Deoxyribonucleic Acid). It also contains structures, which regulates the cell cycle, growth, protein synthesis and reproductive function.

Plastid

Plastids are organelles responsible for the photosynthetic activity and for the manufacture and storage of chemical compounds in plants. Chloroplast is an important form of plastid containing chlorophyll pigment, which helps in harvesting light energy and converting it to chemical energy.



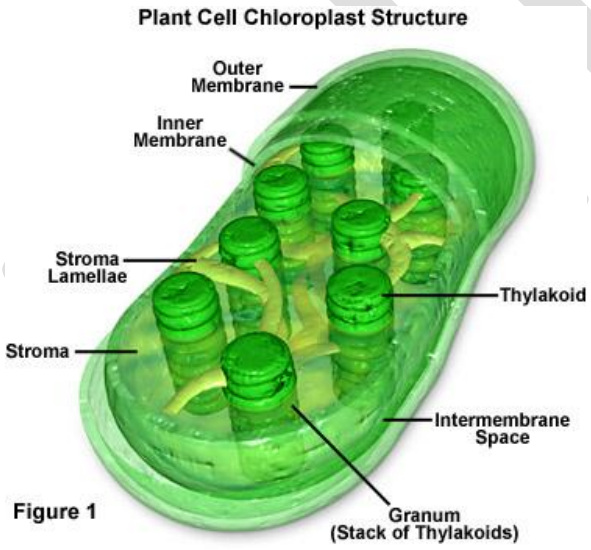


Figure 1

Mitochondria

Mitochondria are oblong shaped organelles that are also known as "the powerhouse of the cell". They are responsible for breaking down the complex carbohydrate and sugar molecules to simpler forms that the plants can use.

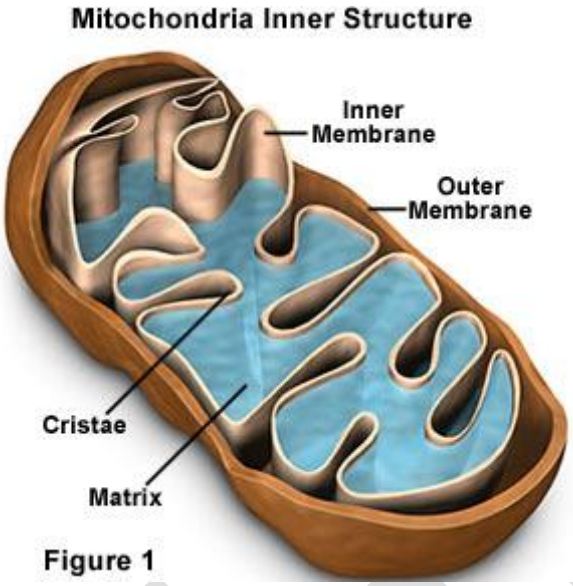


Figure 1

Functions of mitochondria

- The most important function of the mitochondria is to produce energy. The simpler molecules of nutrition are sent to the mitochondria to be processed and to produce charged molecules. These charged molecules combine with oxygen and produce ATP molecules. This process is known as oxidative phosphorylation.
- Mitochondria help the cells to maintain proper concentration of calcium ions within the compartments of the cell.
- The mitochondria also help in building certain parts of blood and hormones like testosterone and estrogen.
- The liver cells mitochondria have enzymes that detoxify ammonia.
- The mitochondria also play important role in the process of apoptosis or programmed cell death. Abnormal death of cells due to the dysfunction of mitochondria can affect the function of organ.

Endoplasmic Reticulum - Endoplasmic reticulum is an organelle responsible for the manufacturing and storage of chemical compounds like glycogen and steroids, translation

and transportation of protein. It is also connected to the nuclear membrane so as to make a channel between the cytoplasm and the nucleus.

Golgi apparatus

Golgi apparatus also known as golgi complex is an organelle responsible for the processing and packaging of macromolecules such as proteins and fats, which are synthesized by the cell and prepares them for transportation.

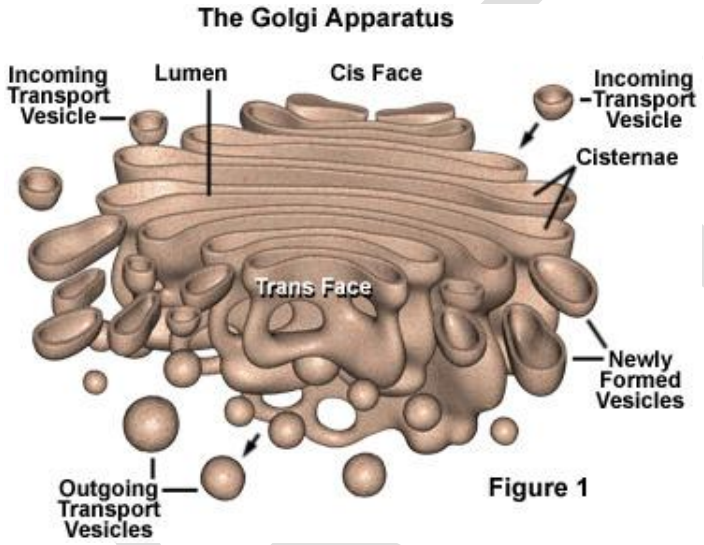


Figure 1

Functions of golgi complex

1. The cell synthesizes a huge amount of variety of macromolecules. The main function of the Golgi apparatus is to modify, sort and package the macromolecules that are synthesized by the cells for secretion purposes or for use within the cell.
2. It mainly modifies the proteins that are prepared by the rough endoplasmic reticulum.
3. They are also involved in the transport of lipid molecules around the cell.
4. They also create lysosomes.
5. The Golgi complex is thus referred as post office where the molecules are packaged, labeled and sent to different parts of the cell.

6. The enzymes in the cisternae have the ability to modify proteins by the addition of carbohydrates and phosphate by the process of glycosylation and phosphorylation respectively.
7. In order to modify the proteins the golgi complex imports substances like nucleotides from the cytosol of the cell. The modifications brought about by the golgi body might form a signal sequence. This determines the final destination of the protein.
8. The Golgi complex also plays an important role in the production of proteoglycans. The proteoglycans are molecules that are present in the extracellular matrix of the animal cells.
9. It is also a major site of synthesis of carbohydrates. These carbohydrates includes the synthesis of glycoasaminoglycans, Golgi attaches to these polysaccharides which then attaches to a protein produced in the endoplasmic reticulum to form proteoglycans.
10. The Golgi complex involves in the sulfation process of certain molecules.
11. The process of phosphorylation of molecules by the Golgi complex requires the import of ATP into the lumen of the Golgi.

Ribosome

Ribosomes are organelles, which are made up of 60% RNA (Ribonucleic Acid) 40% protein and play an important role in protein translation.

Microbodies

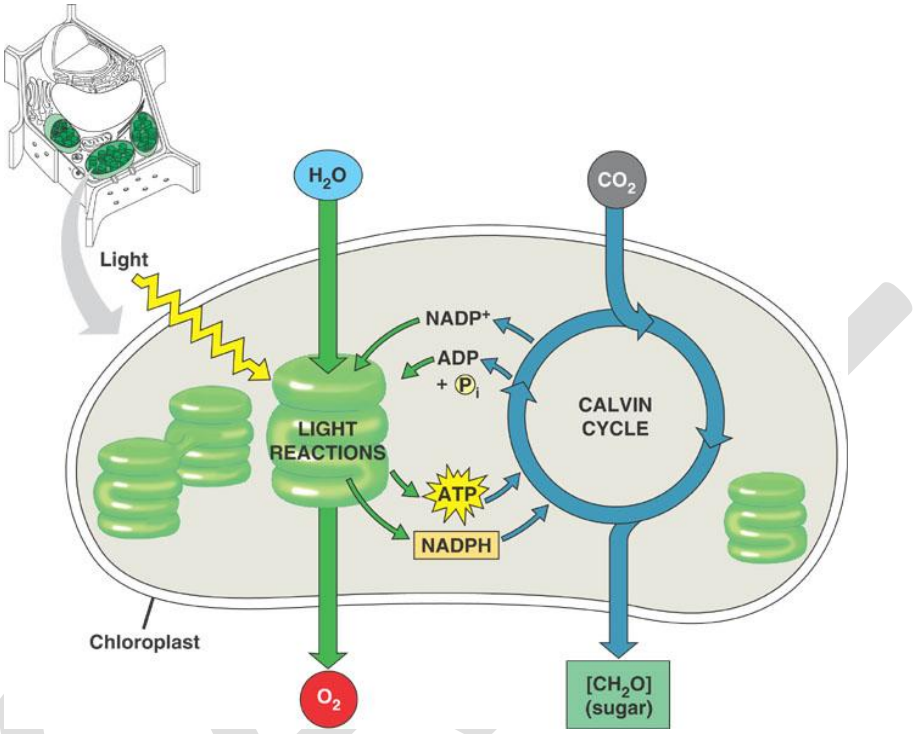
Microbodies are single membrane bound organelles, globular in shape and contains degradative enzymes. Most common microbodies are peroxisomes.

Microtubules

Microtubules are straight, hollow, tubular cylinders, which make up the cytoskeleton. They are responsible for structural support and transport of the cell.

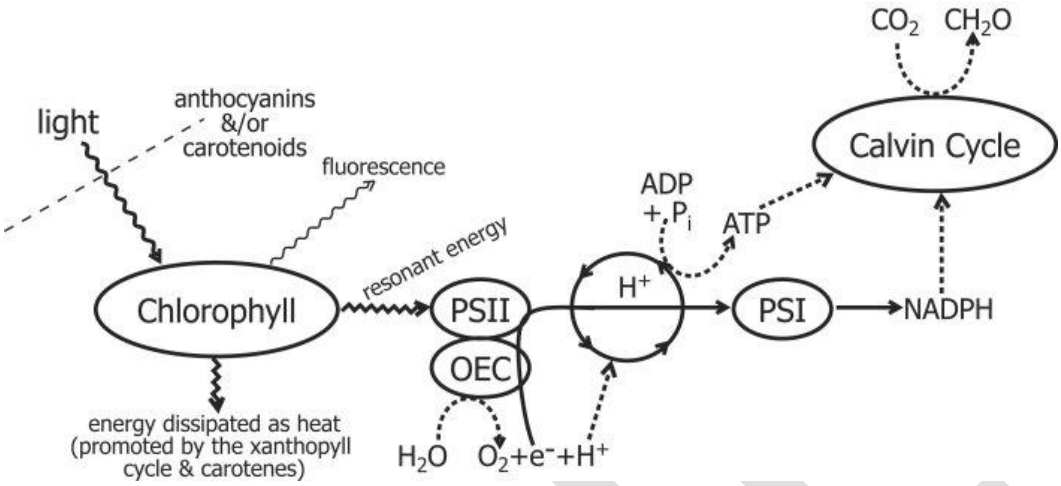
Microfilaments - Microfilaments are thin filaments of the cytoskeleton and are responsible for structural support of the cell.

Photosynthesis-overview



Photosynthetic apparatus

Simplified diagram of photosynthetic apparatus. PS I and II are photosystem I and II, respectively; OEC is the oxygen-evolving complex; P_i is inorganic phosphate; e^- represents electrons; H^+ represents protons; CH_2O represents carbohydrate products of photosynthesis.



Reaction center

A photosynthetic reaction center is a complex of several proteins, pigments and other co-factors assembled together to execute the primary energy conversion reactions of photosynthesis.

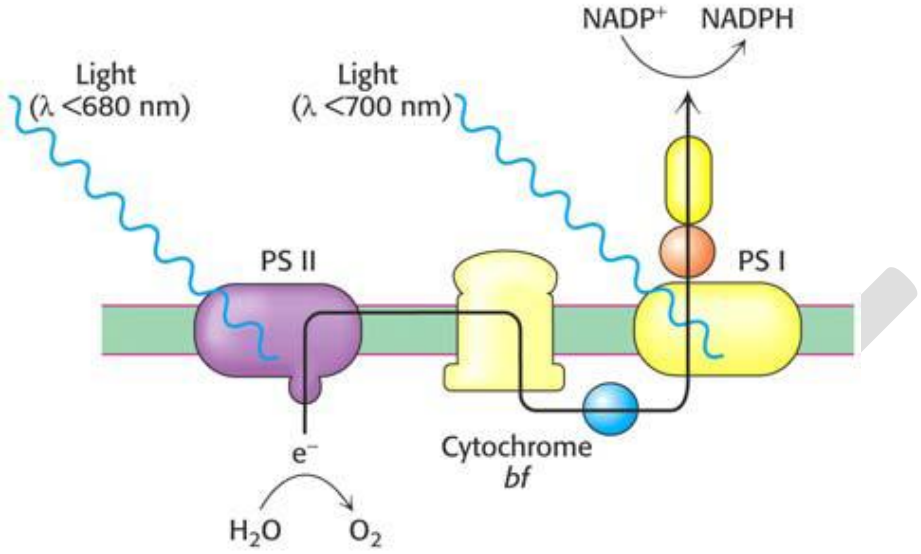
The reaction center of photosystem I is P700

The reaction center of photosystem II is P680

Photosystem I and II

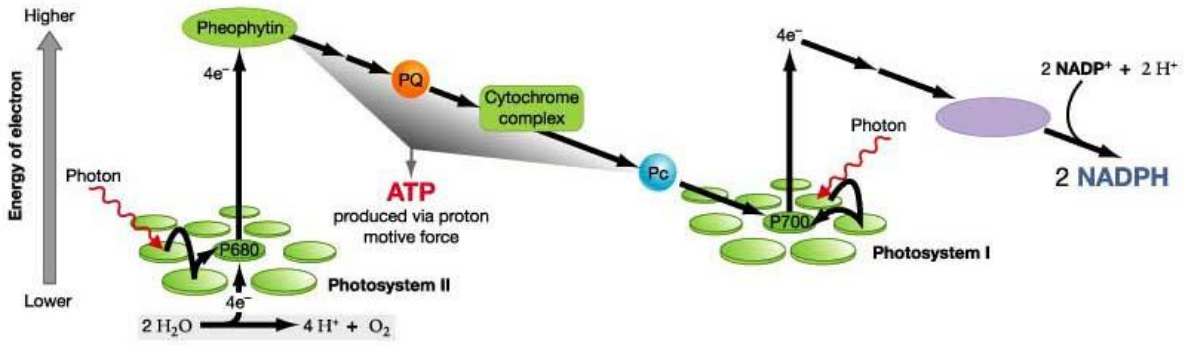
Within the thylakoid membranes of the chloroplast, are two photosystems. Photosystem I optimally absorbs photons of a wavelength of 700 nm. Photosystem II optimally absorbs photons of a wavelength of 680 nm. The numbers indicate the order in which the photosystems were discovered, not the order of electron transfer. Under normal conditions electrons flow from PSII through cytochrome *bf* (a membrane bound protein analogous to Complex III of the mitochondrial electron transport chain) to PSI. Photosystem II uses light energy to oxidize two molecules of water into one molecule of molecular oxygen. The 4 electrons removed from the water molecules are transferred by an electron transport chain to ultimately reduce 2NADP+ to 2NADPH. During the electron

transport process a proton gradient is generated across the thylakoid membrane. This proton motive force is then used to drive the synthesis of ATP. This process requires PSI, PSII, cytochrome *bf*, ferredoxin-NADP+ reductase and chloroplast ATP synthase.

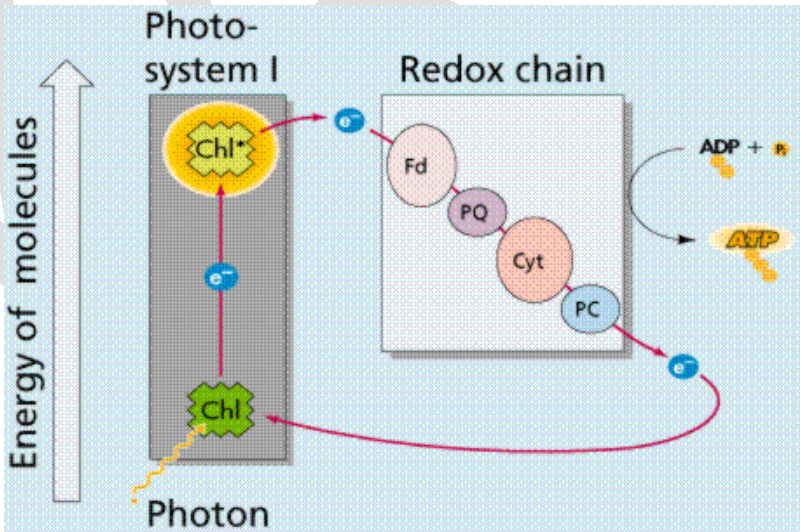


Cyclic and Non Cyclic Photophosphorylation

- Photophosphorylation is the process of creating ATP using a Proton gradient created by the Energy gathered from sunlight. The process of creating the Proton gradient resembles that of the electron transport chain of Respiration. But since formation of this proton gradient is light-dependent, the process is called Photophosphorylation.
- Non-cyclic photophosphorylation really refers to the ATP generated by Protons moved across the thylakoid membranes during the Z-scheme. The Cytb6-f complex acts as an electron transport chain. As the electrons lose energy (during a series of re/dox reactions) protons are moved into the Thylakoid space. This Proton gradient can be used to generate ATP chemiosmotically.



- During Cyclic Photophosphorylation the electrons are recycled, hence the name cyclic photophosphorylation. The excited electrons resulting from the absorption of light in photosystem I are received by the primary electron acceptor and then transferred to the cytb6-f complex which acts as an electron transport chain. The electrons return back to the reaction center of Photosystem I, where the cycle is ready to start all over. The electrons are using to translocate Protons which the ATPase uses to synthesize ATP. No reduction of NADP+ occurs in Cyclic Photophosphorylation.



Evidences in support of light and dark reactions

1. Evidence from intermittent light indicates that the rate of dark reaction is reduced due to continuous supply of light.
2. From temperature coefficient – It also indicates that light and dark reactions are although independent but are interlinked.
3. From CO2 reduction in dark – It indicates that this phase is definitely a dark phase.

POSSIBLE QUESTION

Unit-I

PART A (2 Marks)

1. List out the functions of peroxisomes.
2. Define Red drop and Emerson's enhancement effect.
3. Write a note on vacuoles.
4. List out the functions of power house of the cell.
5. Define reaction center.
6. What are the functions of golgi complex?
7. Define action spectrum and absorption spectrum.
8. List out the functions of plastids.
9. What are the supportive evidences of light and dark reactions?

PART B (8 Marks)

1. How many assimilatory powers are synthesized to assimilate CO2 in plants? Write the mechanism of synthesis.
2. Draw a neat diagram of golgi complex and elaborate its functions.
3. Elaborate on the structure and functions of plastids.
4. Explain in detail the mechanism of cyclic photophosphorylation with supportive evidence of light and dark reactions.
5. Explain photosystem I and photosystem II and its involvement in photosynthesis.

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DEPARTMENT OF BIOCHEMISTRY

III B.SC BIOCHEMISTRY – FIFTH SEMESTER

17BCU503A – PLANT BIOCHEMISTRY

MULTIPLE CHOICE QUESTIONS

Unit 1

Question	Opt A	Opt B	Opt C	Opt D	Answer
The most predominant chemical constituent of life	Water	protein	lipid	carbohydrate	Water
The cellular organelles regarded as the digestive tract of the cell	Nucleus	Golgi apparatus	mitochondria	endoplasmic reticulum	endoplasmic reticulum
Gases such as oxygen and carbon dioxide cross the plasma membrane by	secondary active transport	passive diffusion through the lipid bilayer	specific gas transport proteins	primary active transport	passive diffusion through the lipid bilayer
Which of the following is an example of primary active transport	Cl--HCO_3- exchange	Na^+ - H^+ exchange	Na^+-Ca^{2+} exchange	The Na^+, K^+ ATPase	The Na^+, K^+ ATPase
The sodium pump	Exchanges extracellular Na+ for intracellular K+	Is important for maintaining a constant cell volume	Can only be inhibited by metabolic poisons	Is an ion channel	Is important for maintaining a constant cell volume
A substance can only be accumulated against its electrochemical gradient by	Facilitated diffusion	Passage through ion channels	Diffusion through a uniport	Active transport	Active transport
The movement of molecules from an area of high concentration to an area of lower concentration is known as	Osmosis	Diffusion	Active Transport	Phagocytosis	Diffusion
What is the collective term for all of the chemical processes occurring within a cell?	Anabolism	catabolism	metabolism	synthesis	metabolism
Change in color of particular reactant can be detected in	Spectrometer	calorimeter	colorimeter	all of them	colorimeter

According to the Beer-Lambert Law, on which of the following does absorbance not depend?	Distance that the light has travelled through the sample	Colour of the solution	Solution concentration	Extinction coefficient of the sample	Colour of the solution
What is the name of an instrument used to measure the absorbance of a coloured compound in solution?	Coulometer	Colourmeter	Colorimeter	Calorimeter	Colorimeter
The wavelength of an absorption is 495 nm. In what part of the electromagnetic spectrum does this lie?	Radiowave	Infrared	Ultraviolet-visible	Microwave	Ultraviolet-visible
Aqueous KMnO4 solutions are purple. A plot of absorbance against concentration is	linear with a positive gradient	non-linear	an exponential curve	linear with a negative gradient	an exponential curve
Ribosomes help in	Protein synthesis	Photosynthesis	Lipid synthesis	Respiration	Protein synthesis
Food is converted to energy in	Nucleus	Nucleolus	Chloroplast	Mitochondria	Mitochondria
Extra cellular DNA is found in	Chloroplast	Endoplasmic reticulum	Ribosomes	Nucleus	Chloroplast
Fluid mosaic model was given by	Robertson	Schwann	Dave Donson	Singer and Nicolson	Singer and Nicolson
The cellular organelles called “suicide bags” are	Lysosomes	Ribosomes	Nucleolus	Golgi’s bodies	Lysosomes
The power house of the cell is	Nucleus	Cell membrane	Mitochondria	Lysosomes	Mitochondria
The Golgi complex	Synthesizes proteins	Produces ATP	Provides a pathway for transporting chemicals	Forms glycoproteins	Forms glycoproteins
Plasma membrane is made up of	Protein, lipid, carbohydrate	Lipid, carbohydrate	Protein, lipid	Protein	Protein, lipid, carbohydrate
Plant cell is mainly composed of	Cellulose	Starch	Protein	Lipid	Cellulose
Anabolism and catabolism are types of	chemical reaction	chain reactions	metabolism	complex reactions	metabolism
Overall chemical reaction that takes place with in a cell are collectively called as	metabolism	anabolism	complex reaction	catabolism	metabolism

Study of chemical components as well as chemical processes that takes place in a living organism is	Microbiology	Biochemistry	Fresh water biology	Chemical biology	Biochemistry
Which of the following is a chemical link between catabolism and anabolism?	AMP	ADP	ATP	All of these	ATP
Tunnels which allow specific ions to pass through them are called	selectively permeable tunnels	permeable tunnels	both A and B	channel proteins	channel proteins
Type of transport which always involves a protein is	passive transport	active transport	lateral diffusion	flip flop	active transport
Mitochondrial DNA is	Circular double stranded	Circular single stranded	Linear double helix	None of these	Circular double stranded
Which of these is part of the cell membrane?	triglycerides	phospholipids	ATP	more than one of these	phospholipids
How do fat-soluble molecules normally get into a cell?	they dissolve in the fat layers of the membrane and enter the cell by diffusion	they pass through protein pores in the cell membrane	they are absorbed by phagocytosis	they never get in	they dissolve in the fat layers of the membrane and enter the cell by diffusion
The phospholipids are unusual molecules because:	they have hydrophilic regions	they have hydrophobic regions	they are triglycerides	both A and B	they have hydrophobic regions
Which of the following statements best describes the "fluid mosaic model" of the structure of the cell membrane?	two layers of protein with lipid layers between the protein layers	two layers of lipid with proteins between the lipid layers	a double layer of lipid molecules with protein molecules suspended in the layer	A single layer of protein on the outside and a single layer of lipids on the inside	a double layer of lipid molecules with protein molecules suspended in the layer
The movement of chloride ions from an area where chloride is concentrated to an area where chloride is less concentrated is which of these?	diffusion	active transport	osmosis	exocytosis	diffusion

If a cell has a solute concentration of 0.07% which of the solutions would be hypotonic to the cell?	0.01% solute	0.1% solute	1% solute	10% solute	0.01% solute
Which of the following is necessary in order for osmosis to occur?	a permeable membrane	a semi-permeable membrane	an isotonic solution	ATP	a permeable membrane
Which of these are passive transport mechanisms?	osmosis	diffusion	phagocytosis	both A and B	both A and B
In an isotonic solution there would be:	no net movement of water	net movement of water into the cell	net movement of water out of the cell	bursting of the cell	no net movement of water
The sodium-potassium pump (which carries sodium out of a cell and potassium into a cell) is an example of:	active transport	endocytosis	exocytosis	passive transport	active transport
The process of a cell engulfing a solid object is:	phagocytosis	exocytosis	pinocytosis	diffusion	phagocytosis
What is likely to happen to a plant cell that is placed in pure water?	it becomes turgid	it becomes flaccid	it undergoes plasmolysis	it bursts	it becomes turgid
When a cell bursts due to osmosis, it is in a solution that is:	hypertonic	isotonic	hypotonic	either A or C	hypotonic
Why do plant cells behave differently to animal cells when placed in a hypotonic solution?	Plant cells are permeable to water	Plant cells do not carry out active transport	Plant cells contain a vacuole	Plant cells have a cell wall	Plant cells have a cell wall
Which of these equations is correct?	ATP + inorganic phosphate --> ADP	ADP + inorganic phosphate --> ATP	ATP + ADP --> inorganic phosphate	ATP + ADP --> organic phosphate	ADP + inorganic phosphate --> ATP
Atoms which have same number of protons but different number of neutrons are called	isotopes	isomers	spectators	allotropes	isotopes
To determine mass of other compound by comparing it with mass of carbon-12 atoms is	relative molecular mass	relative atomic mass	relative molecular radius	relative atomic radius	relative atomic mass
Sum of protons (p+) and neutrons (n0) in an atom is called its	atomic number	nucleon number	Avogadro's number	protonic identity	nucleon number
Chloride ion has number of protons of	17	18	24	34	17
Smaller particles in atom are called	atomic particles	sub-atomic particles	smaller particles	neutral particles	sub-atomic particles

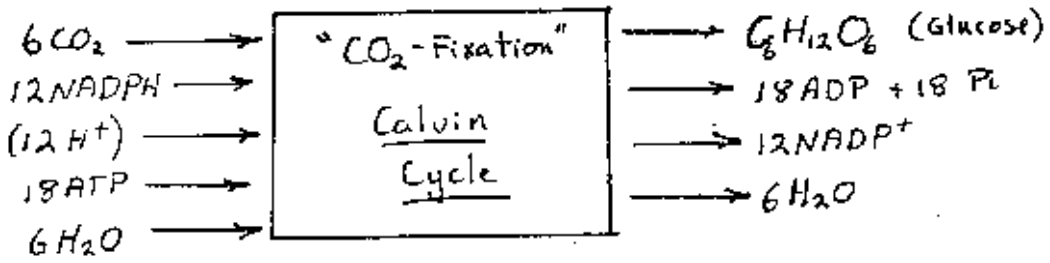
Number of protons and electrons in n atom is	different	same	average	constant	same
Electrons orbit around nucleus and bears	positive charge	negative charge	no charge	neutral charge	negative charge
Positively charged particle of atom is called	protons	neutrons	electrons	charges	protons
Neutrons carry	positive charge	negative charge	no charge	neutral charge	no charge
Total number of protons in an atom of each element is called its	atomic number	atomic mass	molecular mass	atomic scale	atomic number
Nucleus in an atom consists of	protons	neutrons	electrons	a and b	a and b
Molecules which contains fixed number of same type of atoms are molecules or	elements	compounds	mixtures	all of them	elements
If an atoms loses electron ion obtained is charged	positively	negatively	neutral	smaller	positively
If 3 Na+ ions pumped out of cell and 2 K+ pumped in to cell then number of ATP molecules hydrolyzed are	1	2	3	4	1
The filter color used to measure optical density of any blue color solution is	Blue	Yellow	Red	Green	Blue
The cuvette used for analysis of sample at UV range is	Glass cuvette	Quartz	Silica	All	Quartz

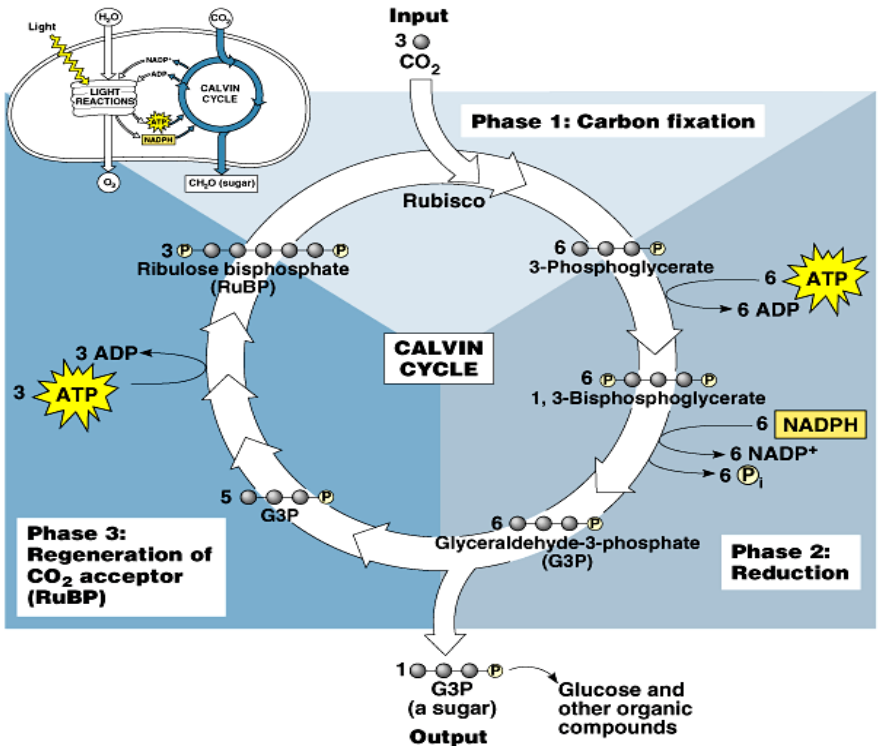
UNIT-II

Assimiliatory mechanisms in plants: Photorespiration and water consumption, CO_2 assimilation by C3 and C4 plants. Nitrogen assimilation; reduction of nitrate, nitrogen fixation in symbiotic and non-symbiotic plants, nitrogen cyclce. Sulphate metabolism in leaf; sulfite reduction and sulphur cycle, glutathione synthesis. Carbon and phosphorus cycles.

The Light Independent Reactions-Dark reactions

The Calvin Cycle (C3 plants)





This part of photosynthesis occurs in the stroma of the chloroplasts called carbon dioxide fixation.

The fixation of the CO_2 is carried out by a giant enzyme ribulose biphosphate carboxylase/oxidase (RUBISCO) which is the most abundant enzyme on earth. This enzyme is very sluggish it works much slower than most other enzymes. (i.e. ~ 3 molecules of substrate per sec. compared with ~1000/sec for others). Therefore, there are many copies of this enzyme in the stroma ~ 50% of chloroplast protein.

The first fixation reaction of the cycle uses a five carbon sugar ribulose 1-5 biphosphate and adds to it a CO_2 molecule to form 2 (3 carbon) molecules of 3-phosphoglycerate. These are rearranged through a series of energy requiring reactions, using up ATP and NADPH to generate 2 molecules of glyceraldehydes 3 - phosphate. (If this were done six (6) times we now would have 12 molecules of glyceraldehydes - 3 - phosphate (G3P). Two (2) of the G3Ps are removed to make one glucose while the rest 10 G3Ps go back into the cycle to regenerate six (6) of the five (5) carbon sugars ribulose 1-5 biphosphate.

The Calvin Cycle and "C4 Plants"

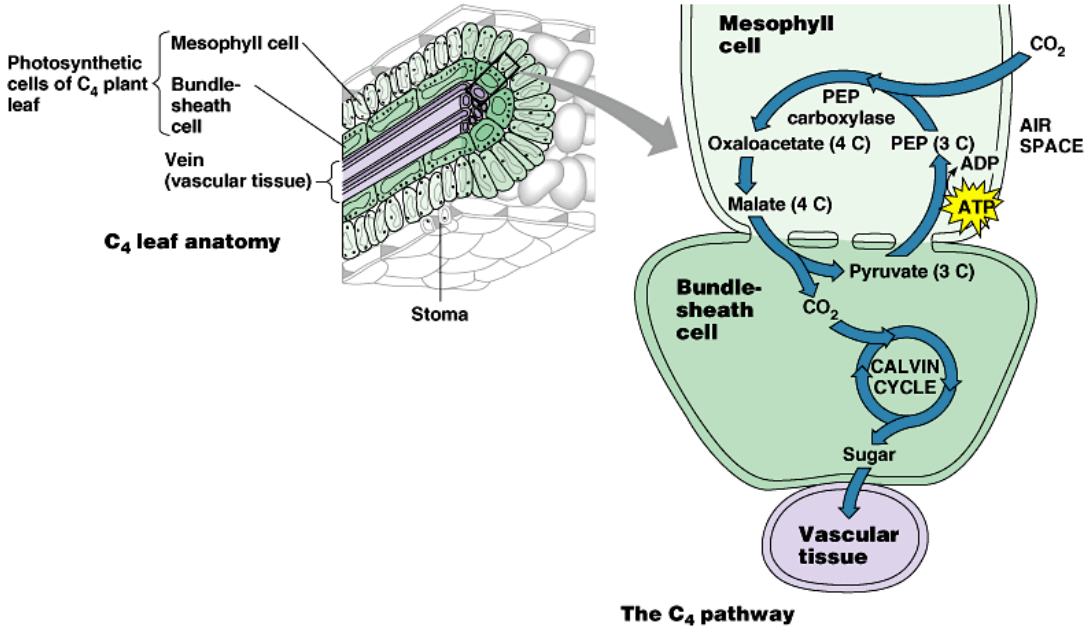
A number of plants display an increased and more efficient net photosynthesis during strong light intensities. Prime examples are the Gramineae of warmer regions like maize or sugar-cane.

At the beginning of the sixties observed that the first product of photosynthesis in sugar-cane is not the C_3 unit 3-phosphoglycerate but a unit with four C-atoms. The Australian plant physiologist MD Hatch and his English colleague CR Slack confirmed this result and identified the compound as oxaloacetate (OAA). It is produced by the addition of one molecule of carbon dioxide to phosphoenolpyruvate (PEP). The cycle is also known as the HATCH- SLACK-cycle or the C_4 cycle. Plants with this cycle are called C_4-plants (and CAM plants, respectively) in contrast to C_3 plants where the carbon dioxide is directly fed into the CALVIN cycle. The oxaloacetate is usually converted into malate of which the

carbon dioxide is split off again with the help of an enzyme. This carbon dioxide is now bound by ribulose-1,5-diphosphate and assimilated *via* the CALVIN cycle. : Some species use malate instead of aspartate

oxaloacetate + L-glutamate > aspartate + *alpha*-ketoglutarate.

The reversible binding of carbon dioxide has the function to accumulate and store CO_2. The process consumes energy, so that it could also be spoken of a carbon dioxide pump. It should be mentioned that the Hatch-Slack cycle requires two molecules of ATP are per fixed carbon dioxide.

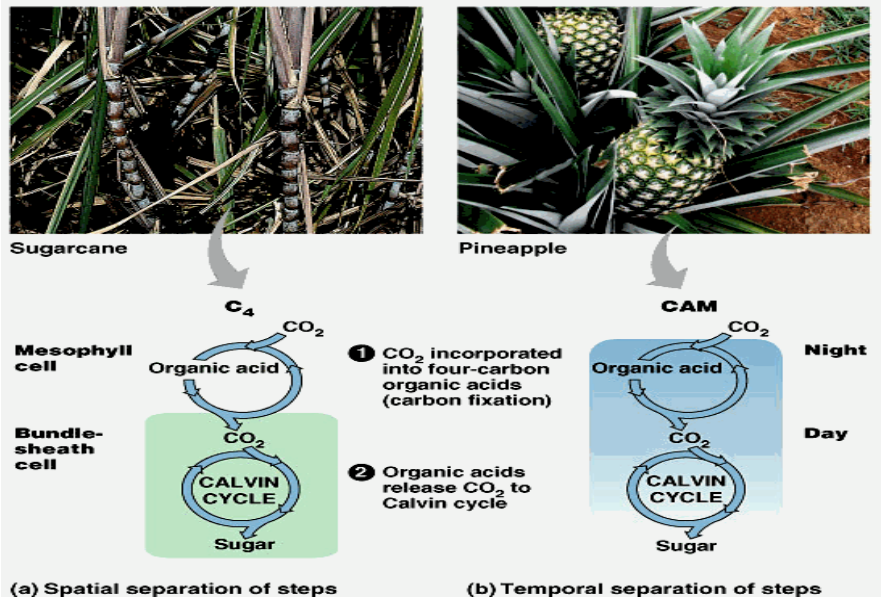


The C_4 pathway

Crassulacean Acid Metabolism

The ultimate prevention of CO_2 loss is found in desert plants like cactus. CAM In these plants the stomata are open at night. The plant fixes CO_2 into C_4 carbon compounds during the night, then transfers the carbon to the Calvin cycle during the daylight hours while the stomata are completely closed therefore reducing H_2O loss. This is all done in the same cell.

C_3, C_4 and CAM. Regulation of the Activity of Photosynthesis



Photosynthesis of C_4 plants

CO_2 is bound to phosphoenolpyruvate (PEP) in mesophyll cells. The product is oxaloacetate. The next step generates malate. In the cells of the vascular bundle sheath, the 'Kranz' cells, is carbon dioxide split off the malate and fed into the CALVIN cycle. The pyruvate is transported back into the mesophyll cells (active transport) and is with the help of additional ATP phosphorylated to PEP.

The anatomy of C_4 leaves with so-called 'Kranz' cells differs fundamentally from that of C_3 plants. The chloroplasts of C_3 plants are of homogeneous structure, while two types of chloroplasts occur in C_4 plants. The mesophyll cells contain normal chloroplasts, that of the vascular bundle sheath have chloroplasts without grana, i.e. they are partially impaired in function. This peculiarity does not affect the CALVIN cycle, it concerns only the light reactions of photosynthesis. The first binding of carbon dioxide (the HATCH-SLACK reaction) occurs in the mesophyll cells, the incorporation into carbohydrates (the CALVIN

cycle) in the cells of the vascular bundle sheath. Both processes of photosynthesis are spatially separated.

The Crassulacean Acid Metabolism (CAM)

CAM is the abbreviation of Crassulacean acid metabolism. The name points at the fact that this pathway occurs mainly in Crassulacean species (and other succulent plants). The chemical reaction of the carbon dioxide accumulation is similar to that of C_4 plants but here are carbon dioxide fixations and its assimilation not separated spatially but in time. CAM plants occur mainly in arid regions. The opening of the stomata to take up carbon dioxide is always connected with large losses of water. To inhibit this loss during intense sun (the transpiration *via* the cuticle remains intact) has a mechanism developed that allows the uptake of carbon dioxide during the night. The prefixed carbon dioxide is stored in the vacuoles as malate (and isocitrate) and is used during the daytime for photosynthesis.

Which Metabolism Goes With Which Conditions?

The enzyme that catalyzes the primary carbon dioxide fixation of C_4 and CAM plants is phosphoenolpyruvate carboxylase (PEPC). Its affinity for carbon dioxide is by far higher than that of Rubisco, the first enzyme of the CALVIN cycle. As a consequence are C_4 plants able to use even trace amounts of carbon dioxide. PEPC occurs in small amounts (roughly 2 - 3 %) also in C_3 plants, where it, too, has a key position in the metabolic regulation.

CAM

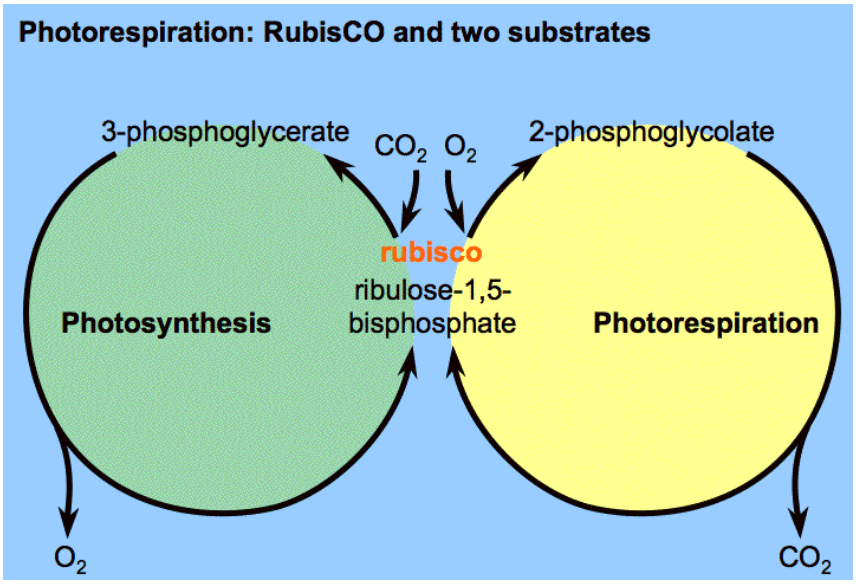
Advantages and Disadvantages

CAM has been detected in more than 1000 angiosperms of 17 different families. It is usually accompanied by succulence, though not all Crassulaceae, for example, display CAM and succulence is no precondition of CAM. *Tillandsia usneoides* of the bromelia family is not succulent, but uses CAM. *Mesemryanthemum crystallinum* (a plant with succulent leaves) can use the C_3 pathway but switches to CAM when growing in saline soils. Under experimental conditions can the shift be achieved by increasing the NaCl concentration of

the nutrient medium While the advantage of C_4 plants comes in useful under high light intensities, is the degree of the CAM influence in CAM plants regulated mainly by temperature, atmospheric humidity and salinity. Both strong and weak CAM plants are known. In weak CAM plants becomes CAM only apparent at certain differences between day and night temperature. CAM plants that store a lot of malate and due to the thus high osmotic value also a lot of water, are usually less frost resistant than C_3 plants. Because of the high concentration of acid are they less heat resistant, too. Species of arid regions are therefore forced to break their pool of malate down during the daytime. Usually do the C_4 pathway and CAM exclude each other. An exception is the succulent C_4 dicotyledon *Portulaca oleracea* that is able to choose the optimal pathway under natural conditions.

Photorespiration

Rubisco also catalyzes photorespiration

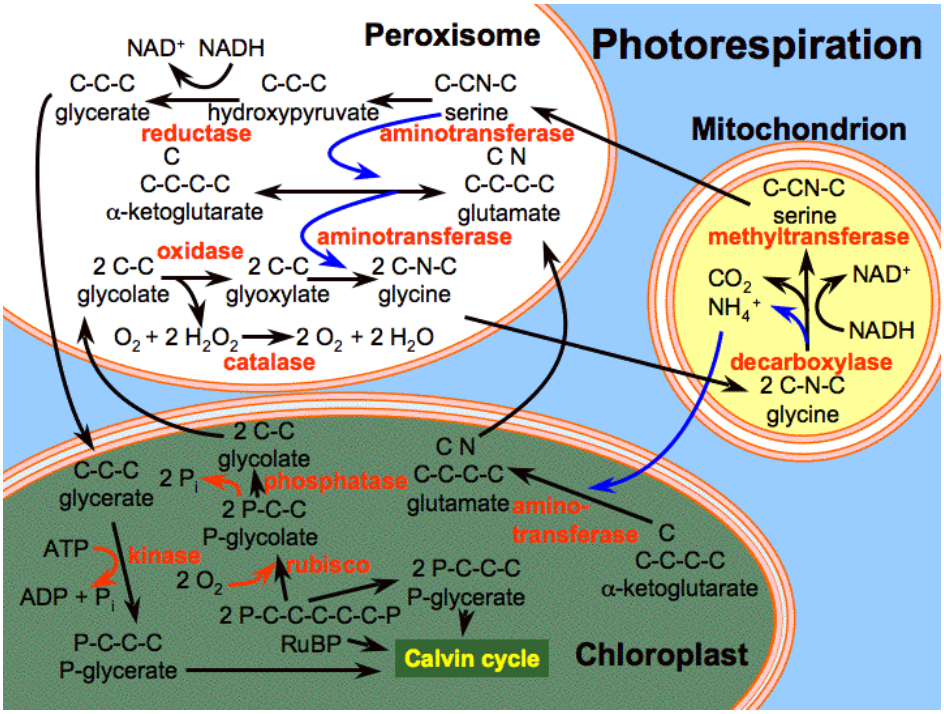


The enzyme, rubisco, not only initiates carbon fixation in the Calvin cycle; it also combines with oxygen to initiate photorespiration. As its name suggests (rubsiCO) the enzyme is both a carboxylase and an oxygenase. The active site of rubisco cannot

distinguish the two similar substrates: O=C=O and O=O. As we shall see, the two reactions catalyzed by the same enzyme are diametrically opposed to each other.

Each reaction pathway undoes the other, and both reactions can operate in a cell simultaneously depending upon the environmental conditions. As both substrates combine with the active site of rubisco, they are competitive inhibitors of each other's reactions. One might recall our earlier discussions about competitive inhibition. The relative concentration of the two substrates and the differential affinity of the enzyme for each substrate will determine which of the reactions (Calvin cycle or Photorespiration) predominate. Fortunately for plants rubisco has an affinity for carbon dioxide that is 80 times higher than its affinity for oxygen. However, the relatively low ratio of CO_2 to O_2 of mesophyll fluids in contact with air (0.04) means that, in a typical plant, the Calvin cycle only occurs about three times faster than photorespiration. Temperature also influences the relative rates of photorespiration and the Calvin cycle. Because increased temperature more efficiently removes carbon-dioxide from solution than it does oxygen, high temperatures favor photorespiration.

The photorespiration pathway is an enzymatic one that is not coupled to any electron transfer system. It does not generate ATP. It does use oxygen and it does produce carbon dioxide, and it uses a sugar-phosphate as its primary fuel. The complete pathway is depicted here.



It is worthy to note that this diagram, as others of its type, show the organelles tightly appressed to each other. Indeed there are some famous electron micrographs (example above) that show this, but other micrographs do not show them this way. I say this just to comment that this positioning may be more an efficient design for communication to students than a realistic portrayal of life in a typical cell.

In the chloroplast, rubisco, combines with ribulose-1,5-bisphosphate (RuBP) and oxygen. The five-carbon RuBP is split into the two-carbon 2-phosphoglycolate and the three-carbon 3-phosphoglycerate (PGA). The enzymes of this pathway are enumerated in the diagram above.

The 2-phosphoglycolate is converted to glycolate by phosphoglycolate phosphatase in the chloroplast. The phosphate liberated is returned to the local phosphate pool. The glycolate is transported from the chloroplast into a nearby peroxisome.

In the peroxisome, the glycolate is oxidized by oxygen gas to glyoxylate and hydrogen peroxide by glycolate oxidase. The peroxide is converted to water and oxygen gas by catalase. So the consumption of oxygen in the oxidation is replaced by catalase activity in the peroxisome.

The glyoxylate is converted to the amino acid glycine in the peroxisome. The amino group is transferred to the glyoxylate from glutamate (another amino acid) by glyoxylate:glutamate aminotransferase. The glutamate is converted to α-ketoglutarate. The glycine is transported to the mitochondrion.

In the mitochondrion, glycine decarboxylase carves off carbon dioxide gas from the glycine. This requires NAD^+ to park the hydrogen atom. It also cleaves off the amino group. If you are paying attention to the chemical structures, you realize that the two-carbon amino acid has had both its amino and acid groups removed! There is only one carbon left! This methylene group is parked on a folate molecule in the mitochondrion.

When a second glycine arrives into the mitochondrion from the peroxisome, it combines with the methylene-folate to release the three-carbon amino acid serine through the action of serine hydroxymethyltransferase. Also released for re-use by this enzyme reaction is the folate. The serine is transported to the peroxisome.

In the peroxisome, the serine loses its amino group to α-ketoglutarate to regenerate the glutamate required in an earlier step in the pathway. This amino-transfer is accomplished by serine aminotransferase. In this reaction the serine is converted to hydroxypyruvate.

The peroxisome reduces the hydroxypyruvate to glycerate by hydroxypyruvate reductase. The reducing power for this comes from NADH; if you recall this was produced in an earlier step in the mitochondrion. The glycerate is transported to the chloroplast.

In the chloroplast, the glycerate is converted by glycerate kinase to 3-phosphoglycerate. The phosphate comes from ATP. Instead of producing ATP, photorespiration uses ATP. The 3-phosphoglycerate from the beginning and this new one

from the end of photorespiration enter the chloroplast pool of PGA that is used to regenerate RuBP.

Photorespiration loses 25% of the carbon it takes from the Calvin cycle

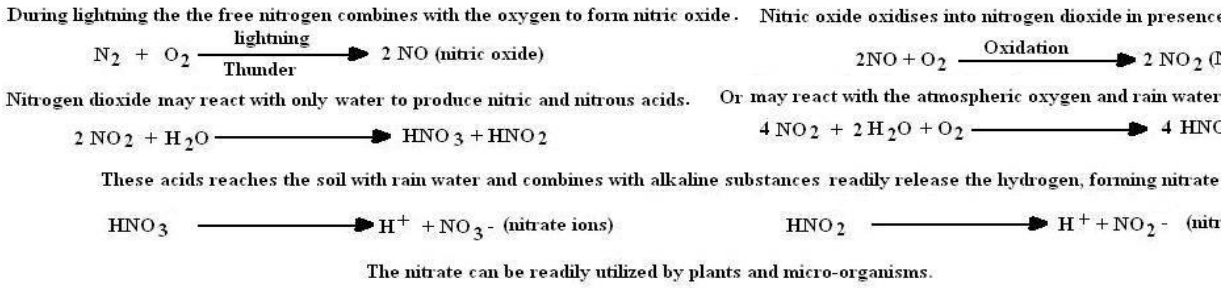
The photorespiration pathway siphons carbon away from the Calvin cycle, but it also returns some of what it takes. Because it takes two glycines in photorespiration to complete the pathway, two glycolates must be taken from the Calvin cycle. Of these four carbons taken, one is lost as carbon dioxide and three are returned to the Calvin cycle. This 25% loss of carbon is going to give measurement errors for photosynthesis in whole-cells or leaves. We are probably under-estimating the photosynthetic rate by 25%.

Rubisco evolution

The wastefulness of photorespiration is probably a consequence of just two factors. Early in the evolution of photosynthesis there was a higher carbon dioxide to oxygen gas ratio in the ancient atmosphere. Indeed the atmosphere was likely anaerobic in the earliest times on Earth. Rubisco evolved its active site when oxygen was rare and carbon dioxide was common. Since ancient times, rubisco has not yet evolved a mechanism to discriminate between the two similar substrates (O=C=O and O=O). The reactions are similar too; the substrate is attached at a point along RuBP resulting in its splitting into organo-monophosphates. 3-phosphoglycerate is a common product of both reactions. So the difficulty of a protein to distinguish such similar molecules and to catalyze one reaction but not the other just has not happened yet. Photorespiration losses have not been intolerable either; the selection pressure is probably not severe in most environments. But in hot, dry, heavily-populated environments where plants effectively reduce the local carbon-dioxide content of the atmosphere, selection should have resulted in a few adaptations to overcome photorespiration. In Nitrogen fixation and nitrogen metabolism

Living organisms need nitrogen because it is a part of the amino acids that make up proteins, and the nucleic acids that make up DNA (deoxyribonucleic acid) and RNA

(ribonucleic acid).Nitrogen (dinitrogen, N2) is highly inter gas. Because the nitrogen atoms in dinitrogen are bound by a very strong triple bond, this gas is very stable and cannot be utilized as a source of nutrition by any but a few highly specialized microorganisms. Nitrogen within living organisms is eventually decomposed and converted to atmospheric nitrogen (N2). Molecular nitrogen (N2) is the major component (approximately 80%) of the earth's atmosphere but most organisms cannot use free nitrogen, to build the chemicals required for growth and reproduction. But it has to be combined with C,H,N,O to form compounds. Before its incorporation into plants, N2 must first be "fixed" (combined) in the



form of ammonium (NH4) or nitrate (NO3) ions. This process of reduction of N2, commonly known as "nitrogen fixation" (N-fixation) .

Nitrogen fixation is the process by which atmospheric nitrogen gas is converted into salts of nitrogen such as, ammonia, nitrate and nitrogen dioxide.

$$N_2 + 16\ ATP + 8e^- + 8H^+ \longrightarrow 2NH_3 + 16\ ADP + 16\ Pi + H_2$$

Although ammonia (NH3) is the direct product of this reaction, it is quickly ionized to ammonium (NH4+). The reaction is mediated by an oxygen-sensitive enzyme nitrogenase and requires energy, as indicated by the consumption of adenosine triphosphate (ATP). This complex process is carried out by nitrogen-fixing bacteria present in the soil.

Nitrogen fixation is of two types

Non Biological Fixation

Atmospheric nitrogen fixation (or Natural) by lightning:- It contributes about 10% of the total nitrogen fixation. Usually atmospheric nitrogen fixation (or Natural) by lightning occurs in rainy season during lightning or thunder storms.

Industrial fixation through the Haber - Bosch process and combustion. Some ammonia is also produced industrially by the Haber-Bosch process. When nitrogen (dinitrogen) combines with hydrogen in the presence of an iron-based catalyst, at a pressures of 35-100 MPa and fairly high temperature. Usually fossil fuels are used both as a source of energy and hydrogen. Most nitrogenous fertilizers are now derived from atmospheric nitrogen through this type of fixation process.

Biological fixation by certain microbes - alone or in a symbiotic relationship with some plants and animals. Biological nitrogen fixation was discovered by the Dutch microbiologist Martinus Beijerinck. It contributes 60% of total nitrogen fixation. But the major conversion of atmospheric N2 into salts of nitrogen, and then into proteins, is achieved by microorganisms (prokaryotes) such as bacteria, fungi and algae in the process called biological nitrogen fixation (or dinitrogen fixation). Microorganisms that fix nitrogen are called diazotrophs.

- Free living or non-symbiotic nitrogen fixation: The fixation of free nitrogen of the soil by all the microorganisms living freely or outside the plant cell is called non-symbiotic biological N2 fixation. It is performed by the aerobic and anaerobic bacteria and blue green algae.
- By bacteria Nitrogen fixing bacteria: which are present in the soil convert free nitrogen into soluble compound which are absorbed from the soil by plants. The nitrogen fixing bacteria are of four types:-
- Free living non-photosynthetic aerobic nitrogen fixing bacteria e.g., Azotobacter, Beijerinckia and Derxia.
- Free living non-photosynthetic anaerobic nitrogen fixing bacteria e.g., Clostridium.

- Free living photosynthetic nitrogen fixing bacteria e.g, Chromatium, Rhodo-pseudomonas, Rhodospirillum.
- Free living chemosynthetic nitrogen fixing bacteria e.g., Desulfovibrio
- By free living nitrogen fixing Blue-Green algae. About 15 genera of photosynthetic cyanobacteria (blue-green algae) are found freely in the soil where they fix free N2 into nitrogenous and ammonium compound. Mostly they are heterocysts. e.g, *Nostoc, Anabaena, Aulosira, Cylindrospernum, Calothrix*. Nitrogen fixation occurs in special thick walled cells termed heterocysts or heterocytes (H) which occur at intervals along the cyanobacterial filaments. This separation of cellular functions is necessary because cyanobacteria have oxygen-evolving photosynthesis but the nitrogen-fixing enzyme, nitrogenase, is unstable in the presence of oxygen. This problem is overcome because the heterocysts contain only part of the photosynthetic apparatus, termed photosystem I, which can be used to generate energy (as ATP). But the heterocysts do not contain photosystem II, which is used to split water into hydrogen (for combination with CO2 to produce organic products) and oxygen. Non-heterocystous nitrogen fixing blue-green algae are less in number e.g., Oscillatoria, Phormidium, Gleocapsa.
- Free-living, non-photosynthetic bacteria depend on soil organic matter as a food source whereas the photosynthetic microorganisms may derive their food from the products of photosynthesis.
- The nitrogen fixing activity of free-living, non-photosynthetic, aerobic bacteria is strongly dependent on favorable moisture conditions, oxygen, and an organic food source. Anaerobic representatives (Clostridium) predominate in grassland and waterlogged soils and soil aggregates where moisture conditions and organic substrates are available but oxygen supply to the micro-environment of the bacteria is severely restricted.

Symbiotic

Some N_2-fixing organisms develops loose (associative) symbiosis with plants or animals (Acetobacter and sugarcane), or establish longer-term relationships within specialized structures provided by their host (Rhizobium and the legume nodule). To provide them with sugars, supplying both a source of energy and a source of carbon for the bacterium's own synthetic reactions. Symbiosis is a close ecological relationship between the individuals of two (or more) different species. Sometimes a symbiotic relationship benefits both species, sometimes one species benefits at the other's expense, and in other cases neither species benefits.

The fixation of free nitrogen of the soil by N_2-fixing organisms living symbiotically inside plants is known as symbiotic biological nitrogen fixation.

Nitrogen fixation through nodule formation in Leguminous plants

The bacteria responsible for the formation of root nodules in leguminous plants belong to the genus Rhizobium. Rhizobium also lives free in soil but only fixes N_2 when inside plant The symbiotic Bacteria Rhizobia (from the Greek words Riza = Root and Bios = Life) are soil bacteria that fix nitrogen (diazotrophy) after becoming established inside root nodules of legumes. According to host specificity and growth of bacteria have been divided into three groups:

Genus	Species	Plant host
Rizobium	leguminosarum	Peas
Rizobium	Meliloti	Lucerne
Rizobium	Trifolii	Clover
Rizobium	phaseoli	Beans
Rizobium	Lupine	Lupins

Bradyrhizobium japonicum is the group of slow growing symbionts of Soybeans (plant host).

Azorhizobium caulinodans is a bacterium that forms stem nodules in Sesbania (plant host).

The bacteria "invade" the plant and cause the formation of a nodule by inducing localised proliferation of the plant host cells. Root nodules acts as a site of Nitrogen fixation. The root nodules contain a pigment called leghaemoglobin (serving the same function as the oxygen-carrying haemoglobin in blood).The heme (oxygen-binding) portion is produced by the bacterium, while the globin (protein) portion is produced by the host plant, again showing the closeness of the symbiotic relationship. The function of this molecule in nodules is to reduce the amount of free oxygen, and thereby to protect the nitrogen-fixing enzyme nitrogenase, which functions only under anaerobic conditions. Nitrogenase is the only enzyme that can split nitrogen molecule for nitrogen fixation.

Nitrogen fixation through nodule formation in None-Leguminous plants :- There are many plants belonging non- Leguminosae families, specially shrubs and plants which produces root nodules. Example: Frankia is a genus of the bacterial group termed actinomycetes - filamentous bacteria.

Frankia form nitrogen-fixing root nodules (sometimes called actinorhizae) with numerous genera of non-leguminous angiosperms, such as alder (Alnus species), sea buckthorn (Hippophaerhamnoides, which is common in sand-dune environments) and Casuarina (a Mediterranean tree genus).

Alder and the other woody hosts of Frankia are typical species that invade nutrient-poor soils. These plants probably benefit from the nitrogen-fixing association, while supplying the bacterial symbiont with photosynthetic products.

Rhizobiums also form nitrogen-fixing root nodules with genus Parasponia.

Sometimes nodules are also formed in the roots of certain gymnosperms e.g., Podocarpus and in the leaves of Pavettazinumermanniana and Chomelia.

Nitrogen fixation through non-nodulation: In some plants root nodules are not formed but symbiotic nitrogen fixation takes place. Examples: Lichens live as symbionts with photosynthetic cyanobacteria (blue green algae or Green chlorophyllous and with fungi.

Anthroceros (Bryophte): It contains Blue green alga Nostoc inside mucilage cavities present on ventral side.

Azolla: The water fern, Azolla lives symbiotically with the nitrogen-fixing cyanobacteria (Anabaena azollae) Azolla is grown in rice paddies early in the season. As the rice grows above the water surface, it shades out the fern, which dies, releasing the stored nitrogen. In this way, the paddy is fertilized without application of chemical fertilizers.

Cycas (gymnosperms) It contains cyanobacteria (blue-green algae) Anabaena or Nostoc. Aerial roots contain a nitrogen-fixing cyanobacterialsymbiont.

Gunneramacrophylla (angiosperms): Its stem contains Nostoc.

Associative Symbiotic Nitrogen Fixation

When bacteria form a close association with the roots of cereals and grasses and fix nitrogen, the association is of loose mutualism type and known as loose (associative) symbiosis and this type of nitrogen fixation is known as associative symbiotic nitrogen fixation. The bacteria grow in the rhizosphere in close contact with the roots, sometimes invade the outer cortical regions of the roots, and fix nitrogen. Azospirillum bransilense (= Spirillumlipoferum) a bacterium discovered J. Dobereiner (Edomonds, 1978), is the bacterium forming associate-symbiosis with the cereal roots. Others are Pseudomonas azotogensis, Enterobacter, Bacillus, Klebsiella etc.

Rates of symbiotic N_2 fixation in legumes vary with plant species and cultivator, growing season, and soil fertility. Some forage legumes can fix 600 kilograms per hectare per year but more common values are 100 to 300 kilograms per hectare per year. Rates for grain legumes are often lower. Inclusion of legumes in crop rotations is generally thought to improve soil nitrogen levels, but benefits depend on the level of N_2 fixed and the amount

of nitrogen removed in grain or forage. A good soybean crop might fix 180 kilograms per hectare but remove 210 kilograms per hectare in the grain. Non-symbiotic bacteria fix only upto 5 kg of nitrogen per hectare in one year

Formation of Nodule

- Rhizobia is the group of genera of alpha-proteobacteria (family Rhizobiaceae) which includes all of the nitrogen-fixing species that produce nodules with legumes such as clover and soybean, Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Phyllobacterium, Rhizobium, and Sinorhizobium, as well as the plant pathogen Agrobacterium.
- Rhizobia produce stem or root nodules on their host(s), and within these nodules receive protection from external stresses and energy for growth and N2 fixation. The host receives most of the nitrogen it needs for growth.
- Only infection via root hairs is considered here. Plants of legume family secrete flavonoids which are recognized by bacterial NodD protein When NodD binds flavonoid it activates other nodulation genes.
- Rhizobium secretes Nod factors (Some nod genes encode enzymes that make Nod factors) the leguminous plant recognizes Nod factors.
- Then growing root hair of a leguminous plant comes in contact with the bacterium - Rhizobium, the growing root hairs curl and form a pocket for the rhizobia. The bacteria invade the plant by a newly formed infection thread growing through it. The root hair cell wall.
- Simultaneously, cortical cells are mitotically activated, giving rise to the nodule primordium.

- lnfection threads grow toward the primordium, and the bacteria are then released into the cytoplasm of the host cells, surrounded by a plant derived peribacteroid

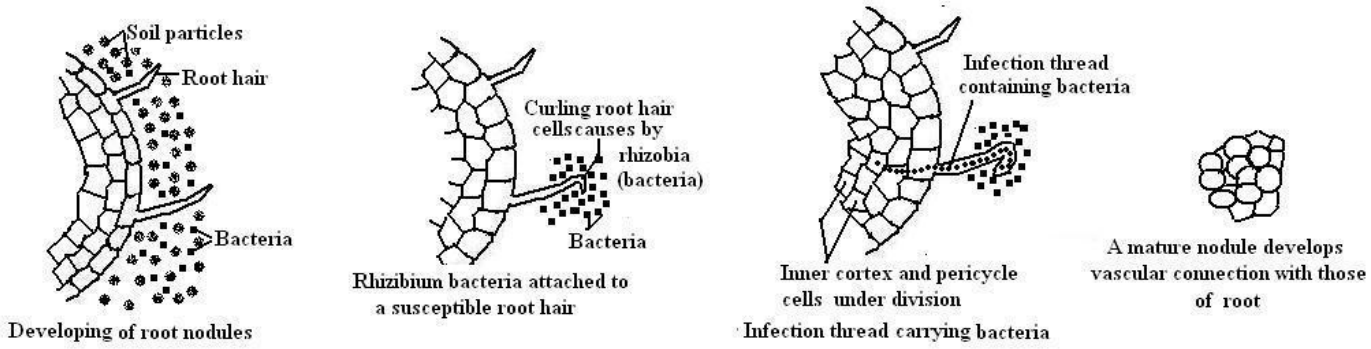


Fig 4. Development of root nodule

membrane (PBM). This separation is usually seen as a mechanism to suppress plant defense responses likely to harm the bacteria.

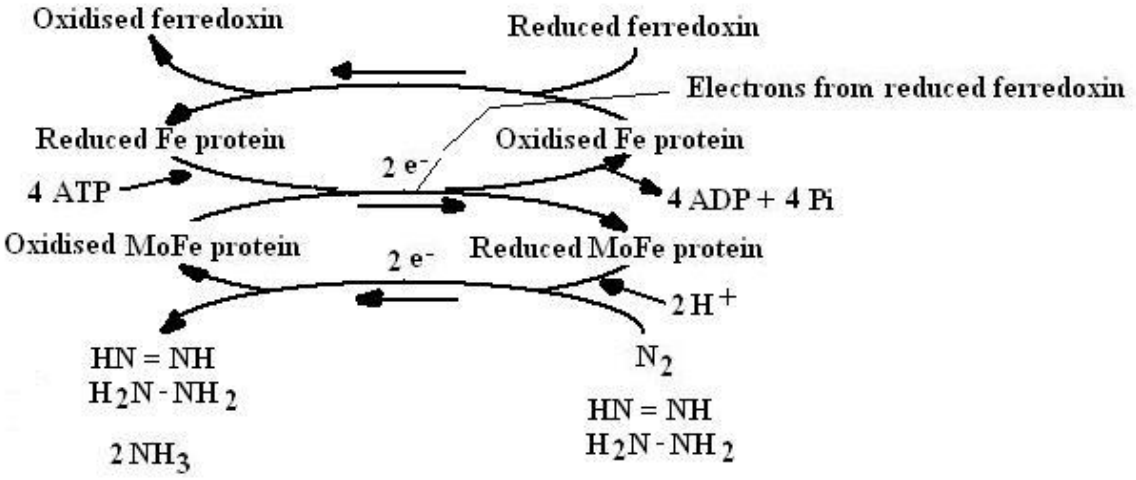
- With the production of the infection threads, bacteria produce cytokinins (type of plant hormone). Cytokinins promote division of plant cells to form nodules and nodules begin to form in the root hairs.
- The nodule primordium thereupon develops into a mature nodule, while the bacteria differentiate into their endosymbiotic form, which is known as the bacteroid. Bacteroids, together with the surrounding PBMs, are called symbiosomes.
- Cell division now sets in, in the infected tissue leading to nodule formation. The area of active N2 fixation is either pink or red in color due to the presence of leghaemoglobin needed for oxygen transport. The nodule thus formed establishes a direct vascular connection with the host for the exchange of nutrients.
- All of the steps of nodule development involve the expression of nodule-specific plant genes, the so called nodulin genes. The early nodulin genes encode products that are expressed before the onset of nitrogen fixation and are involved in infection and nodule development. The products of the late nodulin genes are involved in the interaction with the endosymbiont and in the metabolic specialization of the nodule.

Mechanism of nitrogen fixation

The bacteroids within the nodules formed on legume roots fix nitrogen.

Structure and Operation of Nitrogenase

The nitrogenase is an enzymatic complex, which converts atmospheric nitrogen (N2) to ammonia. Then nitrogenase complex exists in both free living nitrogen fixing organisms as well as in symbiotic nitrogen fixing bacteria. Nitrogenase is a complex of two separately isolated proteins- an iron protein or dinitrogenreductase and a molybdenum-iron protein or dinitrogenase. The proteins have a negative redox potential. The MoFe protein (Iron-Molybdenum protein), is a heterotetramer composed of two alpha subunits and two beta subunits. The protein contains two copies of each of two types of clusters: P clusters and FeMo cofactors. Each P cluster contains 8 iron atoms and 7 sulfides linked to the protein by 6 cysteinate residues. Each FeMo cofactor contains one molybdenum atom, 7 iron atoms, 9 sulfides. This protein is responsible for reducing atmospheric nitrogen to ammonia via a series of electron transfers within the protein to the substrate molecule. The reaction requires the addition of six electrons for each nitrogen molecule that is split into two ammonia molecules. The Fe protein (Nitrogenase Reductase - NR) is a dimer and formed by 2 subunits of polypeptide chains linked by a 4Fe-4S cluster. Each monomer contains an ATP binding site.

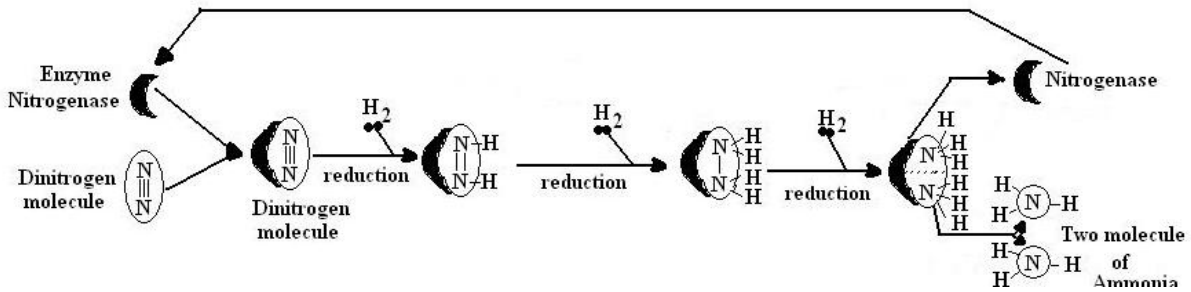


The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin) HN=NH is reduced to H2N-NH2, and this in turn is reduced to 2NH3.The reactions occur while N2 is bound to the nitrogenase enzyme complex.

The favorable condition for nitrogen fixation is

- Presence of enzymes nitrogenase and hydrogenase in the nitrogen fixing cells or organisms.
- Presence of leghaemoglobin which protect the enzyme nitrogenase from oxygen.
- Ferredoxin which supplies electrons for this process.
- A source of hydrogen (strong reducing agent) like NADPH or FMNH2
- Constant supply of ATP to transfer hydrogen atoms to dinitrogen.ATP is provided by aerobic respiration of sugars, ultimately produced by photosynthesis. Phosphorous is an important component of the biochemical energy source, ATP (adenosine triphosphate). Thus, for legumes to fix N, there must be adequate available soil P.
- Presence of coenzymes and cofactors.

- Compounds for trapping ammonia formed by the reduction of dinitrogen (N2). Nitrogen fixation is controlled by plant nod genes and bacterial nod, nif and fix gene cluster. Biological nitrogen fixation by free living and symbiotic bacteria is carried out by step by step progressive reduction of dinitrogen (N2) molecules by the addition of of a pair of hydrogen atoms. Depending on the type of microorganism, the reduced ferredoxin which supplies electrons for this process is generated by photosynthesis, respiration or fermentation. In the heterocystous bacteria the primary electron donor to nitrogenase is also a ferrredoxin, but it receives electrons produced by the action of light on the photosynthetic apparatus. The electrons are supplied via ferredoxin to nitrogenasereductase and then nitrogenase. The reductase donates 8 electrons in succession to the nitrogenase cofactor, a molybdenum-iron containing active center which catalyses the actual reduction of dinitrogen. Iron (Fe+3) and molybdenum (Mo+4) of enzyme nitrogenase takes part in attachment of a dinitrogen molecule (N2) and weaken the bonds between the two atoms of the nitrogen. The weakened molecule of nitrogen is reduced by the reducing agent (NADPH, FMNH2).
  It produces dimide (N2H2), hydrazine (N2H4) and then ammonia (2NH3) Where one molecule of N2 is reduced in the presence of protons to 2 NH3, and H2 as a byproduct. Semi-activated nitrogenase can reduce easy substrates such as acetylene. In the typical reaction, two molecules of ATP are consumed for each electron transferred. 16 ATP's are needed to fix a single nitrogen molecule in nitrogen fixation, the plant regulates the nitrogenase's activity and expression according to reduced nitrogen availability and oxygen presence.



- Molecular oxygen is a strong inhibitor of the nitrogenase Mo-Fe cofactor and is removed by the plant oxygen binding protein leghemoglobin in the root nodules. Some of the cyanobacteria have yet another mechanism for protecting nitrogenase: nitrogen fixation occurs in special cells (heterocysts) which possess only photosystem I (used to generate ATP by light-mediated reactions) whereas the other cells have both photosystem I and photosystem II (which generates oxygen when light energy is used to split water to supply H2 for synthesis of organic compounds). Nitrogenase also converts hydrogen ions to hydrogen gas at the same time thus consuming even more ATP in the process.

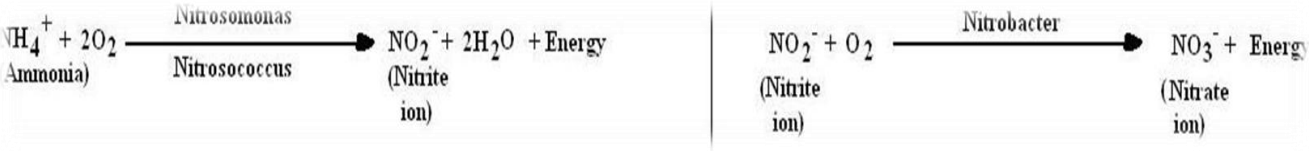
Nitrogen metabolism

Nitrogen metabolism All of the nitrogen in a plant, whether derived initially from nitrate, nitrogen fixation, or ammonium ions, is converted to ammonia, which is rapidly incorporated into organic compounds through a number of metabolic pathways.

Nitrogen reduction or Nitrification

Ammonia formed as a result of nitrogen fixation is used for the synthesis of amino acids. Amino acids are transported through phloem to other parts for the synthesis of proteins .Ammonium ions can be taken by higher plants but plants are more adapted to absorb nitrate ($NO3^-$) than ammonium ions ($NH4^+$) from soil. Nitrification is an aerobic microbial process by which specialized bacteria oxidize ammonium to nitrite and then to nitrate. It is accomplished by nitrifying bacteria like nitrosomonas, nitrosococcus and nitrobacter. Nitrification is a two-step process. The first stage is the oxidation of ammonium (NH4+) to nitrite (NO2-), a function carried out by bacteria in the genus Nitrosomonas. The nitrite formed is rapidly oxidized to nitrate (NO3-) by bacteria in the genus Nitrobacter.

The nitrifying bacteria nitrosomonas, nitrosococcus and nitrobacter are chemoautotrophs. They gain their energy by chemical oxidations (chemo-) and they are autotrophs (self-feeders) because they do not depend on pre-formed organic matter. As



they derive energy for synthesis of organic food by oxidising inorganic materials ammonia. Nitrification is an autotrophic process during which energy is liberated from the oxidation of ammonium with the biosynthesis of simple inorganic molecules such as carbon dioxide and water into organic compounds and oxygen is an electron acceptor. Nitrifying bacteria, gain their energy by oxidising ammonium, while using CO2 as their source of carbon to synthesize organic compounds

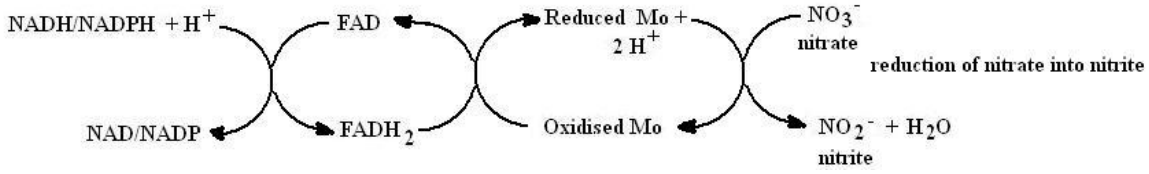
Nitrogen assimilation

Nitrogen assimilation is the conversion of inorganic nitrogen (such as nitrate) into an organic form of nitrogen like, for example, an amino acid. Nitrate is reduced for this purpose by enzymes first to nitrite (by nitrate reductase), then to ammonia (by nitrite reductase). Ammonia is incorporated into amino acids. The process of nitrate reduction to ammonia is accomplished in two steps, each mediated by specific enzymes.

Reduction of nitrate to nitrite

The nitrate serves as a terminal electron acceptor for anaerobic respiration. The nitrate is reduced to nitrite by enzyme nitrate reductase. It is co-enzyme NADH/NADPH-dependent according to organism.

The enzyme is a flavoprotein that contains iron and molybdenum serves as an electron carrier. FAD receives hydrogen from reduced co-enzyme NADH/NADPH +H+ (serves as hydrogen donor) for the reduction of nitrate



Reduction of nitrite to ammonia

Nitrite reductase reduces the nitrite ions to ammonium ions. Nitrite reductase does not require molybdenum and may contain copper and iron. Ferredoxin is the direct source of electrons for nitrite reduction, which occurs in higher plants mostly in the leaves.

The nitrite ions formed in other parts of the plant are also transported to leaves and reduced to ammonia. The reduced coenzyme NADPH +H+ or NAD + H+ serves as hydrogen donor for the reduction of nitrite.

Ammonia thus formed as a result of nitrogen fixation is not given out .It is highly toxic and used for the synthesis of amino acids. Amino acids are the building blocks for the synthesis of proteins. The amino acids are transported through phloem to other parts of the plant for the synthesis of proteins.

Amino acids are the initial products of nitrogen assimilation. An amino acid molecule consists of at least one carboxyl (-COOH) groups and one or several amino (-NH2) groups. Majority of amino acids are synthesized in plants by two main processes.

Reductive animation

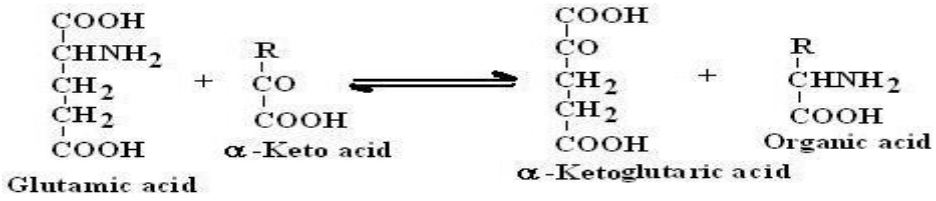
In this process, ammonia reacts with alpha-ketoglutaric acid to form glutamic acid in the presence of enzyme glutamate dehydrogenase. A reduced coenzyme NADPH in leaves, NADH in roots is required.

Transamination

Glutamic acid is the main amino acid from which other 17 amino acids are formed

$$2\,NO_2 + 7NADPH + 7H^+ \xrightarrow[\text{Ferredoxin}]{\text{Nitrite reductase}} 2\,NH_3 + 4\,H_2O + 7\,NADP^+$$

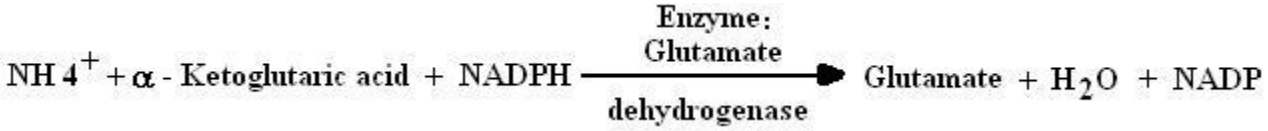
through transamination. With the help of enzyme transaminase, amino group of an amino acid (-CHNH2) is exchanged with keto group (-CO) of ketoacid. Pyridoxal phosphate is required as coenzyme which is obtained from a vitamin.

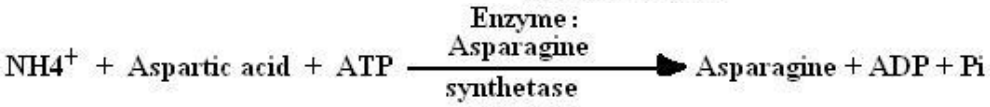


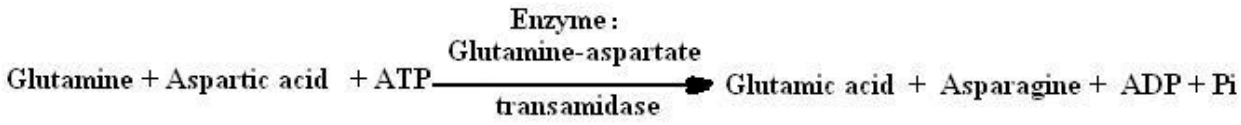
In most of transamination glutamic acid is present as one of the reactants. The other reactant may be any one of a number of alpha-keto acids, which alpha keto acid receives the amino group from glutamic acid is determined by the specificity of the enzyme.

Amides

Amides are nitrogen-containing organic compounds and contain more nitrogen than amino acids. Amides are formed when amino acids bond to each other to form proteins. Amino acids in which hydroxyl group (-OH) of carboxylic group is replaced by amino group (-NH2) from ammonia, or another amino acid. ATP is required. The two important amides found in plants are asparagine and glutamine.



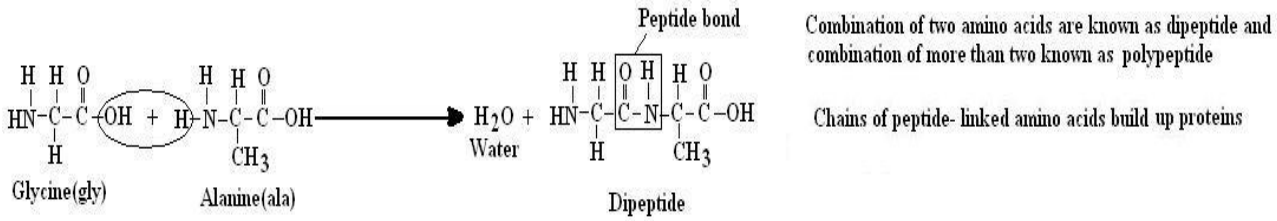




They are formed from two amino acids namely glutamic acid and aspartic acid. This reaction takes place in the presence of the enzymes glutamine synthetase or asparagine synthetase.

Proteins synthesis

Proteins are made up of long chains of amino acids. Proteins are in the form of one or more chain called polypeptide chains. Amino acids bond to each other by peptide or amide bonds. The carboxyl group (-COOH) of one amino acid reacts with the amino group (-NH2) of the next amino acid, releasing a molecule of water and as a result peptide bond (-CONH) is formed. This may be illustrated with the two simplest amino acids, glycine and alanine.

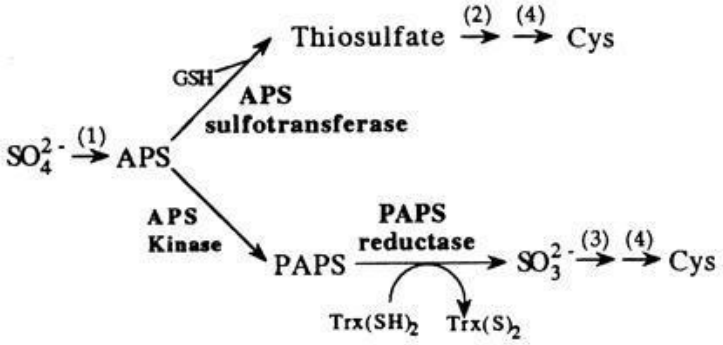


In a polypeptide, amino acids are arranged according to coded information contained in mRNA. Polypeptide synthesis occurs over ribosome where mRNA gets attached. Amino acids are brought there according to codons by means by tRNAs. The number of amino acids varies greatly among proteins and thus differs the molecular weight of proteins also.

Sulphate Metabolism

Sulfur is an essential element for growth and physiological functioning of plants. However, its content strongly varies between plant species and it ranges from 0.1 to 6% of the plants' dry weight. Sulfates taken up by the roots are the major sulfur source for growth, though it has to be reduced to sulfide before it is further metabolized. Root plastids contain all sulfate reduction enzymes, but the reduction of sulfate to sulfide and its subsequent incorporation into cysteine predominantly takes place in the shoot, in the chloroplasts. Cysteine is the precursor or reduced sulfur donor of most other organic sulfur compounds in plants. The predominant proportion of the organic sulfur is present in the protein fraction (up to 70% of total sulfur), as cysteine and methionine (two amino acids) residues. Cysteine and methionine highly significant in the structure,

conformation and function of proteins. Plants contain a large variety of other organic sulfur compounds, as thiols (glutathione), sulfolipids and secondary sulfur compounds (alliins, glucosinolates, phytochelatins), which play an important role in physiology and protection against environmental stress and pests. Sulfur compounds are also of great importance for food quality and for the production of phyto-pharmaceutics. Sulfur deficiency will result in the loss of plant production, fitness and resistance to environmental stress and pests.



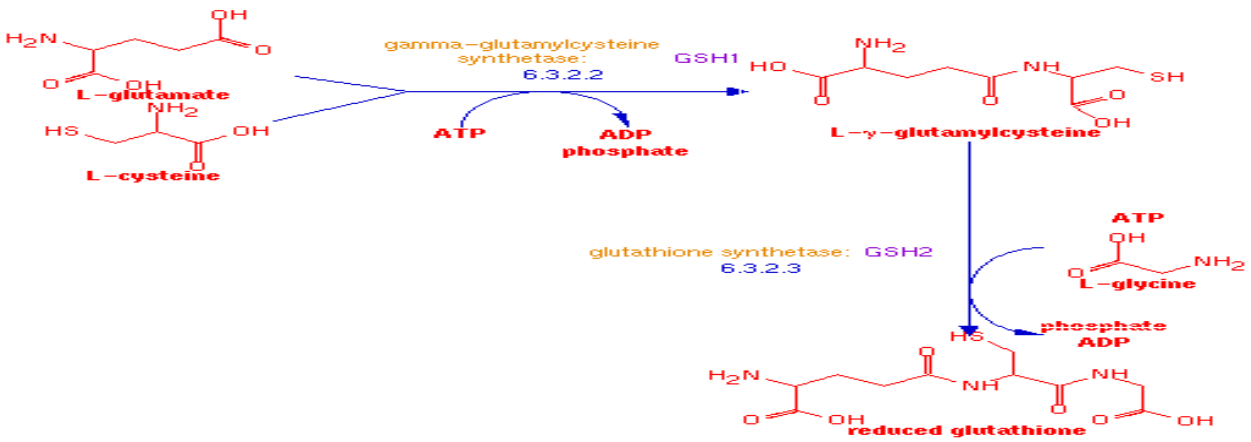
Pathways for sulfate reduction in higher plants.

The upper scheme illustrates the adenosine 5′-phosphosulfate (APS) (5′-adenylylsulfate) sulfotransferase pathway and the lower scheme depicts a microorganism-like APS kinase/3′-phosphoadenosine-5′-phosphosulfate (PAPS) reductase pathway. The numbers refer to the following enzymes 1 = ATP sulfurylase, 2 = thiosulfate reductase, 3 = sulfite reductase, 4 = *O*-acetylserine(thiol)lyase.

Sulfate is taken up by the roots that have high affinity. The maximal sulfate uptake rate is generally already reached at sulfate levels of 0.1 mM and lower. The uptake of sulfate by the roots and its transport to the shoot is strictly controlled and it appears to be one of the primary regulatory sites of sulfur assimilation. Sulfate is actively taken up across the plasma membrane of the root cells, subsequently loaded into the xylem vessels and transported to the shoot by the transpiration stream. The uptake and transport of sulfate is

energy dependent (driven by a proton gradient generated by ATPases) through a proton/sulfate co-transport. In the shoot the sulfate is unloaded and transported to the chloroplasts where it is reduced. The remaining sulfate in plant tissue is predominantly present in the vacuole, since the concentration of sulfate in the cytoplasm is kept rather constant. Distinct sulfate transporter proteins mediate the uptake, transport and subcellular distribution of sulfate.

Glutathione Synthesis:



Glutathione (GSH) is a tripeptide that contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamateside-chain. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides.

Thiol groups are reducing agents, existing at a concentration of approximately 5 mM in animal cells. Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form glutathione disulfide (GSSG). Glutathione is found almost exclusively in its reduced form, since the enzyme that reverts it from its oxidized form, glutathione

reductase, is constitutively active and inducible upon oxidative stress. In fact, the ratio of reduced glutathione to oxidized glutathione within cells is often used scientifically as a measure of cellular toxicity.

Carbon Cycle

Carbon-based molecules are crucial for life on earth, because it is the main component of biological compounds. Carbon is also a major component of many minerals. Carbon also exists in various forms in the atmosphere. Carbon dioxide (CO_2) is partly responsible for the greenhouse effect and is the most important human-contributed greenhouse gas

In the past two centuries, human activities have seriously altered the global carbon cycle, most significantly in the atmosphere. Although carbon dioxide levels have changed naturally over the past several thousand years, human emissions of carbon dioxide into the atmosphere exceed natural fluctuations. Changes in the amount of atmospheric $CO_2$are considerably altering weather patterns and indirectly influencing oceanic chemistry. Records from ice cores have shown that, although global temperatures can change without changes in atmospheric CO_2 levels, CO_2 levels cannot change significantly without affecting global temperatures. Current carbon dioxide levels in the atmosphere exceed measurements from the last 420,000 years and levels are rising faster than ever recorded, making it of critical importance to better understand how the carbon cycle works and what its effects are on the global climate.

Main reservoir

- The global carbon cycle is now usually divided into the following major reservoirs of carbon interconnected by pathways of exchange:
- The atmosphere
- The terrestrial biosphere

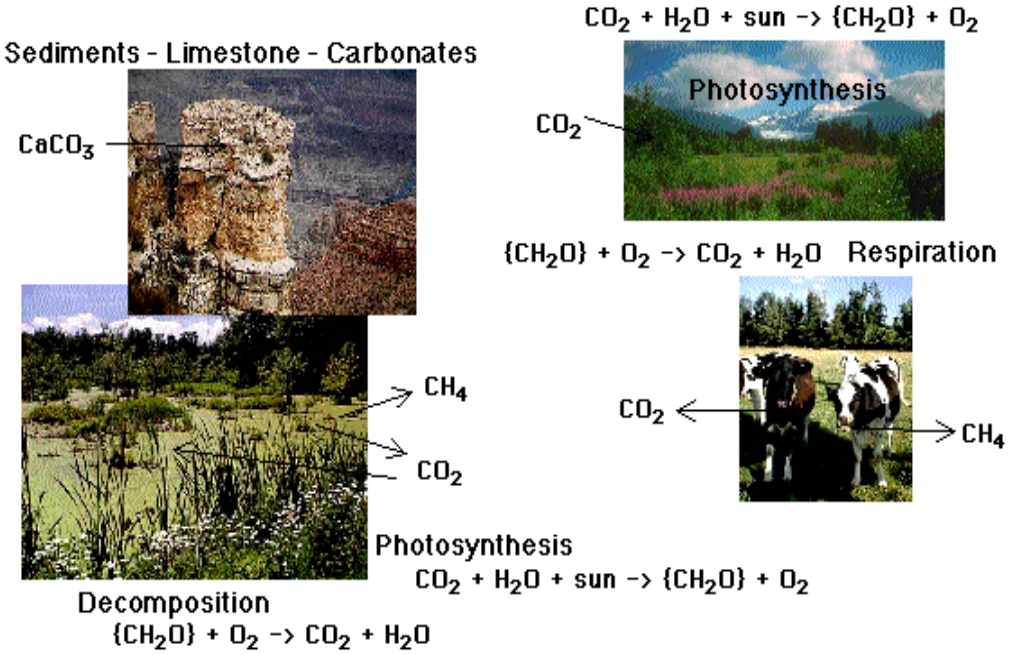
- The oceans, including dissolved inorganic carbon and living and non-living marine biota
- The sediments, including fossil fuels, fresh water systems and non-living organic material, such as soil carbon

The Earth's interior, carbon from the Earth's mantle and crust. These carbon stores interact with the other components through geological processes The carbon exchanges between reservoirs occur as the result of various chemical, physical, geological, and biological processes. The ocean contains the largest active pool of carbon near the surface of the Earth.

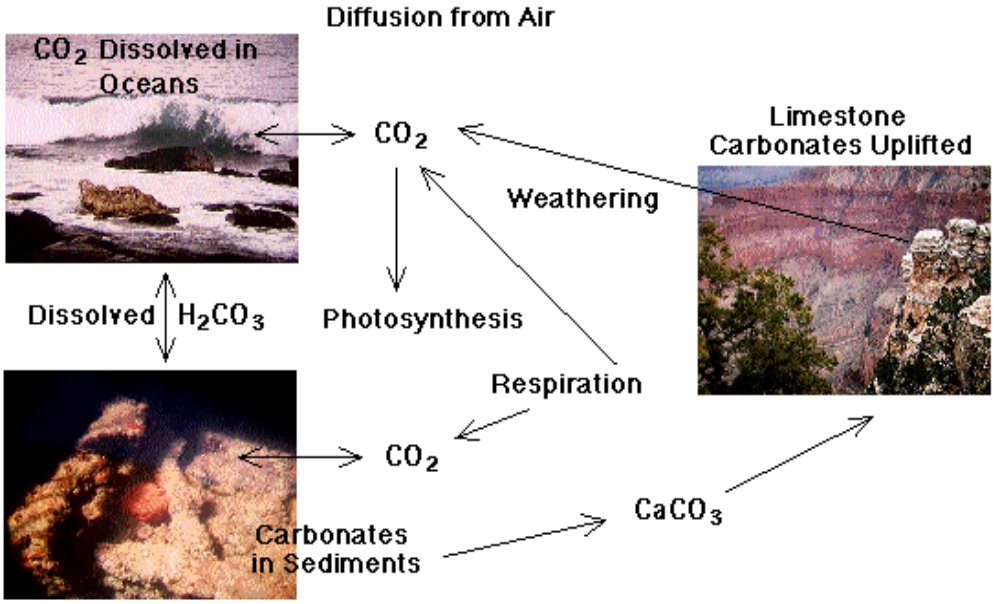
Photosynthesis is a complex series of reactions carried out by algae, phytoplankton, and the leaves in plants, which utilize the energy from the sun. The simplified version of this chemical reaction is to utilize carbon dioxide molecules from the air and water molecules and the energy from the sun to produce a simple sugar such as glucose and oxygen molecules as a by product. The simple sugars are then converted into other molecules such as starch, fats, proteins, enzymes, and DNA/RNA i.e. all of the other molecules in living plants. All of the "matter/stuff" of a plant ultimately is produced as a result of this photosynthesis reaction.

An important summary statement is that during photosynthesis plants use carbon dioxide and produce oxygen.

Carbon Cycle



Carbon Cycle - Ocean/Sedimentation



Phosphorus cycle

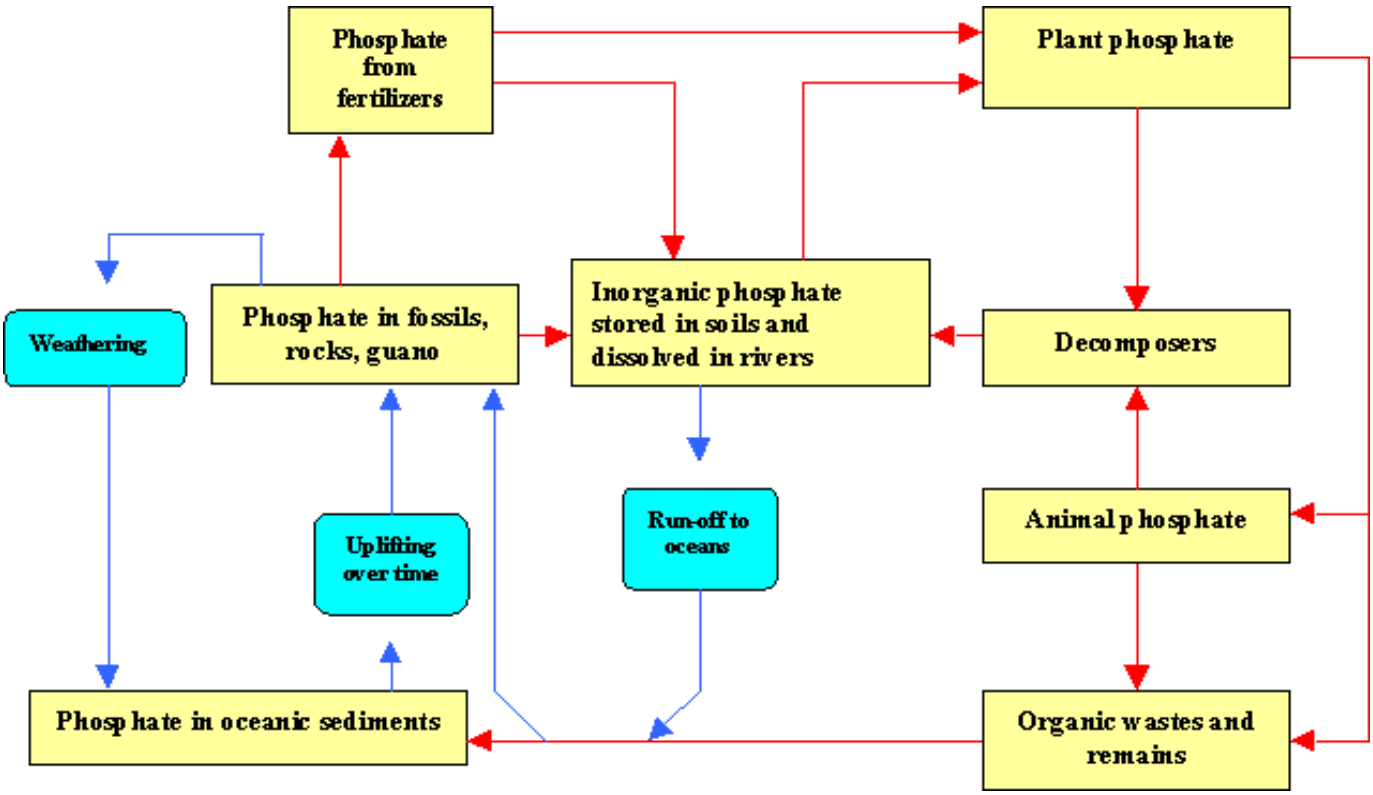
Phosphorus is an essential nutrient for plants and animals in the form of ions PO_4^{3-} and HPO_4^{2-}. It is a part of DNA-molecules, of molecules that store energy (ATP and ADP) and of fats of cell membranes. Phosphorus is also a building block of certain parts of the human and animal body, such as the bones and teeth.

Phosphorus can be found on earth in water, soil and sediments. Unlike the compounds of other matter cycles phosphorus cannot be found in air in the gaseous state. This is because phosphorus is usually liquid at normal temperatures and pressures. It is mainly cycling through water, soil and sediments. In the atmosphere phosphorus can mainly be found as very small dust particles. Phosphorus moves slowly from deposits on land and in sediments, to living organisms, and than much more slowly back into the soil and water sediment.

Phosphorus is most commonly found in rock formations and ocean sediments as phosphate salts. Phosphate salts that are released from rocks through weathering usually dissolve in soil water and will be absorbed by plants. Because the quantities of phosphorus in soil are generally small, it is often the limiting factor for plant growth. That is why humans often apply phosphate fertilizers on farmland. Phosphates are also limiting factors for plant-growth in marine ecosystems, because they are not very water-soluble. Animals absorb phosphates by eating plants or plant-eating animals.

Phosphorus cycles through plants and animals much faster than it does through rocks and sediments. When animals and plants die, phosphates will return to the soils or oceans again during decay. After that, phosphorus will end up in sediments or rock formations again, remaining there for millions of years. Eventually, phosphorus is released again through weathering and the cycle starts over.

A schematic representation of the phosphorus cycle


Phosphate from fertilizers
Plant phosphate
Weathering
Phosphate in fossils, rocks, guano
Inorganic phosphate stored in soils and dissolved in rivers
Decomposers
Uplifting over time
Run-off to oceans
Animal phosphate
Phosphate in oceanic sediments
Organic wastes and remains

POSSIBLE QUESTION

Unit-II

PART A (2 Marks)

1. Write the significance of C4 cycle.
2. Write a note on sulphite reduction.
3. Draw a neat sketch of glutathione synthesis.
4. Differentiate Calvin cycle and Hatch-Slack cycle.
5. Write a note on CAM plants.
6. What is symbiotic nitrogen fixation?
7. What is kranz anatomy?
8. Draw a neat sketch of carbon cycle.
9. Define photorespiration. Write a note on the site of photorespiration.

PART B (8 Marks)

1. Draw a neat sketch of C_3 cycle and explain in detail.
2. Explain in detail the nitrogen assimilation process.
3. What will happen in plants if CO_2 concentration is less than 1%? Explain
4. How is nitrogen fixed in symbiotic plants? Explain.
5. Explain in detail the C4 pathway. Write its significance and characteristics.
6. Explain the biochemistry of nitrogen fixation.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed University established Under Section 3 of UGC Act 1956)

DEPARTMENT OF BIOCHEMISTRY

III B.SC BIOCHEMISTRY – FIFTH SEMESTER

17BCU503A – PLANT BIOCHEMISTRY

MULTIPLE CHOICE QUESTIONS

Unit II

SL.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4		ANSWER
1	Both Photo systems I and II are involved in	Cyclic photophosphorylation	Non-cyclic Photophosphorylation	Pseudophotophosphorylation	photorespiration		Non-cyclic Photophosphorylation
2	Chlorophyll a and b can be excited to the second excited electronic states by wavelength	650-700nm	420-460	700-740nm	250-300nm		650-700nm
3	The reaction center of Photo system I is	P680	P700	P800	P750		P700
4	Which of the following chlorophylls is found in all plants and cyanobacteria?	Chl a	Chl b	Chl c1	Chl c2		Chl b
5	Chlorophyll b differs from chlorophyll a	in having a formyl group on ring II	in having a formyl group on ring I	in having a formyl group on ring III	in having a methyl group on ring II		in having a formyl group on ring II
6	The source of oxygen evolved during photosynthesis is	water	CO2	Organic acid	glucose		water
7	Photosynthetic enhancement is referred as	Red drop	Quantum yield	Emerson effect	Hills reaction		Emerson effect
8	The central atom that is covalently and coordinately bo	Mg++	Fe++	Fe3+	K+		Mg++
9	Chlorophyll is found in	Plasma membrane	Thylakoid membrane	Peroxisomes	Glyoxysomes		Thylakoid membrane
10	One of the following is not a photo synthetic pigment	Chlorophyll	Carotenoid	Phycobilin	xanthin		xanthin
11	The Chlorophylls absorb light of wavelength	650 – 700nm	450 – 500nm	500 – 600 nm	350-400 nm		650 – 700nm
12	The Carotenoids absorb light in the wavelength	450 – 500nm	650 – 700nm	500 – 600nm	350-400nm		450 – 500nm
13	The Phycobilns absorb light in the wavelength	500 – 600nm	450 – 500nm	650 – 700nm	~ 450nm		500 – 600nm

14	The most potent reagents which block non-cyclic photo	CMU & DCMU	Octyl guanidine & grameodine	urea	cyanogen bromide		CMU & DCMU
15	The primary electron donar in PS II is	P700	Cyt C	P680	Chl a		P680
16	In cyclic photo phosphorylation	Only PS I is functional	Both PSI and PSII are functional	O2 is evolved	NADPH2 is formed		Only PS I is functional
17	The main function of light harvesting complex is to	Increase the temperature	Capture solar energy	Enhance ATP formation	transfer of electrons		Capture solar energy
18	The skeleton of chlorophyll is made of a	pentanone	Tetrapyrrole	Per hydro phenenthrene	Hexagon		Tetrapyrrole
19	The following are C4 plants except	Chlorella	Sugarcane	maize	sorghum		Chlorella
20	Which of the following is the substrate for photorespiration	Oxaloacetate	PEP	succinate	glycollate		PEP
21	The stomata of CAM plants remain	Opened during the day and night	closed during day and opened at night	closed during the day and night	opened during the day and closed at night		closed during day and opened at night
22	The following organelles are involved in photo respiration except	mitochondria	chloroplasts	peroxisomes	golgi bodies		golgi bodies
23	Kranz anatomy is a special feature in the leaves of	C3 plants	C4 plants	C2 plants	CAM plants		C4 plants
24	In C_3 plants the primary co_2 acceptor is ----------	ribulose 1,5 bis phosphate	Ribulose-5-phosphate	ribulose-1-phosphate	ribose -1-phosphate		ribulose 1,5 bis phosphate
25	The sequence of dark reaction in photosynthesis was established by	A.A Bensen	J.Bassham	Melvin Calvin	J.C.Bose		Melvin Calvin
26	The Benson – Calvin cycle takes place in	Chloroplasts	etioplasts	Mitochondria	cytoplasm		Chloroplasts
27	Sugarcane & cynodon dactylon are	C4 plants	C3 Plants	C2 plants	CAM plants		C4 plants
28	The primary acceptor of CO_2 in C_4 plant is	PEP	Pyruvate	Alanine	oxaloacetate		PEP
29	In CAM plants CO_2 assimilation occurs during	Day	Night	Day & Night	Evening		Night
30	In C_4 plants Pyruvate, Phosphate dikinase is located mainly in the	mesophyll cells of chloroplast	bundle sheath cells of chloroplast	mitochondria	peroxisomes		mesophyll cells of chloroplast
31	Plants in which the Hatch slack pathway takes place are called as	C2 plants	C3 plants	C4 plants	CAM plants		C4 plants
32	In bundle sheath cells, malate is decarboxylated to form	oxaloacetate	citrate	pyruvate	PEP		pyruvate

33	The calvin cycle enzymes are present in	Stroma	Thylakoid lumen	Grana	Thylakoid membrane		Stroma
34	During photophosphorylation the NADPH and ATP are	Absorbed	Released	Reduced	Oxidised		Released
35	Thylakoids of grana possess the	enzymes of calvin cycle	enzymes of photophosphorylation	enzymes for C3 cycle	enzymes of C4 cycle		enzymes of photophosphorylation
36	Hydrogen peroxide is formed during	Calvin cycle	Hatch-Slack cycle	CAM cycle	photorespiration		photorespiration
37	ATP molecules are synthesized in all except	cyclic photophosphorylation	non cyclic photophosphorylation	dark respiration	photorespiration		photorespiration
38	The optimum temperature of photorespiration is	10 - 20°C	25 - 35°C	40 - 60°C	35 - 45°C		25 - 35°C
39	The nocturnal opening of stomata is the characteristic feature of	water plants	C4 plants	CAM plants	C3 plants		CAM plants
40	The first stable compound formed in C_3 cycle is	DHAP	phosphoglyceric acid	oxalo acetic acid	glycolic acid		phosphoglyceric acid
41	The first stable compound formed in C_4 cycle is	DHAP	phosphoglyceric acid	oxalo acetic acid	glycolic acid		oxalo acetic acid
42	The optimum temperature for the growth of C_4 plants is	30 - 45°C	25 - 35°C	0 - 20°C	40 - 60°C		30 - 45°C
43	For the synthesis of each molecule of glucose from CO_2 in a photosynthesis how many ATP molecules are required?	18	12	3	2		18
44	For the synthesis of each molecule of glucose from CO_2 in a photosynthesis how many NADPH molecules are utilized	18	12	3	2		12
45	Number of ATP molecules synthesized in non cyclic photophosphorylation is	2	3	1	4		1
46	Number of ATP molecules synthesized in cyclic photophosphorylation is	2	3	1	4		2

47	The following statement is true with cyclic photo phosphorylation	PS I is involved	PS II is involved	photo oxidation of water takes place	NADP+ is reduced to NADPH + H+		PS I is involved
48	Carotenes are usually found in	PS I	PSII	Both PS I and PSII	neither PS I nor PSII		PS I
49	Xanthophylls are usually found in	PS I	PSII	Both PS I and PSII	neither PS I nor PSII		PSII
50	In C_4 cycle oxalo acetic acid is converted to malic acid by the enzyme	malic dehydrogenase	malate decarboxylase	malic oxidase	transaminase		malic dehydrogenase
51	In C_4 plants malic enzyme is present in	mesophyll cells	bundle sheath cells	xylem	phloem		bundle sheath cells
52	Photosynthetic yield is more in	C4 plants	C3 plants	C2 plants	CAM plants		C4 plants
53	CAM cycle is observed in all the plants except	cactus	orchid	chlorella	bryophyllum		chlorella
54	In C_4 cycle the enzyme involved in conversion of oxalo acetic acid to malic acid is	malic enzyme	malate dehydrogenase	transaminase	malate decarboxylase		malate dehydrogenase
55	Only mesophyll cells are involved in	C3 cycle	C4 cycle	CAM cycle	C2 cycle		C3 cycle
56	In CAM plants carbohydrate synthesis takes place during	night time	day time	both night and day time	only in the evening		day time
57	Which of the following is formed during photo respiration	H2O2	O2	OH- ions	O3		H2O2
58	In photo respiration glyoxylic acid is converted to glycine by	transaminase	decarboxylase	dehydrogenase	reductase		transaminase
59	In photo respiration the enzyme involved in detoxification of H_2O_2 is	catalase	decarboxylase	transaminase	dehydrogenase		catalase
60	When the atmospheric CO_2 is less than 1% ribulose di phosphate is converted to	3 phospho glyceric acid	glycolic acid	glyoxylic acid	glycine		glycolic acid

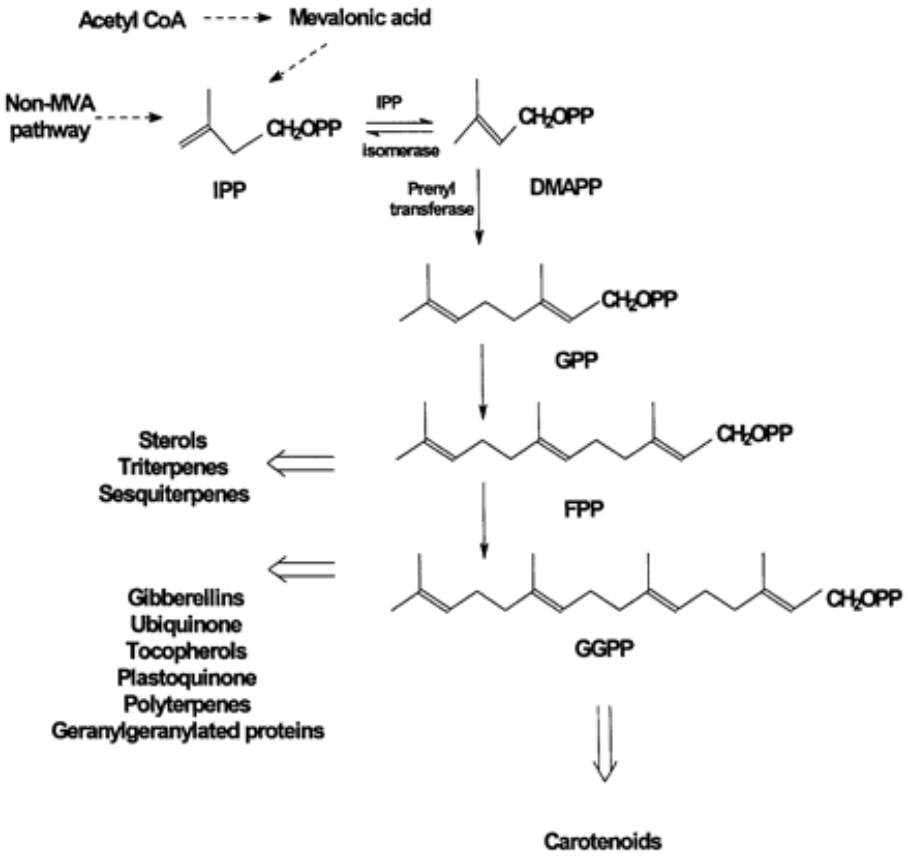
UNIT-III

Lipid metabolism in plants: Biosynthesis of fatty acids in plastids, synthesis of waxes, triacylglycerols and glycolipids. Synthesis of chlorophyll. Carotenoid formation. Synthesis of nitrogenous compounds: caffeine synthesis, ureide synthesis in nodulated legumes.
Secondary oxidative mechanisms: β-oxidation, ω-oxidation, glyoxylate pathway.

Biosynthesis of chlorophyll

In plants, chlorophyll may be synthesized from succinyl-CoA and glycine, although the immediate precursor to chlorophyll a and b is protochlorophyllide. In Angiosperms, the last step, conversion of protochlorophyllide to chlorophyll, is light-dependent and such plants are pale (etiolated) if grown in the darkness. Non-vascular plants and green algae have an additional light-independent enzyme and grow green in the darkness as well. Chlorophyll itself is bound to proteins and can transfer the absorbed energy in the required direction. Protochlorophyllide, occurs mostly in the free form, and, under light conditions, acts as photosensitizer, forming highly toxic free radicals. Hence, plants need an efficient mechanism of regulating the amount of chlorophyll precursor. In angiosperms, this is done at the step of aminolevulinic acid (ALA), one of the intermediate compounds in the biosynthesis pathway. Plants that are fed by ALA accumulate high and toxic levels of protochlorophyllide; so do the mutants with the damaged regulatory system.

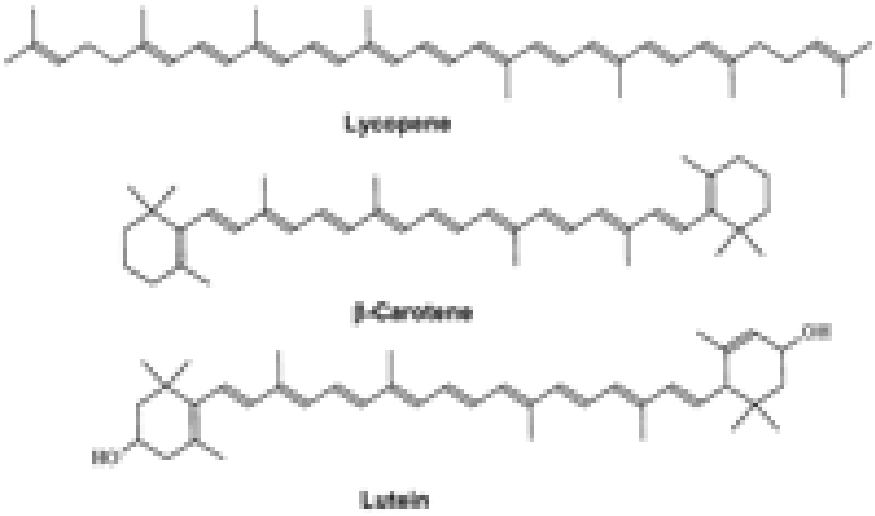
Carotenoid formation



The isoprenoid biosynthetic pathway

Biological and nutritional importance of carotenoids

Carotenoids are isoprenoid molecules that are common to all photosynthetic tissues. They are divided into the hydrocarbon carotenes, such as lycopene and β-carotene or xanthophylls, typified by lutein. Coloured carotenoids are also found in fruits, flowers and roots, where they probably act as attractants to pollinators and for seed dispersal. In the chloroplast they participate in light harvesting in photosynthetic membranes and also protect the photosynthetic apparatus from excessive light energy by quenching triplet chlorophylls, superoxide anion radicals and singlet oxygen. Furthermore, they are essential components of some pigment–protein complexes and are precursors of abscisic acid.



Structures of typical carotenoids

Dietary carotenoids fulfill essential requirements for human and animal nutrition. β-Carotene is the most potent dietary precursor of vitamin A, the deficiency of which leads to xerophthalmia, blindness and premature death . Vitamin A deficiency has been reported as the most common dietary problem affecting children worldwide, with some 1.2 million deaths annually among children aged 1–4 years.

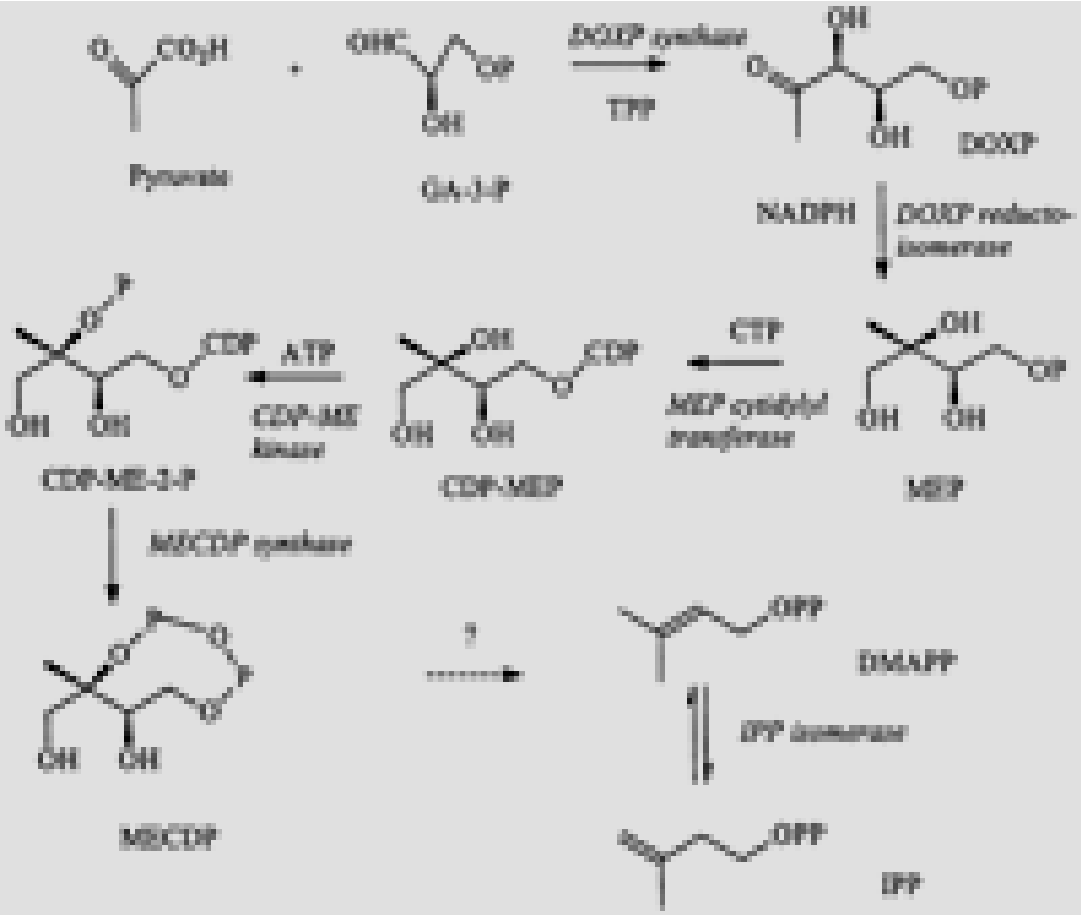
In this context, efforts to manipulate rice genetically in order to produce β-carotene have received considerable attention Other carotenoids have been shown to alleviate age-related diseases when taken in sufficient quantities in the diet, probably because of their powerful properties as lipophilic antioxidants. For example, zeaxanthin and lutein offer protection against macular degeneration, whilst there is a considerable body of evidence to link a high intake of tomatoes (and presumably lycopene) to a reduced incidence of prostate cancer. More recently, evidence has been presented to show that tomato sauce reduces the amount of DNA damage in white blood cells and prostate tissues of prostate cancer victims. Since the tomato fruit is virtually the sole dietary source of

lycopene, its formation in the tomato has been the subject of considerable attention, as has attempts to increase the levels by genetic manipulation or conventional plant breeding.

Carotenoid biosynthesis in higher plants

Early studies on the biosynthesis of carotenoids in plants used biochemical approaches and the analysis of intermediates in naturally occurring mutants, especially the tomato. These pioneering studies have been reviewed comprehensively. All carotenoids are derived from isopentenyl diphosphate and are produced in plastids. Genetic and molecular studies have established that nuclear genes encode all the enzymes of the pathway. This multidisciplinary approach has led to the cloning of most of the genes from higher plants. The experimental approaches to cloning the carotenoid genes have been reviewed by.

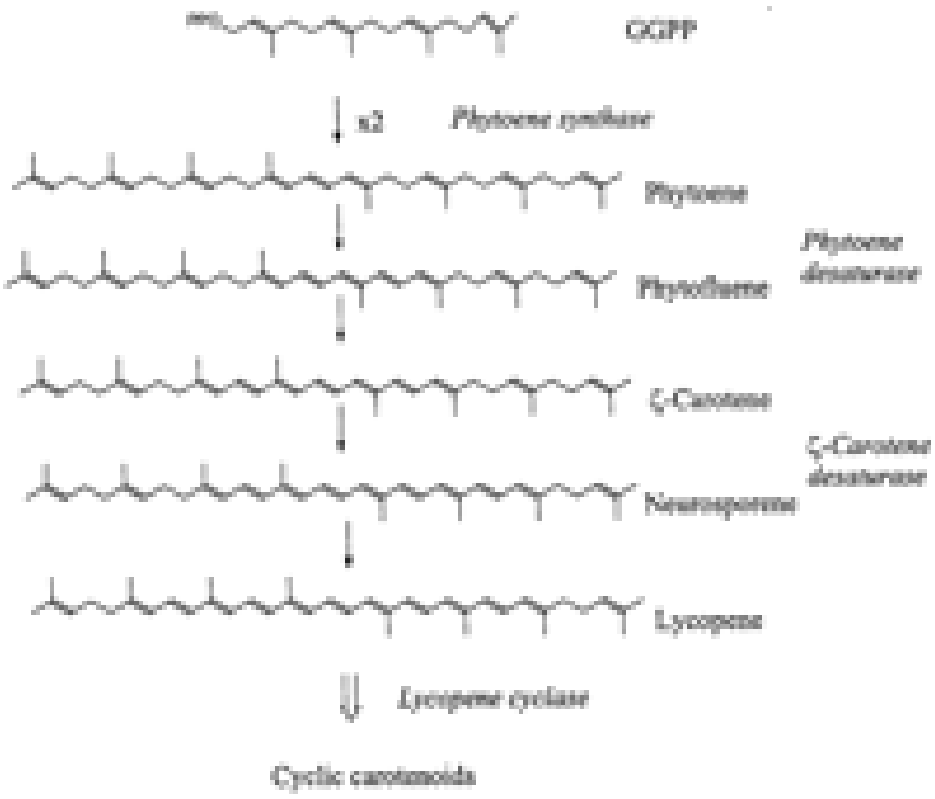
Plants synthesize carotenoids via the recently identified 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway rather than the mevalonic acid pathway as was assumed for many years. Whilst both pathways produce IPP, the latter is responsible for the formation of sterols, sesquiterpenoids and triterpenoids in the cytosol, whilst the DOXP pathway leads to the formation of plastidic isoprenoids, such as carotenoids, phytol, plastoquinone-9, and diterpenes.



The 1-deoxy- D-xylulose 5-phosphate (DOXP) biosynthetic pathway. This is also called the non-mevalonate or the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway. Abbreviations: GA-3-P, glyceraldehyde 3-phosphate; MEP, 2C-methyl-D-erythritol-4-phos phate; CDP-MEP, 4-diphosphocytidyl-2C-methylerythritol; CDP-ME-2-P, 4-diphosphocytidyl-2C-methylerythritol 2-phosphate; MECDP, 2C-methyl-D-erythritol 2,4-cyclodiphosphate; DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate.

IPP is isomerized to its allylic isomer dimethylallyl diphosphate (DMAPP), the activated substrate for the formation of the C_{20} geranylgeranyl diphosphate (GGPP), the precursor of the first C_{40} carotenoid, phytoene. IPP isomerase is found in both the cytosol

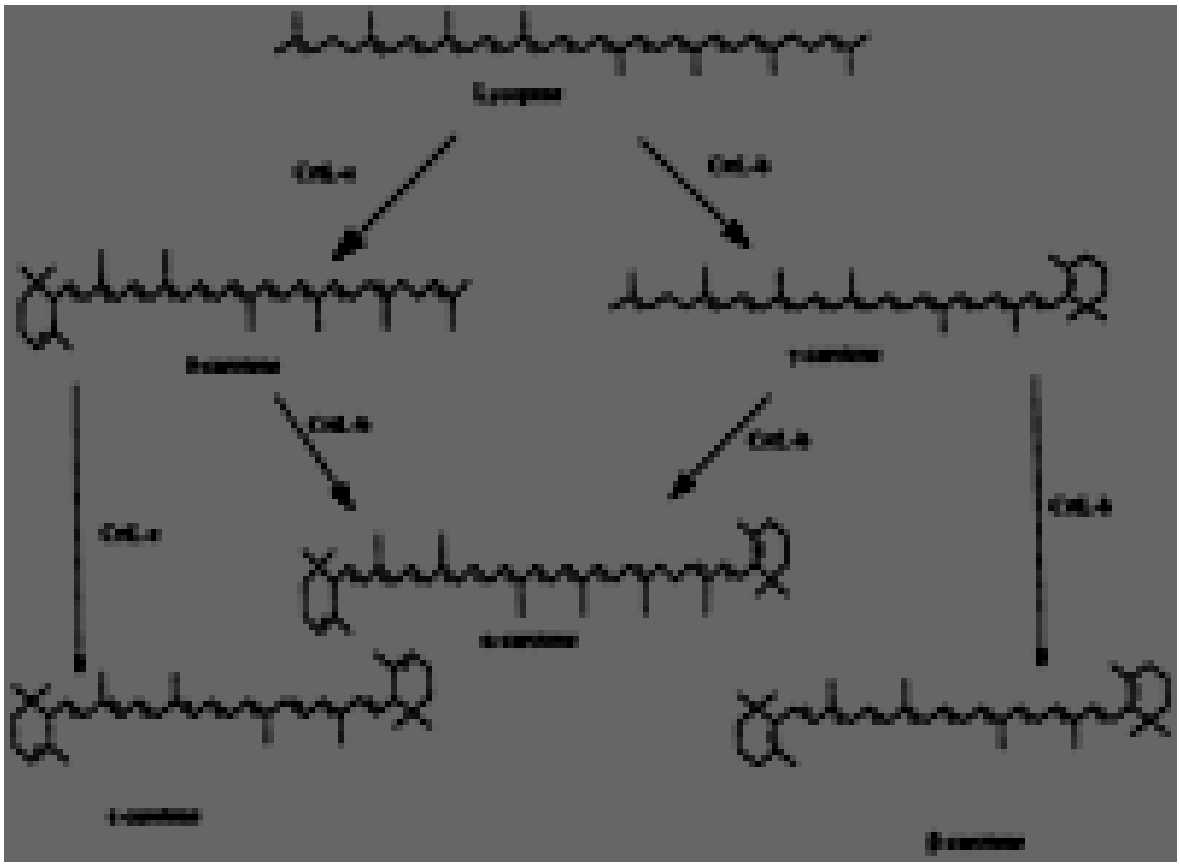
and plastid and there are two *IpI* genes in plants. A single enzyme, GGPP synthase (Ggps), catalyses the formation of GGPP from IPP and DMAPP. At least five *Ggps* genes are expressed in different tissues of *Arabidopsis*, but it is not known how many are linked to carotenoid biosynthesis. It is tempting to consider that different GGPP synthases are responsible for the branches from GGPP to each isoprenoid class. The condensation of two molecules of GGPP to form 15-*cis* phytoene is catalysed by phytoene synthase, PSY. The enzyme is very well conserved among archea, bacteria and eukaryotic organisms. The tomato contains two genes, *Psy*-1 and *Psy*-2. The former encodes the fruit-ripening-specific isoform, whilst *Psy*-2 predominates in green tissues, including mature green fruit and has no role in carotenogenesis in ripening fruit. A mutation in *Psy*-1 causes a yellow flesh phenotype (the *r,r* mutant) and an absence of carotenoids in ripe fruit, an effect that can be mimicked with an antisense *Psy*-1 transformation.



Phytoene formation and desaturation reactions to form lycopene.

Two structurally and functionally similar membrane-bound enzymes, phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS), convert phytoene into lycopene, via ζ-carotene. These two FAD-containing enzymes require at least plastoquinone and a plastid terminal oxidase as electron acceptors. By contrast, the bacterial *crtI* gene encodes a single desaturase that converts phytoene into all-*trans* lycopene. The phylogenetic relationships between the various carotene desaturases have been reviewed. The isolation of a carotene isomerase gene from *Synechocysti* and tomato has finally established the mechanism by which *cis-trans* isomerizations occur during the desaturation of phytoene into lycopene.

The cyclization of lycopene creates a series of carotenes that have one or two rings of either the β- or ϵ- type. Lycopene β-cyclase (LCY-B/CRTL-E) catalyses a two-step reaction that leads to β-carotene (two β-rings, whereas lycopene ϵ-cyclase (LCY-E/CRTL-E) creates one ϵ-ring to produce δ-carotene. It is assumed that α-carotene (β, ϵ-carotene, the precursor of the major leaf xanthophyll, lutein) is formed by the action of both enzymes. These enzymes in tomato show a large amount of structural resemblance and both contain FAD/NAD(P)-binding sequences at the amino termini. Unusually, tomato contains two lycopene β-cyclases, LCY-B, as described above, and also CYC-B, a chromoplast-specific cyclase. They show a 53% identity at the amino acid level. Intriguingly, CYC-B shows a far greater identity to CCS of pepper, leading to speculation of a common ancestral gene. Another carotenoid gene, neoxanthin synthase (NSY) from tomato is closely related to LCY-B and CCS. This enzyme catalyses the conversion of violaxanthin to neoxanthin. LCY catalyses a simplified version of the reaction catalysed by NSY and CCS, suggesting that these enzymes were remodelled from LCY during higher plant evolution to create novel oxidized carotenoids. The importance of CYC-B in regulating lycopene accumulation in ripening tomato fruit will be described later.



Cyclization reactions from lycopene. Abbreviations: CrtL-e, lycopene ϵ-cyclase; CrtL-b, lycopene β-cyclase.

Xanthophylls are formed by the oxygenation of carotenes, typically by the addition of hydroxyl, epoxy or keto groups. Hydroxylation at 3C and 3C′ positions is carried out by two types of enzymes; one specific for β-rings and one for ϵ-rings. The β-carotene hydroxylases require ferredoxin and iron. There are two β-carotene hydroxylases in tomato, one expressed in green tissue and one in the flower. Subsequent reactions to form other xanthophylls, and their interconversion in the xanthophyll cycle,. The formation of ABA from the oxidative cleavage of 9-*cis*-epoxy carotenoids is catalysed by dioxygenases .

Regulation of carotenoid biosynthesis during tomato fruit development and ripening

Since carotenoids are just one class of isoprenoids, the regulation of their formation must involve the co-ordinated flux of isoprenoid units into the C_{40} carotenoids as well as the other branches of the isoprenoid pathway. The discovery of gene families for several of the steps in these pathways (e.g. HMG CoA reductase, GGPP synthase, phytoene synthase) implies unique roles for each member of the family. This has been well documented for multiple forms of HMG CoA reductase, but an understanding of the functions of isoenzymes in later steps remains fragmentary. However, the traditional view of subcellular compartmentation of isoprenoid formation is probably an oversimplification. Although carotenoids are formed in plastids, it is likely that exchanges of cytoplasmic and plastidic metabolites occur and that these exchanges vary depending upon the type and developmental stage of the tissue.

Since carotenoids are an essential part of the pigment–protein complexes in thylakoids, the regulation of carotenogenesis in green tissues must be linked to the formation of chlorophylls, proteins, lipids, and to chloroplast development itself. This highly regulated process is poorly understood. It is known that light, and its intensity, are involved in the regulation of carotenoid formation in the chloroplast. Although expression of carotenoid genes does occur in etiolated plants, their synthesis is stimulated on transfer to light. It has been reported that IPP isomerase activity increases when maize etioplasts are transferred to the light, and *Psy* mRNA levels increase in the light due to a phytochrome-mediated regulation. By contrast, the expression of *Ggps* and *Pds* remain constant. The concentration and composition of xanthophylls, especially those of the violaxanthin cycle, is affected by light intensity reports that shifting either *Arabidopsis* or tomato plants from low light to strong light caused a 5-fold increase in the ratio of *Lcy-b* mRNA and *Lcy-e* mRNA, suggesting that xanthophyll composition can be modulated by the fluxes in the carotene pathway. This supports the results of studies with mutants of

Arabidopsis that lack *Lcy-e* and have no lutein in the light-harvesting antenna. The lutein is replaced by other carotenoids, with no apparent detrimental effects to the plant.

Carotenogenesis in ripening fruit (and flowers) is controlled by regulatory mechanisms that are distinct from those in photosynthetic tissues. Carotenoid formation during tomato fruit ripening has been studied extensively and has become the best model system for other chromoplast-containing tissues. During ripening the concentration of carotenoids increases between 10- and 14-fold, due mainly to the accumulation of lycopene. The tomato is unusual in this respect, as very few other fruit accumulate lycopene. At the breaker stage of ripening, the red colour of lycopene begins to appear, the chlorophyll content decreases and the organoleptic properties of the fruit change. Higher expression of isoprenoid genes in the central pathway has been found at this stage of fruit development, notably DOXP synthase. This has led to the suggestion that the DOXP pathway may be crucial in the overall regulation of lycopene formation in tomato fruit. At this same stage, mRNA levels of *Psy-1* and *Pds* increase significantly. At the same time, the mRNAs of both lycopene cyclases (*Lcy-b* and *-e*) disappear. These changes in gene expression show that transcriptional regulation is involved in the accumulation of lycopene in tomato fruit. Differential gene expression has also been implicated in the accumulation of δ-carotene in fruits of the *Delta* tomato mutant, which results from increased transcription of *Lcy-* and in the formation of β-carotene rather than lycopene in the high-β mutant due to the up-regulation of the *Cyc-b* gene. The high pigment (*hp*) locus in tomato also affects the levels of total carotenoids. Analysis of the *hp-2* mutant has shown that it is involved in phytochrome signalling pathways.

Although control of gene expression at the transcriptional level is a key regulatory mechanism controlling carotenogenesis in chromoplasts, it is not the only one. Post-transcriptional regulation of carotenogenic enzymes has been found in chromoplasts of *Narcissus*. Both PSY and PDS were detected in inactive forms in the soluble fraction, but in active forms when membrane-bound. In addition, substrate specificity of the β- and ϵ-

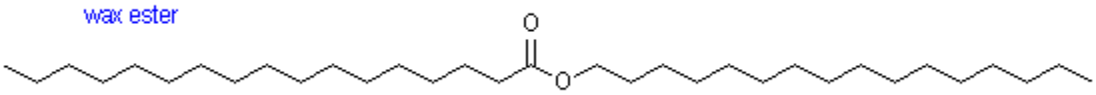
lycopene cyclases may control the proportions of the cyclic carotenoids in plants. It has also been established that sequestration of carotenoids in non-photosynthetic tissues is important in their accumulation, as opposed to their synthesis.

The pathway may also be regulated by feedback inhibition by end-products. Inhibition of lycopene cyclization in tomato leaves causes increased expression of both *Pds* and *Psy-1*. This hypothesis is supported by studies using carotenoid biosynthesis inhibitors in which treated tissue accumulated more total carotenoids than controls. The higher concentration of lycopene in *old-gold* and *old-gold crimson* mutants of tomato, compared to the wild type, may be a consequence of the lack of β-carotene due to the mutated second β-cyclase gene and thus an increase in enzyme activity of earlier enzymes in the pathway. In all of these examples, the molecular mechanism remains to be established. ABA has been implicated, although *Arabidopsis* mutants, impaired in ABA synthesis, do not show elevated levels of carotenoids. Finally, it is likely that metabolite channeling and functional complexes of protein partners are also involved in the efficient flux of metabolites in to the carotenoid pathway, as evidenced by the properties of the two phytoene synthases in tomato.

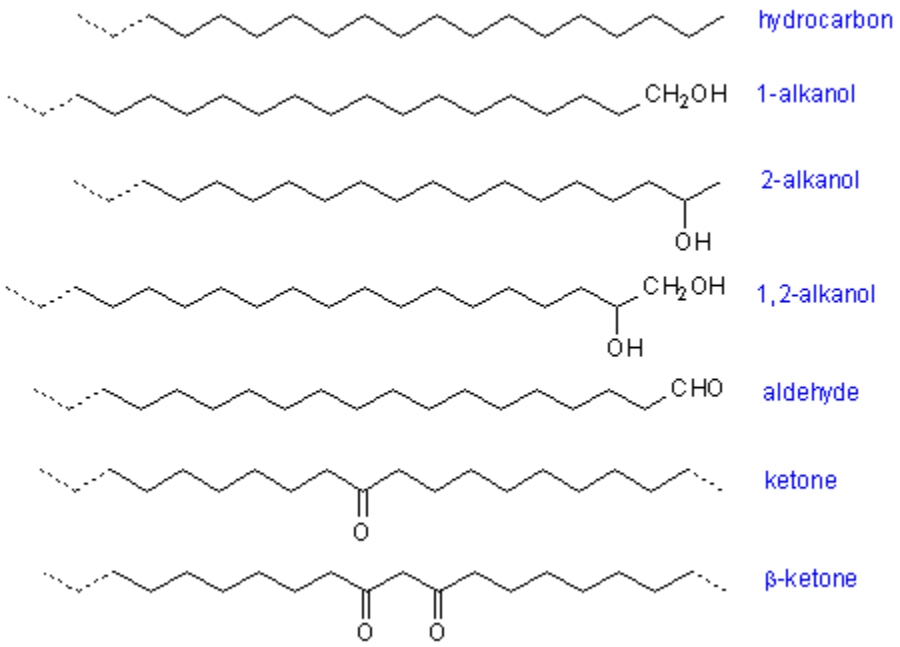
Waxes

Structure, composition, occurrence and analysis

There is no satisfactory definition of the word "wax" in chemical terms. It is derived from the Anglo-Saxon word "weax" for beeswax, so a practical definition of a wax may therefore be "a substance similar in composition and physical properties to beeswax". Technologists use the term for a variety of commercial products of mineral, marine, plant and insect origin that contain fatty materials of various kinds. Biochemists link waxes with the thin layer of fatty constituents that cover the leaves of plants or provide a surface coating for insects or the skin of animals. All of these tend to contain wax esters as major components, *i.e*. esters of long-chain fatty alcohols with long-chain fatty acids.



The nature of the other lipid constituents can vary greatly with the source of the waxy material, but they include hydrocarbons, sterol esters, aliphatic aldehydes, primary and secondary alcohols, diols, ketones, β-diketones, triacylglycerols, and many more.



Also, the chain-length and degree of unsaturation and branching of the aliphatic constituents will vary with the origin of the wax, but other than in some waxes of marine origin or from some higher animals, the aliphatic moieties tend to be saturated or monoenoic.

Plant Surface Waxes

Plant leaf surfaces are coated with a thin layer of waxy material that has a myriad of functions. This layer is microcrystalline in structure and forms the outer boundary of the cuticular membrane; it is the interface between the plant and the atmosphere. It serves

many purposes, for example to limit the diffusion of water and solutes, while permitting a controlled release of volatiles that may deter pests or attract pollinating insects. The wax provides protection from disease and insects, and helps the plants resist drought. As plants cover much of the earth's surface, it seems likely that plant waxes are the most abundant of all natural lipids.

The range of lipid types in plant waxes is highly variable, both in nature and in composition, and Table 1 illustrates some of this diversity in some of the main components.

Table 1. The major constituents of plant leaf waxes.

n-Alkanes	$CH_3(CH_2)_xCH_3$	21 to 35C - odd numbered
Alkyl esters	$CH_3(CH_2)_xCOO(CH_2)_yCH_3$	34 to 62C - even numbered
Fatty acids	$CH_3(CH_2)_xCOOH$	16 to 32C - even numbered
Fatty alcohols (primary)	$CH_3(CH_2)_yCH_2OH$	22 to 32C - even numbered
Fatty aldehydes	$CH_3(CH_2)_yCHO$	22 to 32C - even numbered
Ketones	$CH_3(CH_2)_xCO(CH_2)_yCH_3$	23 to 33C - odd numbered
Fatty alcohols (secondary)	$CH_3(CH_2)_xCHOH\ (CH_2)_yCH_3$	23 to 33C - odd numbered
β-Diketones	$CH_3(CH_2)_xCOCH_2CO(CH_2)_yCH_3$	27 to 33C - odd numbered
Triterpenols	Sterols, α-amyrin, β-amyrin, uvaol, lupeol, erythrodiol	
Triterpenoid acids	Ursolic acid, oleanolic acid, etc	

In addition, there may be hydroxy-β-diketones, oxo-β-diketones, alkenes, branched alkanes, acids, esters, acetates and benzoates of aliphatic alcohols, methyl, phenylethyl and triterpenoid esters, and many more.

The amount of each lipid class and the nature and proportions of the various molecular species within each class vary greatly according to the plant species and the site of wax deposition (leaf, flower, fruit, *etc.*) and some data for some well-studied species are listed in Table 2.

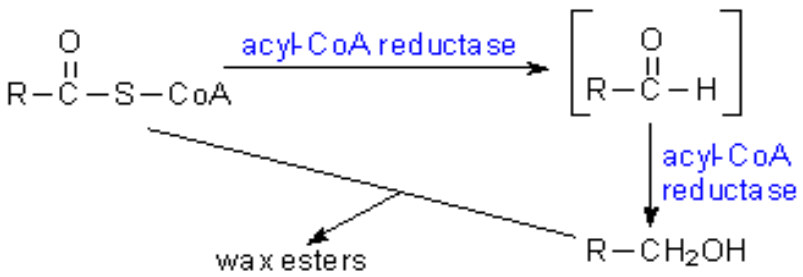
Table 2. Relative proportions (wt %) of the common wax constituents in some plant species.

	Grape leaf	Rape leaf	Apple fruit	Rose flower	Pea leaf	Sugar cane stem
Hydrocarbons	2	33	20	58	40-50	2-8
Wax esters	6	16	18	11	5-10	6
Aldehydes	6	3	2	-	5	50
Ketones	-	20	3		-	-
Secondary alcohols	-	8	20	9	7	-
Primary alcohols	60	12	6	4	20	5-25
Acids	8	8	20	5	6	3-8
Other components present include various diol types and triterpenoid acids						

Biosynthesis of Plant Waxes

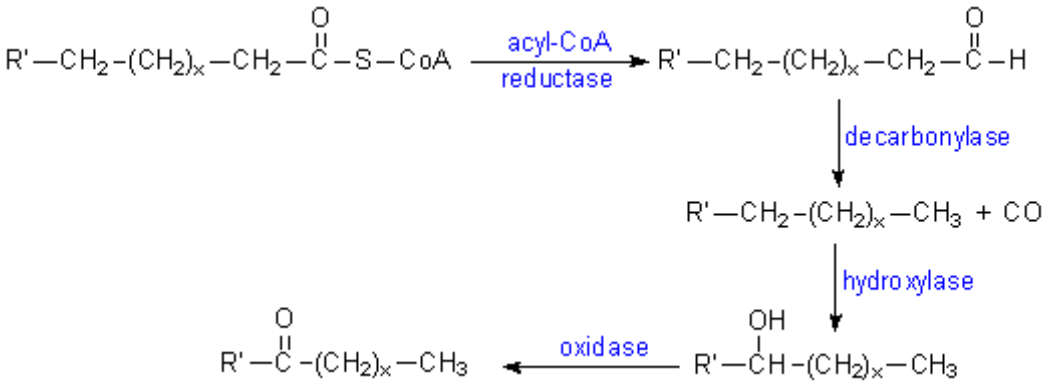
Because of their biochemical importance and relative ease of study, the waxes of the plant cuticle have received most study. All the aliphatic components of plant waxes are synthesized in the epidermal cells from saturated very-long-chain fatty acids (commonly C_{20} to C_{34}). 16:0 and 18:0 fatty acids are first synthesized in the stroma of plastids by the soluble enzymes forming the fatty acid synthase complex The second stage involves multiple elongation steps and is catalysed by membrane-associated multi-enzyme complexes, known as fatty acid elongases out with the plastids. As in fatty acid synthesis *de novo*, each two-carbon extension of the chain involves four reactions: condensation between a CoA-esterified fatty acyl substrate and malonyl-CoA, followed by a β-keto reduction, dehydration and an enoyl reduction to produce saturated very-long-chain fatty acids with 24 to 36 carbon atoms. Many different forms of the elongases have been identified, and these must interact in some manner to produce the chain-length specificity observed.

There are then two main pathways for biosynthesis of wax components: an acyl reduction pathway, which yields primary alcohols and wax esters, and a decarbonylation pathway that result in synthesis of aldehydes, alkanes, secondary alcohols and ketones. In the reductive pathway, acyl-CoA esters produced by chain elongation are reduced in a two-step process via a transient aldehyde intermediate, catalysed by a single enzyme, an acyl-CoA reductase (though it was once thought that two distinct enzymes were involved).



The fatty alcohol produced can then be esterified via an acyl-CoA alcohol transacylase to form a wax ester. Similar mechanisms have been observed in studies with insects, algae and birds (uropygial glands). It seems probable that wax diols are produced by insertion of a hydroxyl group into the alkyl chain of an acyl-CoA precursor.

In the decarbonylation pathway for the synthesis of wax constituents, the first step is again believed to be the reduction of acyl-CoA ester to an aldehyde by means of an acyl-CoA reductase. Removal of the carbonyl group by an aldehyde decarbonylase yields an alkane, with one fewer carbon atom than the fatty acid precursor. However, the enzymes involved have not been characterized.



Further metabolism of the hydrocarbon is then possible, for example by insertion of a hydroxyl group into the chain via a hydroxylase or mixed-function oxidase to form a secondary alcohol. The position of the substitution depends on the species, and the specificities of the enzymes involved. Secondary alkanols can in turn be esterified to form a wax ester. Alternatively, the hydroxyl group can be oxidized with formation of a long-chain ketone. An associated pathway leads to the formation of β-diketones and 2-alkanols. Again, these processes have been studied most in plants, but similar biochemical reactions appear to occur in insects and birds.

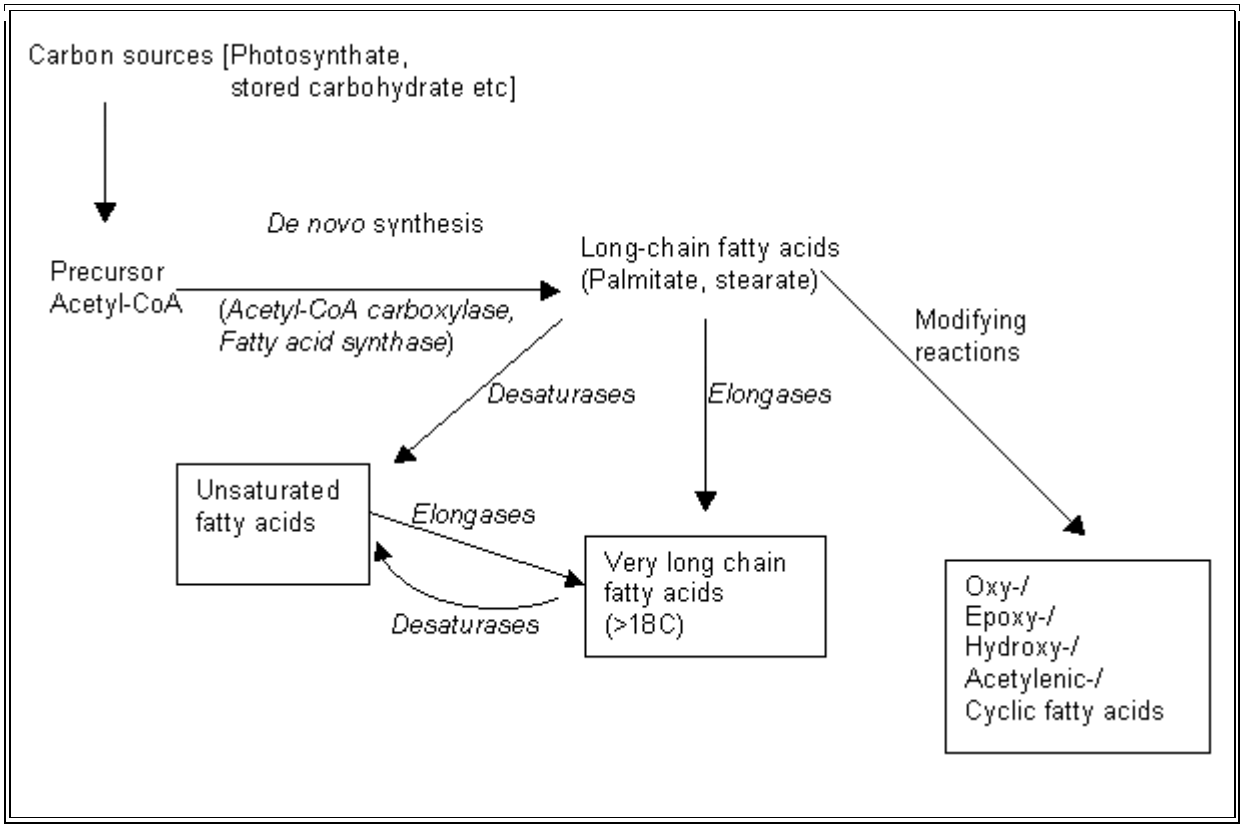
The final step in the production of wax esters from long-chain alcohols and fatty acids involves the action of an acyl-CoA:alcohol transacylase. In animal tissues, this is an enzyme that is also required for triacylglycerol biosynthesis, i.e. an acyl CoA:diacylglycerol acyltransferase (DGAT) or more specifically an isoform of the enzyme known as DGAT1. The same enzyme synthesises retinyl esters also. Similarly, in prokaryotes, an enzyme that is structurally distinct from that in animals is both a diacylglycerol acyltransferase and a wax ester synthase. In plants, C_{16} and C_{18} fatty acids from the fatty acid synthetase are converted to CoA esters and subjected to chain elongation prior to wax formation. An active fatty acyl CoA:fatty alcohol acyltransferase has been isolated from microsomal fractions of seeds of the jojoba plant that is responsible for production of the storage wax, but also appears to be structurally related to the wax synthases involved in the synthesis of the epicuticular waxes.

Once synthesised, the wax components must be exported from the sites of lipid synthesis in the plastid and the endoplasmic reticulum to the plasma membrane and through the cell wall. They must then pass into the cutin layer that provides a matrix within and upon which the waxes are deposited. Very little is known of how wax is exported, but two groups of transport molecules have been identified recently that are known to facilitate this process.

Plant Fatty Acid Synthesis

Plants synthesize a huge variety of fatty acids although only a few are major and common constituents. Broadly speaking, long chain fatty acids are synthesised *de novo* from small precursors ultimately derived from photosynthate. Two enzyme systems are utilised, acetyl-CoA carboxylase and fatty acid synthase. The end products of this synthesis are usually the saturated fatty acids palmitate and stearate with the latter predominating (in most plants by 2-3 times that of palmitate). Once the long chain acids have been produced they can be subject to elongation, desaturation and further modifications. Unlike acetyl-CoA carboxylase and fatty acid synthase, which are soluble enzymes, the elongases

are membrane-bound and sited in the endoplasmic reticulum. Only recently have the details of such reactions started to be elucidated at the molecular level. Elongases are coded by *FAE* genes while the desaturases are coded by *FAD* genes.



Fatty acid desaturases are usually membrane-bound and utilize complex lipid substrates such as phosphatidylcholine or monogalactosyldiacylglycerol. An exception is the stearoyl-acyl carrier protein (ACP) Δ9-desaturase that is present in the chloroplast stroma and converts stearate to oleate. When desaturases produce polyenoic fatty acids, the latter usually have a methylene-interrupted structure, such as linoleic (*cis, cis* Δ9,12-octadecadienoic) acid or α-linolenic (all *cis* Δ9,12,15-octadecatrienoic) acid.

Some plants can produce unusual fatty acids in their seed oils, many of which have useful industrial applications. These include hydroxyl fatty acids, cyclopropane fatty acids, epoxy fatty acids and conjugated unsaturated fatty acids. It is noteworthy that these

unusual fatty acids accumulate preferentially in triacylglycerols and are essentially excluded from membrane acyl lipids – presumably because they would impair function.

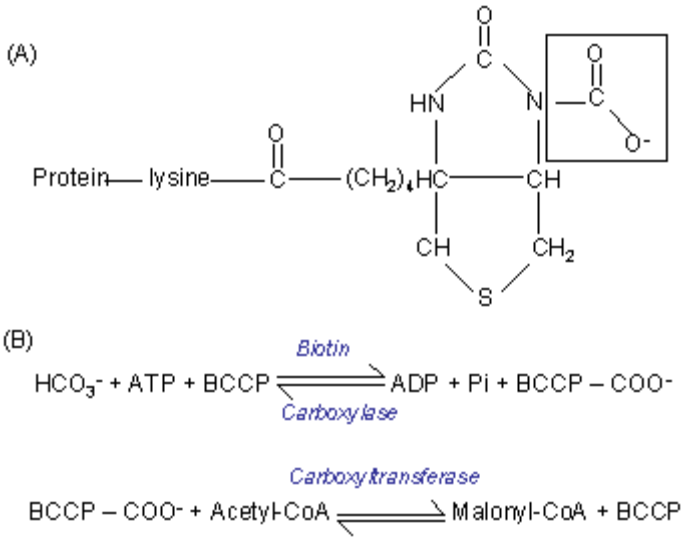
De novo Synthesis

Source of carbon

Apart from germination, photosynthate provides the source of carbon for *de novo* fatty acid synthesis. Early work highlighted a controversy as to how much carbon could be derived directly from plastid pyruvate dehydrogenase. More recent studies have concluded that this enzyme provides most of the acetyl-CoA needed for leaf fatty acid biosynthesis. In contrast, the situation maybe more complex in developing oil seeds such as *Brassica napus* (oilseed rape).

Acetyl-CoA carboxylase

The first committed step in fatty acid biosynthesis is catalysed by acetyl-CoA carboxylase (EC 6.4.1.2). This belongs to the group of soluble Class 1 biotin-containing enzymes which use ATP and bicarbonate to carboxylate a biotin prosthetic group. The carboxy group is then transferred to the acceptor acetyl-CoA to form malonyl-CoA. The initial partial reaction is catalysed by biotin carboxylase, uses bicarbonate (rather than carbon dioxide) as the source of carbon and acts via a carboxyphosphate intermediate. The second partial reaction is catalysed by carboxyltransferase. During the reaction, electron transfer is thought to allow direct reaction of carbon dioxide with the incoming acetyl-CoA to yield malonyl-CoA. While the various Class 1 biotin-containing carboxylases share a very similar sequence identity for their biotin carboxylases, the carboxyltransferases are distinct and give the specificity to the overall reaction.



(A) The biotin prosthetic group which is attached to biotin carboxyl carrier protein (BCCP) via a lysine residue. The carboxylation takes place on a nitrogen in the upper ring (as shown).
(B) The partial reactions of acetyl-CoA carboxylase.

There are two distinct molecular forms of acetyl-CoA carboxylase in plants – multiprotein complexes and multifunctional proteins. Furthermore, plants also have isoforms in two subcellular sites. A plastid-localised isoform is used for *de novo* synthesis of fatty acids while an extra-plastid isoform (presumed to be cytosolic) provides malonyl-CoA for fatty acid elongation as well as other functions. The different susceptibility of grasses and dicotyledons to various herbicides belonging to the aryloxypropionate and cyclohexanedione chemical groups (which both inhibited fatty acid synthesis in grasses) led to the discovery that, while grasses had two different multifunctional protein forms of acetyl-CoA carboxylase (mol. masses of 220-230 kDa), the dicotyledons have a multifunctional protein in the cytosol but a multienzyme complex in the stroma. The latter

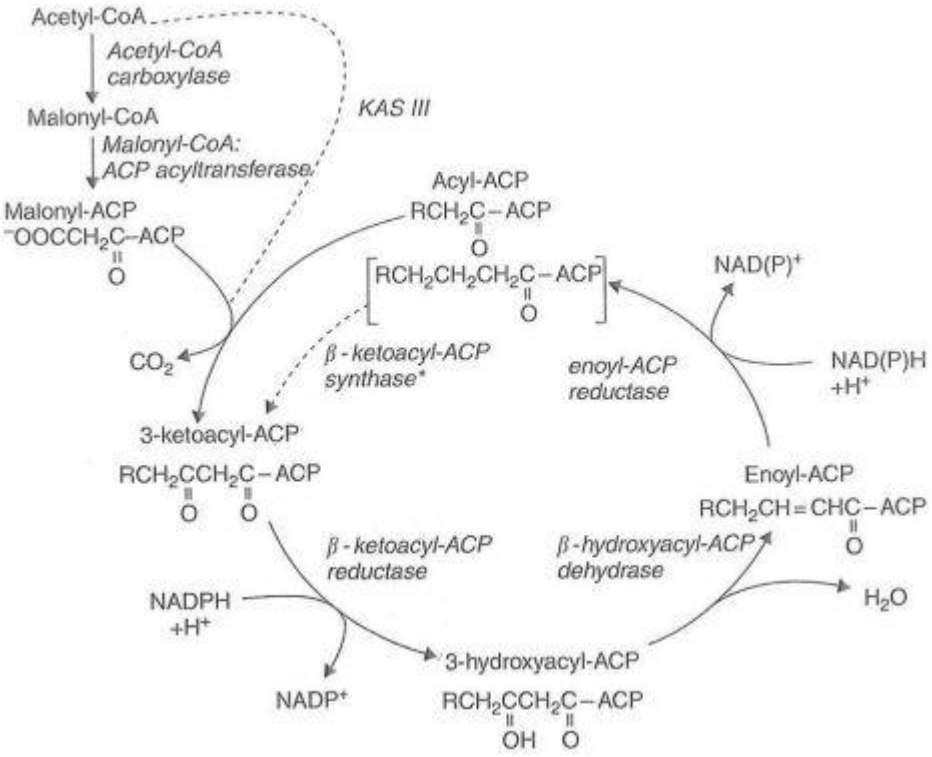
was coded by 4 separate genes. Three of these genes (biotin carboxylase, biotin carboxyl carrier protein, α-subunit of carboxyltransferase) are nuclear-encoded. The fourth gene coding for the β-subunit of carboxyltransferase is plastid-located.

Regulation of acetyl-CoA carboxylase

Since acetyl-CoA carboxylase catalyses the first committed reaction in fatty acid synthesis, it might be thought a good candidate for important regulation – as revealed in animal tissues. Two types of experiment confirmed that the regulation of acetyl-CoA carboxylase was important for the control of lipid synthesis – at least in leaf tissues. First, The data provided experimental evidence pointing to the importance of acetyl-CoA carboxylase in regulating overall synthesis. By making use of the specific action of grass-selective herbicides, they were able to measure the flux control exerted by the enzyme for lipid synthesis in barley or maize leaves. Their results showed that the acetyl-CoA carboxylase reaction alone controlled about 55% of the total flux, thus demonstrating clearly its important role in regulation.

Fatty acid synthase

The second enzyme complex involved in *de novo* synthesis is fatty acid synthase (FAS). In plants this is a Type II FAS consisting of a multiprotein complex. There are a number of enzymes involved in FAS. These are used for acyl-transfer, the four sequential reactions involved in 2-carbon addition and in termination of the overall reaction. In addition, acyl carrier protein (ACP) is used as the acyl carrier for the various intermediate reactions. Although *de novo* synthesis is located in the stroma, plant mitochondria are capable of limited fatty acid synthesis. This is probably mainly used for lipoic acid formation. Nevertheless, three genes for mitochondrial ACP have been detected in *Arabidopsis*. In addition, this plant has five deduced genes for plastid ACP.



* The first condensation reaction is catalysed by β-ketoacyl-ACP synthase III (KAS III), which uses acetyl-CoA and malonyl-ACP substrates. The next six condensations are catalysed by KAS I while the final reaction between palmitoyl-ACP and malonyl-ACP utilises KAS II. Figure taken from reference 8 with permission from the author and the publisher, Blackwell Publishing Ltd.

Although acetyl-CoA:ACP acyltransferase has been studied in plants, its function has been put in doubt by the clear demonstration of a short-chain condensing enzyme (KAS III) in plants. However, the importance of malonyl-CoA:ACP acyltransferase is not in doubt.

For the successive addition of two-carbon units four enzyme reactions are needed. These are catalysed by a condensing enzyme (β-ketoacyl-ACP synthase, KAS), first

reductase (β-ketoacyl-ACP reductase), dehydrase (β-hydroxyacyl-ACP dehydrase) and a second reductase (enoyl-ACP reductase). Three different condensing enzymes are found generally in plants – called KAS I, II and III. A fourth one may be present in some tissues. The initial condensation of two 2-carbon units is catalysed by KAS III which uses acetyl-CoA and malonyl-ACP substrates. After the two reductions and dehydration reactions a 4-carbon fatty acid, butyrate, is produced. This is poorly condensed by KAS III but is a good substrate for KAS I, which acts to elongate 4- to 14- carbon chains. The final condensation is catalysed by KAS II which is mainly responsible for the production of stearate. KAS I and KAS II can be distinguished from each other by their differential sensitivity to cerulenin and arsenite, respectively. As one might anticipate, the plant FAS and, particularly its condensing enzyme isoforms, has many parallels with the classic Type II FAS from *Escheria coli*.

Moreover, there are also some KAS enzymes with unusual properties which are able to cope with particular fatty acid substrates (unsaturated or very long chain) in specific tissues. The crystal structure of *E. coli* KAS III has been deduced and allowed, by sequence comparison, some speculation as to the active site of the plant KAS III. This showed that key site residues (Cys-His-Asn triad) were completely conserved and allowed preferred conformations of other parts of the adjacent protein structure to be deduced. Although it has been suggested that condensing enzymes may be important for regulating carbon flux, experiments to demonstrate this have not been convincing.

After condensation, the intermediate is reduced by β-ketoacyl-ACP reductase. Usually this is regarded as a NADPH-utilising enzyme. It has been purified from *Brassica napus* (subunit mol. mass 28kDa) and functions as a tetramer. The reductase from oilseed rape has also been crystallised and features of its substrate binding and catalysis deduced. At certain developmental stages, this reductase may exert significant influence on the rate of oil accumulation in *Brassica*.

The third enzyme in the elongation cycle is β-hydroxyacyl-ACP dehydrase, which has been purified from spinach leaves. It is encoded by two genes in Arabidopsis and is specific for the D (-) substrate stereoisomer.

The fourth enzyme is enoyl-ACP reductase. Depending on the plant source, the reductase may be specific for NADH or may be able to utilise NADPH as well. The reductase from *Brassica* has a subunit molecular mass of 35KDa. Quite a lot is known about the gene (or genes) coding for the enoyl reductase, as well as the reaction mechanism which involves a compulsory-ordered ternary complex.

Enoyl reductase is a target for a metabolite of isoniazid, which is used for the treatment of tuberculosis. In addition, the commonly-used antibiotic, triclosan targets the enzyme both in *E. coli* and plants. Some carotenoid synthesis herbicides may inhibit the enzyme as a secondary mode of action.

Termination mechanisms

In plants the process of *de novo* fatty acid synthesis can be halted in various ways. For complex lipid synthesis within the plastid (especially the phosphatidylglycerol component of the thylakoid membranes), acyl-ACPs such as palmitoyl-ACP or stearoyl-ACP, can be used directly by acyltransferases of the Kornberg-Pricer pathway producing phosphatidate. This usage is favoured because β-ketoacyl-ACP synthase II (KAS II) uses myristoyl-ACP and palmitoyl-ACP (but not stearoyl-ACP) preferentially as substrates.

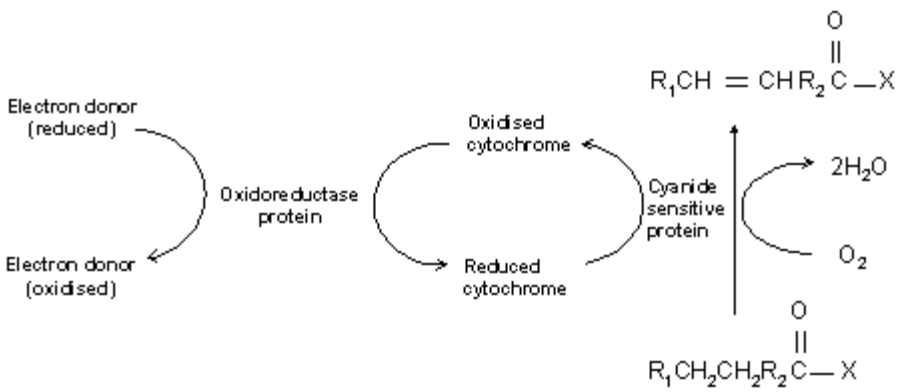
Alternatively, the acyl-ACP products of fatty acid synthase can be hydrolysed by thioesterase(s). The release of unesterified fatty acids allows them to be exported outside the plastid to undergo modifications on the endoplasmic reticulum or to be used for complex lipid biosynthesis in the extra-plastidic compartment. Fatty acids which are exported take part in the 'eukaryotic' pathway of lipid synthesis whereas fatty acids retained in the plastid are used for the 'prokaryotic' pathway. Lipids made by the former pathway characteristically are enriched at both the *sn*-1 and *sn*-2 positions with 16C fatty

acids whereas the prokaryotic pathway produces thylakoid lipid molecules with 18C acids at the *sn*-2 position.

Two classes of fatty acyl-ACP thioesterases have been described (FATA and FATB). FATA preferentially hydrolyses oleoyl-ACP whereas FATB has highest activity with saturated acyl-ACPs. In Arabidopsis there are two genes for FATA and one for FATB. Although most plants contain FATB enzymes that have good activity with substrates in the 14-18C range, some species have a particular need for thioesterases that can act on shorter chains. Thus, tissues such as coconut, California bay, palm kernel or developing *Cuphea* seeds produce oils with short or medium acyl chains. The medium-chain FATB from California bay was the first to be cloned and has been used to transform oilseed rape. Seeds from the latter could produce up to 60% of their total fatty acid contents as laurate.

Fatty Acid Desaturases

The introduction of double bonds into the fatty acid chain relies on the activity of desaturases. In plants, such desaturases work via an aerobic mechanism with oxygen being reduced by 4H, two from the substrate fatty acid and two from the reductant.

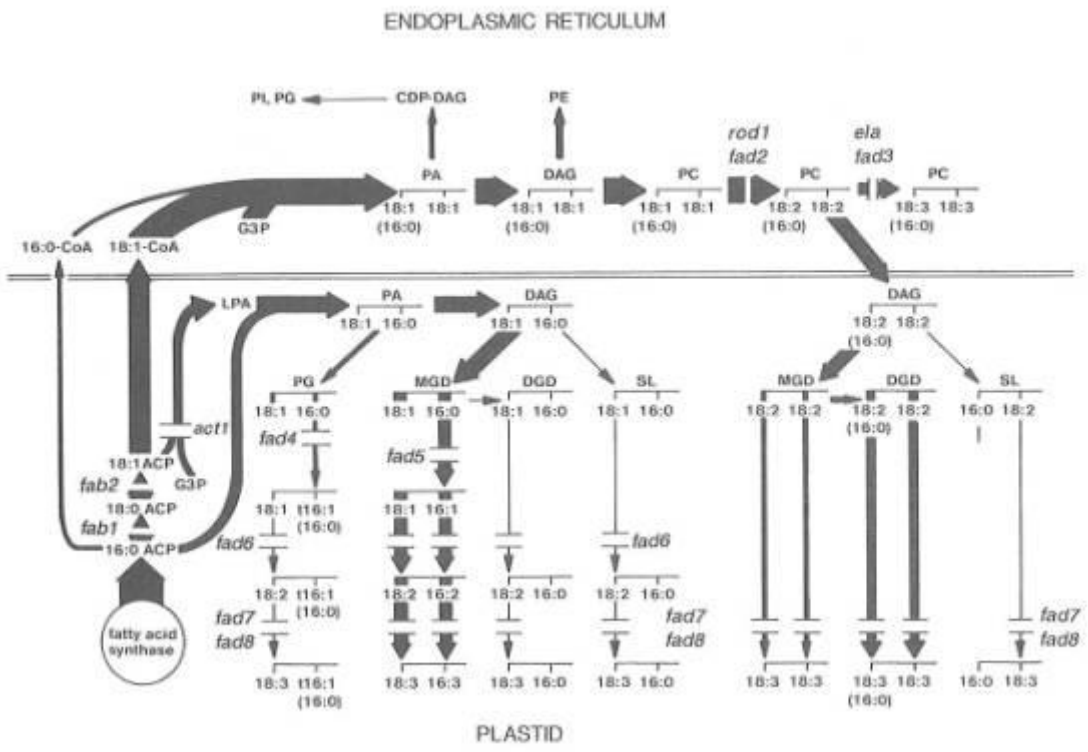


A simplified depiction of the aerobic desaturation mechanism in plants.

Electron donors, cytochromes and substrates can all vary depending on the desaturase. Examples of donors could be NADH, NADPH or reduced ferridoxin

while cytochrome b5 is the usual cytochrome used. Substrates include acyl-ACPs, acyl-CoAs, phosphatidylcholine and monogalactosyldiacylglycerol. Fatty acids can be saturated, monoenoic or polyenoic and the position of the new double bond varies.

Genes coding for desaturases are abbreviated as *FAD* or *fad*. These are numbered generally according to the position of the double bond introduced as well as the nature of the substrate used.



The two-pathway scheme for membrane glycerolipid synthesis in Arabidopsis leaves

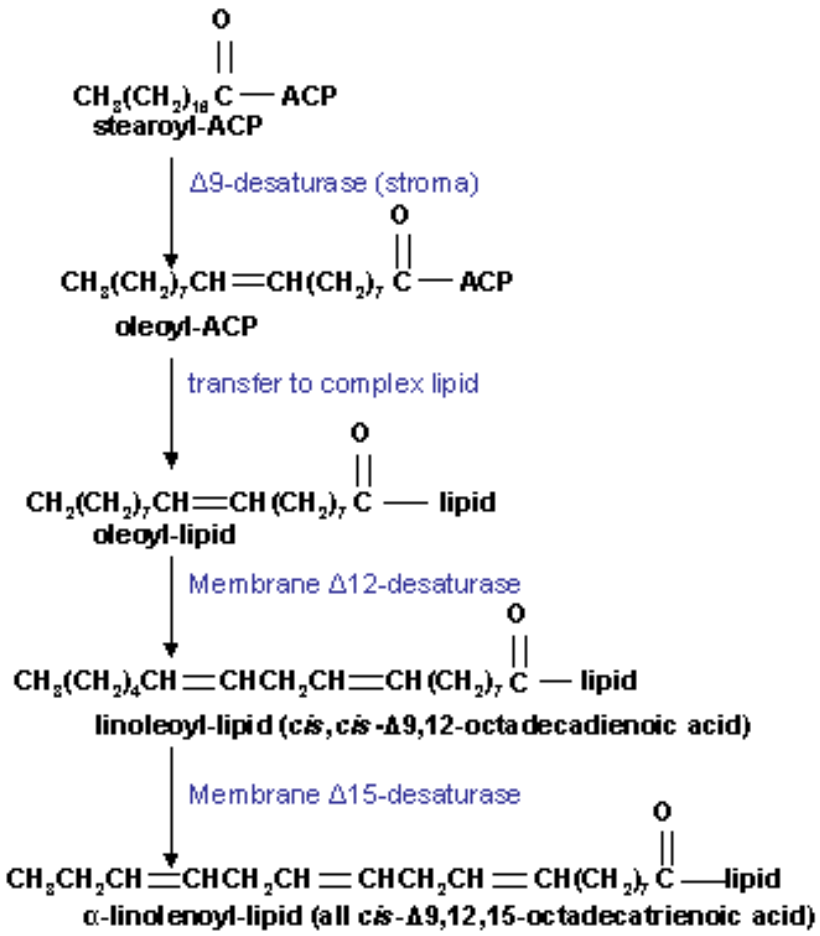
Widths of the lines show the relative fluxes through different reactions. The breaks show the putative enzyme deficiencies in various known mutants.

Stearoyl-ACP Δ9-desaturase

In contrast to other desaturases, this is a soluble enzyme in the plastid stroma that converts stearate into oleate. Its soluble nature has allowed structural studies to be performed. Genes for the enzyme have been identified in a whole variety of plants. Moreover, in addition to the usual stearoyl/palmitoyl-ACP Δ9-desaturases, there are other soluble acyl-ACP desaturases in different plants. For example, Δ4 and Δ6 palmitoyl-ACP desaturases and a Δ9-myristoyl-ACP have been reported. They all have considerable sequence homology which is notably different from acyl-lipid desaturases or to the acyl-CoA desaturase of mammals, yeast or the red alga *C. merolae*. The soluble Δ9-desaturases can be engineered with amino acid substitutions or chimeric proteins produced which have novel properties.

Δ12-Desaturase

The most abundant plant fatty acids are linoleic and α-linolenic acids and these are produced by further desaturation of oleate with the introduction of methylene-interrupted double bond arrangements. Earlier work on the Δ12-desaturase enzyme producing linoleate. Complex lipids are substrates and the desaturation can take place on the endoplasmic reticulum (FAD 2 on phosphatidylcholine) or within the plastid (FAD 6 on glycosylglycerides). For seed oils FAD 2 is the main pathway while in leaves the eukaryotic pathway utilising FAD 2 will operate to varying extents. In most species of higher plants phosphatidylglycerol is the only exclusive product of the prokaryotic pathway and the other thylakoid lipids are produced by the eukaryotic pathway. In other species such as spinach or Arabidopsis, these latter are made equally by the two pathways.



The sequential desaturation of stearate to α-linolenate.

Stearoyl-ACP produced by fatty acid synthase is the substrate for the Δ9-desaturase (also in the stroma) which forms oleoyl-ACP. In the prokaryotic pathway, oleate is then incorporated into chloroplast membrane lipids for further desaturation at the Δ12-(FAD 6 enzyme) and Δ15-(FAD 7, FAD 8 enzymes) positions. For the eukaryotic pathway, oleoyl-ACP is hydrolysed by FATA and/or FATB and the unesterified oleate used to form oleoyl-CoA by acyl-CoA synthase on the plastid envelope. The oleoyl-CoA can then be incorporated into phosphatidylcholine by various reactions where

it forms a substrate for the Δ12-desaturase (FAD 2) to form linoleate and then a Δ15-desaturase (FAD 3) to yield α-linolenate. The diacylglycerol from phosphatidylcholine can be released to be incorporated into chloroplast lipids for further desaturation by FAD 7/8.

POSSIBLE QUESTION

Unit-III

PART A (2 Marks)

1. What is the role of light in chlorophyll synthesis?
2. Write a note on ω oxidation.
3. How are caffeine synthesized?
4. What are glycolipids? Give two examples.
5. Write the reaction of dihydroxy acetone phosphate to glyceraldehydes 3 phosphate.
6. Add a note on desaturase system in fatty acid synthesis.
7. Uses of any two commercially important waxes.
8. Reaction of glyceraldehydes 3 phosphate to lysophosphatidate.
9. Structure of any two glycolipids.
10. How is chlorophyll synthesis regulated?

PART B (8 Marks)

1. Elaborate the biosynthesis of fatty acids in plastids.
2. How are ureides synthesized? Explain.
3. Comment on the synthesis of chlorophyll.
4. What are waxes? How are they synthesized? Write their uses.
5. How are the nitrogenous compounds synthesized? Explain.
6. Explain the secondary oxidative mechanisms in plants.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed University established Under Section 3 of UGC Act 1956)

DEPARTMENT OF BIOCHEMISTRY

III B.SC BIOCHEMISTRY – FIFTH SEMESTER

17BCU503A – PLANT BIOCHEMISTRY

MULTIPLE CHOICE QUESTIONS

Unit III

SL.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4		ANSWER
1	Chlorosis of leaves is due to the deficiency of	Carbon	nitrogen	sulphur	phosphorus		nitrogen
2	The release of NH^{4+} ion in the soil through the decompo	Nitrification	biological nitrogen fixation	Ammonification	denitrification		Ammonification
3	The conversion of ammonium ions into nitrites is performed by	Nitrobacter	nitrococcus	azotobacter	clostridium		nitrococcus
4	Oxidation of nitrites into nitrates is performed by	Nitrobacter	nitrosomonas	nitrococcus	clostridium		Nitrobacter
5	The process by which NH3 is converted to nitrates is called as	Nitrification	denitrification	ammonification	nitrogen fixation		Nitrification
6	Nitrate reductase is found in	Mitochondria	cytoplasm	vacuoles	golgi complex		cytoplasm
7	The electron transfer in the enzyme nitrite reductase is mediated by	Mo	FAD	sirohaem	cyt b557		sirohaem
8	Which of the following is a free living chemo synthetic bacteria?	Chlorobium	clostridium	azotobacter	desulphovibrio		desulphovibrio
9	Which of the following is a free living anaerobic nitrogen fixing bacteria?	Desulphovibrio	clostridium	chlorobium	azotobacter		clostridium
10	The growth factor secreted by the roots of pisum sativum is	Serine	homoserine	glycine	alanine		homoserine
11	Which is the symbiotic nitrogen fixing bacteria?	Rhizobium	azotobacter	clostridium	nitrosomonas		Rhizobium
12	The pink colour of nitrogen fixing nodule is due to the presence of	Xanthophylls	carotenoids	leghaemoglobin	bacteroids		leghaemoglobin
13	Nutrients that are required by plants in smaller quantities are considered as	micronutrients	macronutrients	mega nutrients	chemical nutrients		micronutrients

14	Nutrients that are required by plants in large quantities are considered as	mega nutrients	chemical nutrients	micronutrients	macronutrients		macronutrients
15	Micronutrient which is important in transport of sugar, synthesis of enzymes and cell division is	phosphorus	boron	potassium	Sulphur		boron
16	Insectivorous plants use _____ as nitrogen source	Nitrate	molecular nitrogen	ammonia	nitrite		molecular nitrogen
17	Distinctive odour and flavor to garlic, onion and mustard oil is due to	phosphorus	boron	potassium	Sulphur		Sulphur
18	Suppression of fruit formation and delaying in ripening is due to deficiency of	phosphorus	boron	potassium	Sulphur		Sulphur
19	Reduction in the number of stroma lamellae and increa	phosphorus	boron	potassium	Sulphur		Sulphur
20	Promotion of fruit ripening is due to the deficiency of	phosphorus	boron	potassium	Sulphur		phosphorus
21	Accumulation of carbohydrates is due to the deficiency of	phosphorus	boron	potassium	Sulphur		phosphorus
22	Toxic effect of calcium is antagonized by	phosphorus	boron	potassium	Sulphur		potassium
23	Mottled chlorosis of leaves is due to the deficiency of	phosphorus	boron	potassium	Sulphur		potassium
24	The main constituent of middle lamellae of the cell is	phosphorus	boron	calcium	Sulphur		calcium
25	Oxalic acid is neutralized by	phosphorus	boron	calcium	Sulphur		calcium
26	Arginine kinase is activated by	phosphorus	boron	calcium	Sulphur		calcium
27	Phospho lipase is activated by	phosphorus	boron	calcium	Sulphur		calcium
28	Indole acetic acid is oxidized by	phosphorus	boron	manganese	Sulphur		manganese
29	Enzymes of nitrogen metabolism is activated by	phosphorus	boron	manganese	Sulphur		manganese
30	_____ acts as a cofactor in oxidative phosphorylation	phosphorus	boron	manganese	Sulphur		manganese
31	Formation of seeds is slowed down by the deficiency of	phosphorus	boron	manganese	Sulphur		manganese
32	Retardation in nitrogen assimilation is due to the deficiency of	phosphorus	boron	manganese	Sulphur		manganese
33	Chlorosis in pine apple and citrus is caused by the deficiency of	phosphorus	manganese	calcium	Sulphur		manganese

34	_____ participates in the synthesis of tryptophan and auxin	phosphorus	boron	zinc	Sulphur		zinc
35	Formation of carbonic anhydrase is enhanced by	zinc	boron	calcium	Sulphur		zinc
36	Metabolism of alcohol dehydrogenase is activated by	zinc	boron	calcium	Sulphur		zinc
37	Rosette disease of walnut is due to the deficiency of	zinc	boron	calcium	Sulphur		zinc
38	White bud disease of maize is due to the deficiency of	zinc	boron	calcium	Sulphur		zinc
39	Antibiotic production in *Fusarium* is promoted by	zinc	boron	calcium	Sulphur		zinc
40	Nicotine production in tobacco plants is promoted by	zinc	boron	calcium	Sulphur		zinc
41	Easy transport of sugar in phloem is helped by	zinc	boron	calcium	Sulphur		boron
42	“Top sickness” disease in tobacco is caused due to the deficiency of	zinc	boron	calcium	Sulphur		boron
43	“heart rot disease of sugar beet” is due to the deficiency of	zinc	boron	calcium	Sulphur		boron
44	_____ participates in the formation of phenolase lactase and ascorbic acid oxidase	zinc	boron	copper	Sulphur		copper
45	Black pigmentation in the spores of *Aspergillus niger* is imparted by	zinc	boron	copper	Sulphur		copper
46	____ acts as fungicide to prevent the disease “late blight of potato”	copper	boron	calcium	Sulphur		copper
47	Nitrate reductase enzyme is activated by	molybdenum	boron	calcium	Sulphur		molybdenum
48	“Whip tail” disease of cauliflower is due to the deficiency of	zinc	boron	calcium	molybdenum		molybdenum
49	Cysteine desulfurase converts	Cysteine into pyruvate and H2S	Cysteine into carbohydrate	Cysteine into sulfur	cysteine into cystine		Cysteine into pyruvate and H2S
50	When chlorophyll is burst, which one is obtained?	iron	Magnesium	calcium	Manganese		Magnesium
51	The element which is required in largest quantities by plant is	nitrogen	calcium	sulphur	phosphorus		nitrogen

52	Which of the following elements is responsible for maintaining turgor?	sodium	potassium	Calcium	Magnesium		potassium
53	Which of the following is not an essential element for plants?	iron	zinc	potassium	iodine		iodine
54	Ascorbic acid synthesis is controlled by	Molybdenum	copper	Boron	Zinc		Molybdenum
55	Carbohydrate metabolism is increased by	Molybdenum	copper	Boron	Zinc		Molybdenum
56	Auxin synthesis is enhanced by	Molybdenum	copper	Boron	Zinc		Zinc
57	Toxic effect of NaCl is antagonized by	calcium	copper	Boron	Zinc		Calcium
58	Phospholipase is activated by	calcium	copper	Boron	Zinc		Calcium
59	More lignifications of cells occurs due to deficiency of	Potassium	copper	Boron	Zinc		Potassium
60	Binding of nucleic acid with protein is enhanced by	Calcium	copper	Boron	Zinc		Calcium

UNIT-IV

Plant growth substances: Chemistry, biosynthesis, mode of action and physiological role of auxins, gibberellins, cytokinins, abscisic acid and ethylene. Factors influencing endogenous growth-biotic and abiotic factors. Phytochromes: molecule, biological display, functions as light sensor. Senescence: biochemical changes, regulation.

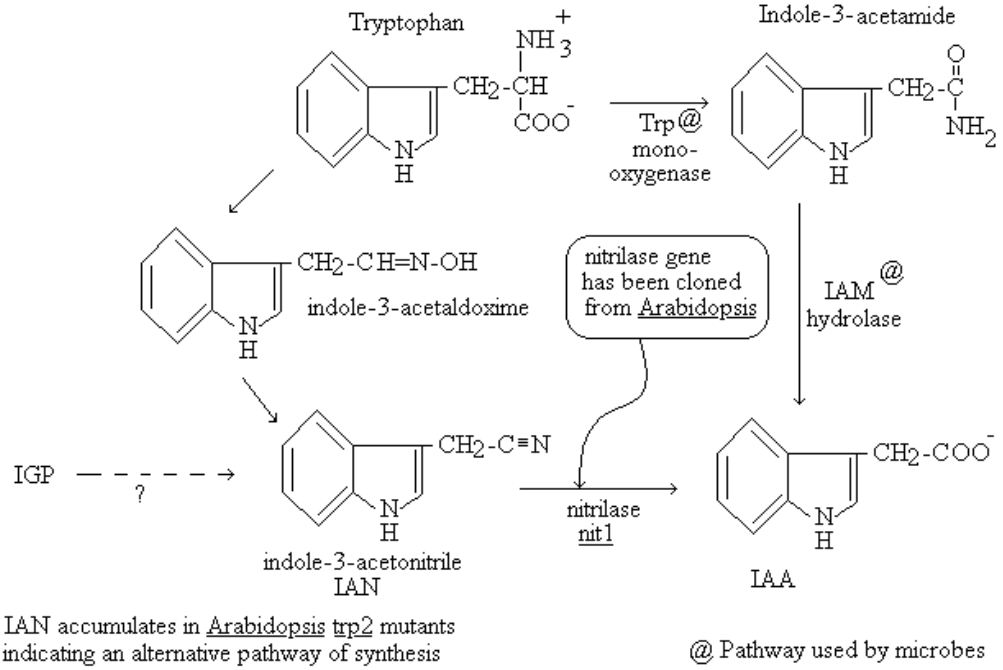
Plant growth substances

Plant hormones are a group of naturally occurring, organic substances which influence physiological processes at low concentrations. The processes influenced consist mainly of growth, differentiation and development, though other processes, such as stomatal movement, may also be affected. Similarly, the effects of plant hormones depend largely on the target tissues and the chemical environment in which these tissues find themselves.

Auxin

Chemistry: Indole-3-acetic acid (IAA) is the main auxin in most plants.

Biosynthesis: IAA is synthesized from tryptophan or indole primarily in leaf primordia and young leaves, and in developing seeds.



Mechanism of Action

The mechanism by which the plant hormone auxin regulates plant growth has puzzled scientists since Darwin's time. Auxin is known to regulate gene expression by binding to its receptor TIR1 and promoting ubiquitin-dependent degradation of Aux/IAA repressor proteins. Now the determination of the crystal structures of TIR1 in complexes with three different auxins and an Aux/IAA peptide shows auxin to act as a 'molecular glue' promoting interactions between the receptor and proteins targeted for degradation. As well as revealing auxin's mechanism, this work establishes the first structural model of a plant hormone receptor. Also, the discovery that a small molecule like auxin can regulate ubiquitin ligases suggests a novel strategy for developing therapeutics for human disorders associated with ubiquitin ligase defects. On the cover, auxin (shown as a spacefilling model) is seen in the cavity between TIR1 (blue) and IAA7 peptide (orange).

Physiological Effects

- Cell enlargement - auxin stimulates cell enlargement and stem growth.
- Cell division - auxin stimulates cell division in the cambium and, in combination with cytokinin, in tissue culture.
- Vascular tissue differentiation - auxin stimulates differentiation of phloem and xylem.
- Root initiation - auxin stimulates root initiation on stem cuttings, and also the development of branch roots and the differentiation of roots in tissue culture.
- Tropistic responses - auxin mediates the tropistic (bending) response of shoots and roots to gravity and light.
- Apical dominance - the auxin supply from the apical bud represses the growth of lateral buds.
- Delayed leaf senescence.
- Leaf and fruit abscission - auxin may inhibit or promote (via ethylene) leaf and fruit abscission depending on the timing and position of the source.
- Delayed fruit ripening.
- In several systems (e.g., root growth) auxin, particularly at high concentrations, is inhibitory. Almost invariably this has been shown to be mediated by auxin-produced ethylene. If the ethylene synthesis is prevented by various ethylene synthesis inhibitors, then auxin is no longer inhibitory.

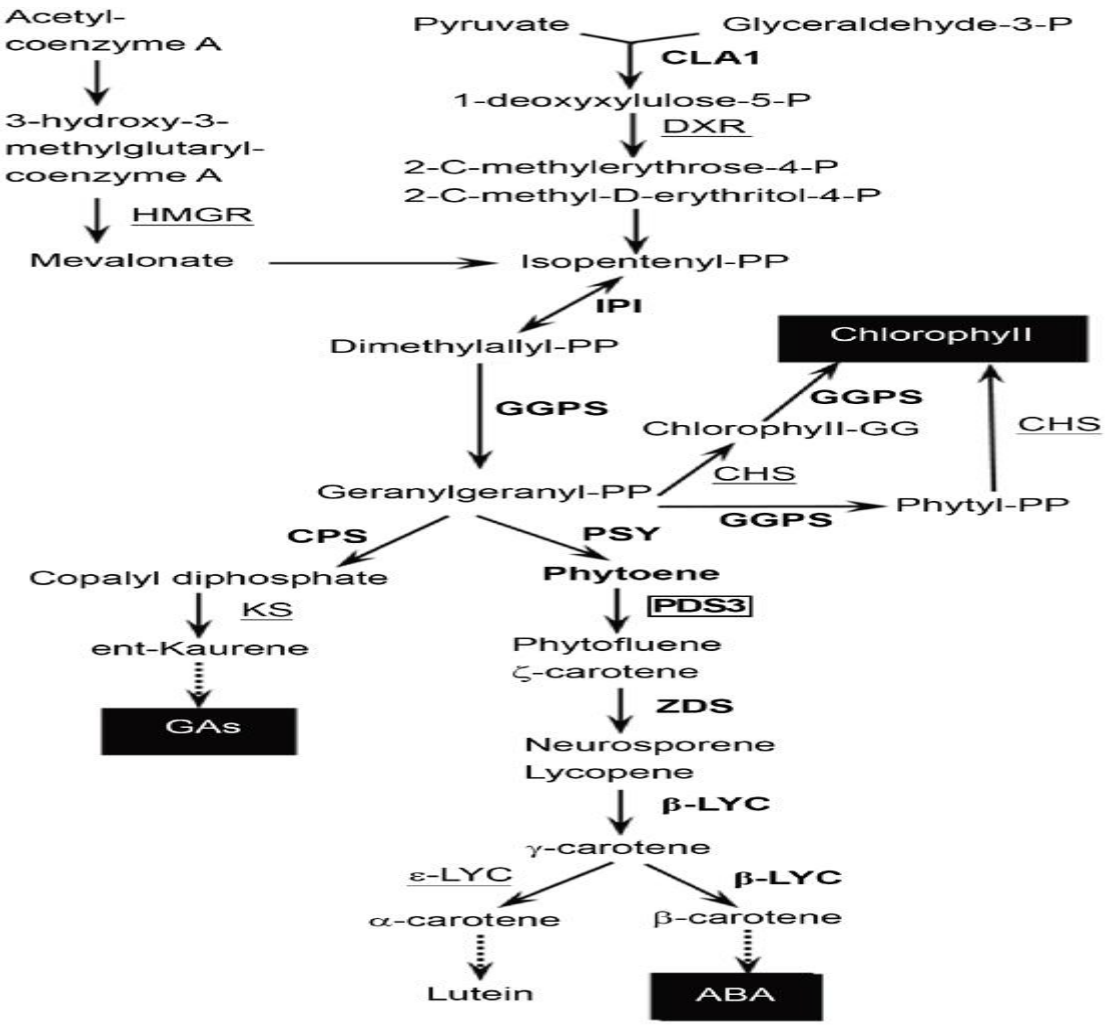
Gibberillin

Gibberellins are a plant growth substance (phytohormone) involved in promotion of stem elongation, mobilisation of food reserves in seeds and other processes. Its absence results in the dwarfism of some plant varieties. Chemically all known gibberellins are gibberellic acids, a family of diterpene acids that are synthesized by the terpenoid pathway in plastids and then modified in the endoplasmic reticulum and cytosol until they reach

their biologically active form. Gibberellin was first isolated in 1926 by Japanese scientists. It was derived from the Gibberella fungus.

Chemistry: The most widely available compound is GA3, or gibberellic acid, which is a fungal product. The most important GA in plants is GA1, which is the GA primarily responsible for stem elongation.

Biosynthesis: GAs are synthesized from mevalonic acid in young tissues of the shoot (exact location uncertain) and developing seed. Gibberellin biosynthesis can be divided into three parts: The first part is localized in proplastids and forms a C_{20}-precursor (*ent*-kaurene). The second part covers oxidation reactions that are located at the endoplasmic reticulum. Finally, in the cytoplasm of the plant cell gibberellin plant hormones are formed by 2-oxoglutarate dependent dioxygenases, enzymes that are also involved in inactivation of the plant hormone.



Physiological Effects

- Stimulates shoot and cell elongation
- Delays senescence of leaves
- Inhibits root growth
- Inhibits adventitious root growth
- Produces seed germination
- Antagonist promotes root growth and GA reverses this
- Promotes root initiation in low concentration in pea cuttings

- Stimulates bolting and flowering in biennials
- Regulates production of hydrolytic enzymes for digesting starches
- Inhibits CK bud growth on calluses
- Inhibits bud formation
- Inhibits leaf formation

Cytokinins

Nature: CKs are adenine derivatives characterized by an ability to induce cell division in tissue culture (in the presence of auxin). The most common cytokinin base in plants is zeatin.

Biosynthesis: CK biosynthesis is through the biochemical modification of adenine. It occurs in root tips and developing seeds.

Mode of action: The action of CKs is still poorly understood and insufficient evidence exists to conclusively identify any biochemical point of action.

Transport: CK transport is via the xylem from roots to shoots.

Physiological Effects

- Cell division - applications of CKs induce cell division in tissue culture in the presence of auxin.
- The presence of CKs in tissues with actively dividing cells (e.g., fruits, shoot tips) indicates that CKs may naturally perform this function in the plant. Morphogenesis - in tissue culture, CKs promote shoot initiation.
- Growth of lateral buds - CK applications can cause the release of lateral buds from apical dominance. Leaf expansion - resulting solely from cell enlargement.
- This is probably the mechanism by which the total leaf area is adjusted to compensate for the extent of root growth, as the amount of CKs reaching the shoot will reflect the extent of the root system.
- CKs delay leaf senescence.
- CKs may enhance stomatal opening in some species.

- Chloroplast development - the application of CK leads to an accumulation of chlorophyll and promotes the conversion of leukoplasts into chloroplasts.

Abscisic Acid

Nature: The name abscisic acid is rather unfortunate. The first name given was "abscisin II" because it was thought to control the abscission of cotton bolls. By a compromise the name abscisic acid was coined. It now appears to have little role in either abscission or bud dormancy, but we are stuck with this name. As a result of the original association with abscission and dormancy, ABA has become thought of as an inhibitor. While exogenous applications can inhibit growth in the plant, ABA appears to act as much as a promoter (e.g., storage protein synthesis in seeds) as an inhibitor, and a more open attitude towards its overall role in plant development is warranted.

Biosynthesis: ABA is synthesized from mevalonic acid in roots and mature leaves, particularly in response to water stress. Seeds are also rich in ABA which may be imported from the leaves or synthesized.

Transport: ABA is exported from roots in the xylem and from leaves in the phloem. There is some evidence that ABA may circulate to the roots in the phloem and then return to the shoots in the xylem.

Physiological Effects

- Stomatal closure - water shortage brings about an increase in ABA which leads to stomatal closure.
- ABA inhibits shoot growth (but has less effect on, or may promote, root growth).
- This may represent a response to water stress. ABA induces storage protein synthesis in seeds.
- ABA counteracts the effect of gibberellin on a-amylase synthesis in germinating cereal grains.
- ABA affects the induction and maintenance of some aspects of dormancy in seeds.

- It does not, however, appear to be the controlling factor in "true dormancy" or "rest," which is dormancy that needs to be broken by low temperature or light.

Ethylene

Nature: The gas ethylene (C_2H_4) is synthesized from methionine in many tissues in response to stress. It does not seem to be essential for normal vegetative growth. It is the only hydrocarbon with a pronounced effect on plants.

Sites of Biosynthesis: Ethylene is synthesized by most tissues in response to stress. In particular, it is synthesized in tissues undergoing senescence or ripening.

Transport: Being a gas, ethylene moves by diffusion from its site of synthesis.

Physiological Effects

- Release from dormancy.
- Shoot and root growth and differentiation.
- Adventitious root formation.
- Leaf and fruit abscission.
- Flower induction in some plants.
- Induction of femaleness in dioecious flowers.
- Flower opening.
- Flower and leaf senescence.
- Fruit ripening.

Factors influencing endogenous growth

Endogenous growth theory holds that economic growth is primarily the result of endogenous and not external forces. Endogenous growth theory holds that investment in human capital, innovation, and knowledge are significant contributors to economic growth. The theory also focuses on positive externalities and spillover effects of a knowledge-based economy which will lead to economic development. The endogenous growth theory primarily holds that the long run growth rate of an economy depends on policy measures.

For example, subsidies for research and development or education increase the growth rate in some endogenous growth models by increasing the incentive for innovation.

Abiotic and Biotic Factors

Abiotic Factors	Biotic Factors
1. Nonliving	1. living
2. Have never lived	2. Lived before
3. Are not dead	3. Can be dead
4. Are not parts of living things	4. Can be parts of living things
Examples:	Examples:
a. water	a. plants
b. sunlight	b. animals
c. minerals	c. fungi (fungus)
d. temperature	d. bacteria
e. carbon dioxide	e. leaves
f. air	f. trees
g. lightening	g. acorns
h. landforms	h. fur

Man made items are not found in nature. They are not abiotic or biotic because they are not found in natural ecosystems. Abiotic and biotic factors have to be made by nature.

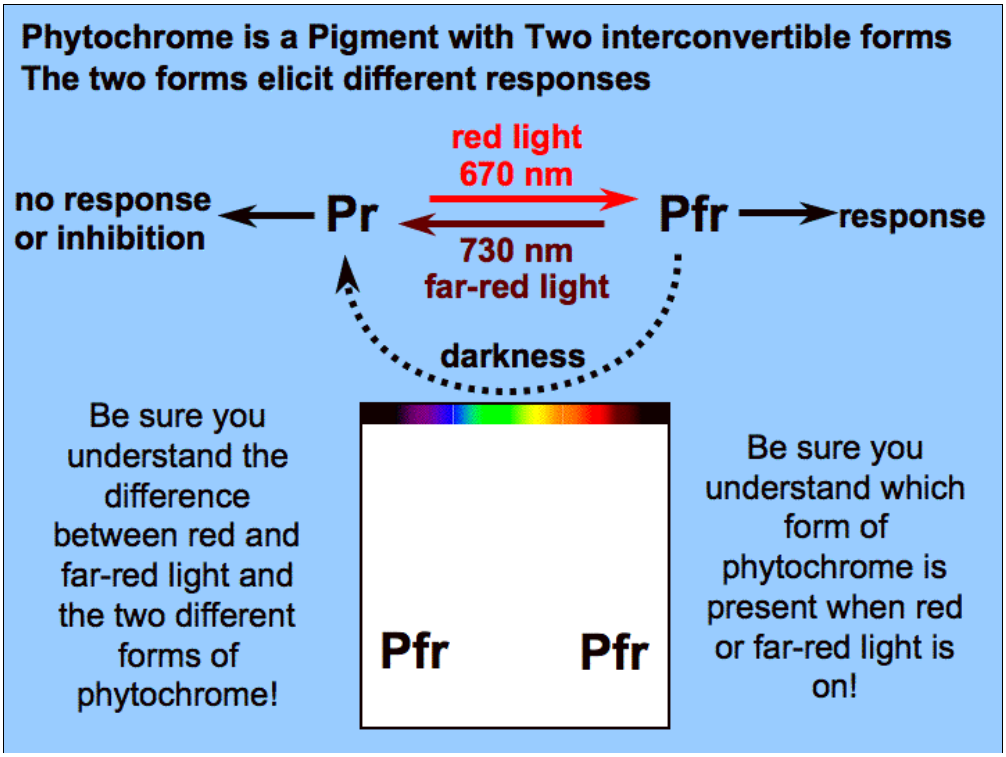
a. batteries
b. video games
c. patio furniture
d. clothes

Phytochrome

Phytochrome is a photoreceptor, a pigment that plants use to detect light. It is sensitive to light in the red and far-red region of the visible spectrum. Many flowering plants use it to regulate the time of flowering based on the length of day and night (photoperiodism) and to set circadian rhythms.

Unlike other pigments you have met so far, phytochrome has two different chemical structures that are inter-convertible. The forms are named by the color of light that they absorb maximally: Pr is a blue form that absorbs red light (660 nm) and Pfr is a blue-green

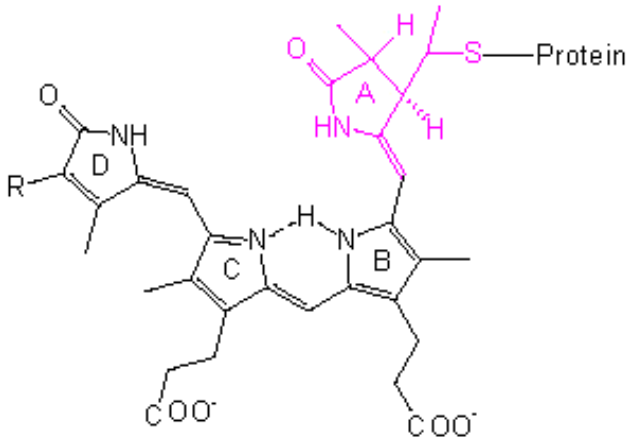
form that absorbs far-red light (730 nm). What is strange about these pigments is that when they DO absorb these photons, they change chemically into the other form.



Obviously Pr absorbs red light (660 nm) very strongly, while Pfr absorbs far-red light (730 nm) very strongly. Both have some absorption in the blue-end of the spectrum. These differences in absorption have to do with differences in the chemical structures of these two forms.

The Phytochrome Molecule

It is attached to the phytochrome protein through a sulfur linkage.



P_{FR}

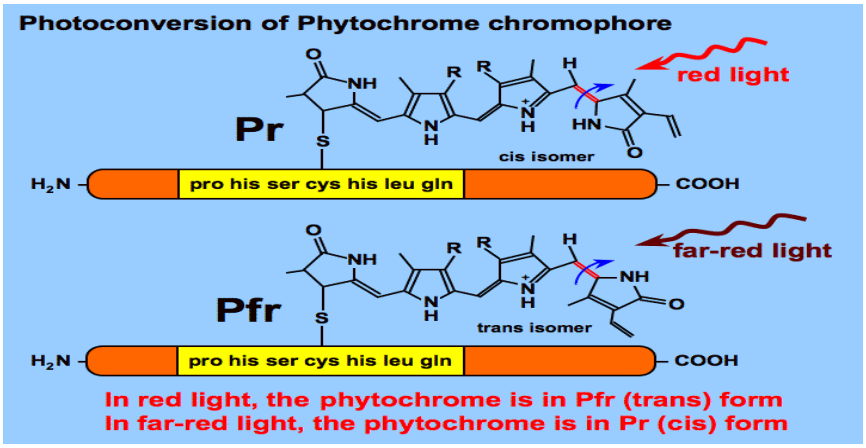
Phytochrome Genes and Proteins

- **There are five phytochrome genes in dicots**, corresponding to the *Arabidopsis* genes termed phyA, phyB, phyC, phyD, phyE. Monocots, like duckweeds, have fewer phytochrome genes, homologs of phyA, phyB, phyC.
- **Phytochrome A** (PhyA), present only in angiosperms, is responsible for early events in germination and seedling de-etiolation. It is powerfully down-regulated in light both at the transcriptional and post-translational levels. In darkness it accumulates to (comparatively) high levels.

  Expression of the other phytochrome types (B to E in angiosperms) is independent of light and both Pr and Pfr forms are stable.
- **Phytochrome B** (PhyB) is probably the photoreceptor involved in shade detection and avoidance. This response allows many species to greatly increase their stem extension rate when they become shaded by competitors. The relative amount of P_{fr} is reduced strongly by the presence of chlorophyll-bearing leaves that filter-out red light but not far-red. The absolute irradiance is irrelevant. Through this red/far-red sensitivity, phytochrome provides the plant with a degree of color perception.

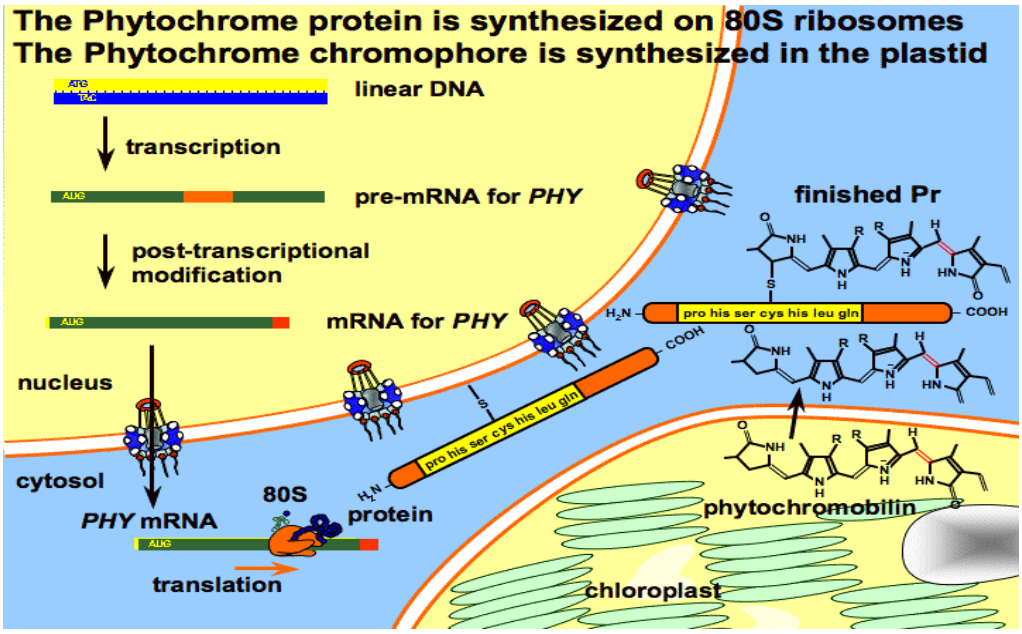
PhyB also is considered responsible for daylength detection in flowering and for tuberization in the potato, though the mechanisms are not understood.

- **Phytochrome C** (phyC) is a low-abundance member of the five-membered phytochrome family of photoreceptors in Arabidopsis. Experimental data indicate that phyC may have some physiological roles that are different to those of phyA and phyB in the control of seedling responses to light signals.

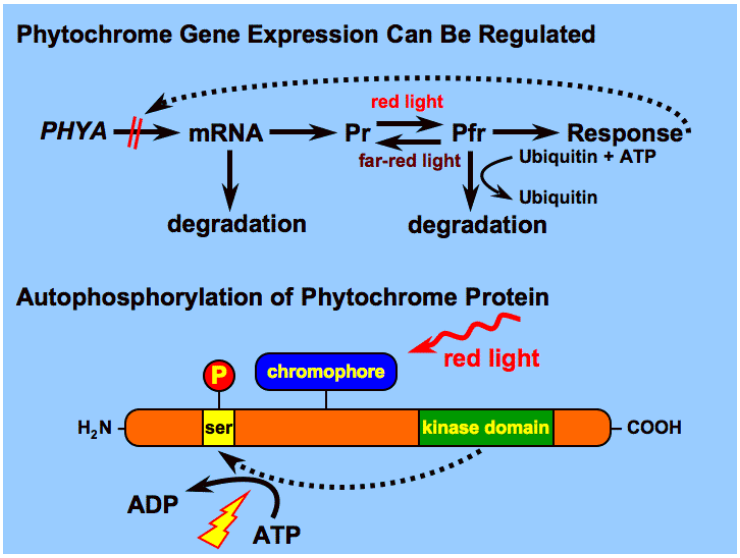


Biosynthesis of phytochrome

Phytochrome is produced in different parts of the cell and assembled from those parts:



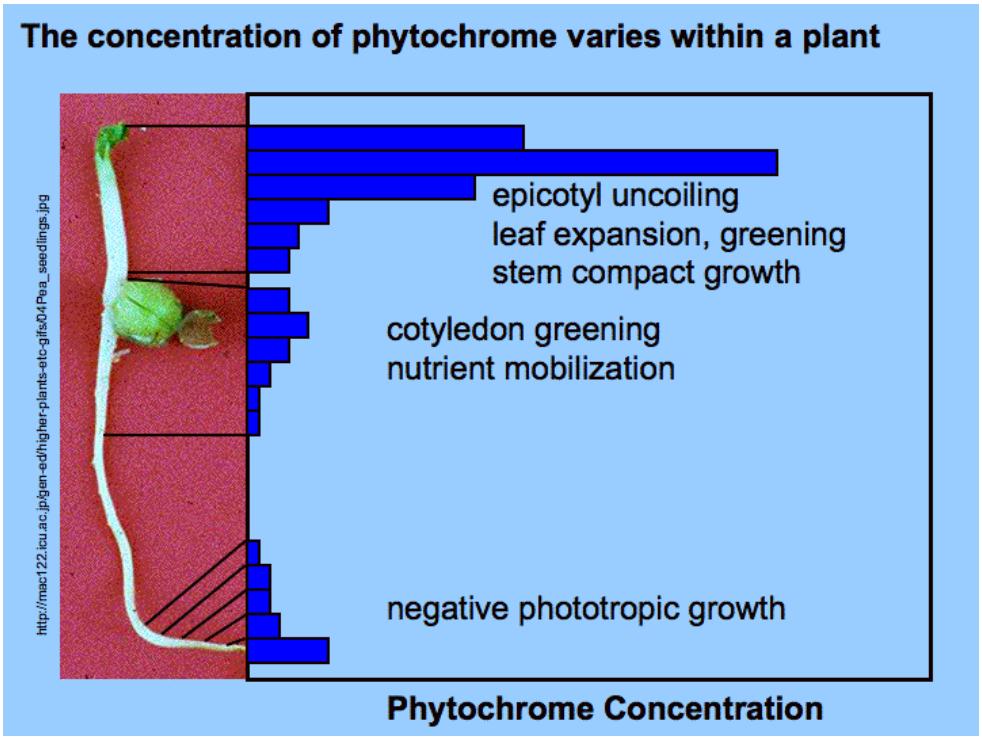
The phytochrome binding protein is coded in nuclear genes, transcribed in the nucleus and translated on cytosolic ribosomes. The phytochrome chromophore is produced in the plastid. These are assembled in the cytosol. However, phytochrome has been found to be associated with plastids in terms of final destination. The regulation of the "central dogma" for phytochrome is shown below. Obviously there are feedback mechanisms so that phytochrome levels are kept to essential and not excessive levels.



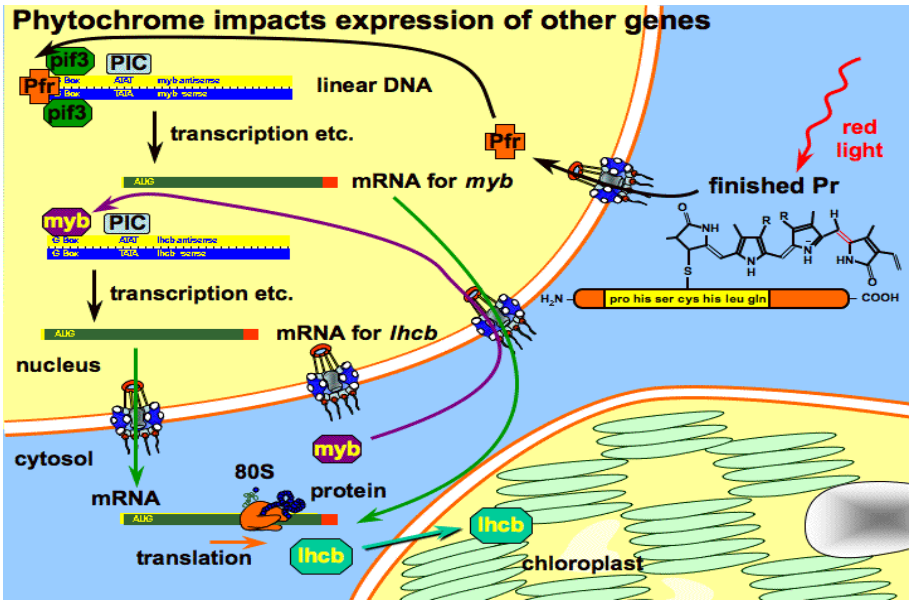
The phytochrome protein includes a kinase domain that, after exposure to red light (i.e. when the chromophore is in Pfr form), allows the protein to phosphorylate itself. This way the auto phosphorylation of phytochrome protein activates it. It may not proceed to somewhere else in the cell to activate other proteins that need to be phosphorylated to become activated. This is the beginning of the response part of our phytochrome mediated physiology.

Phytochrome concentrations vary within the plant

The homeostatic regulation of amount of phytochrome can be observed by measuring its level throughout the plant. The famous example of pea seed germination is shown below.



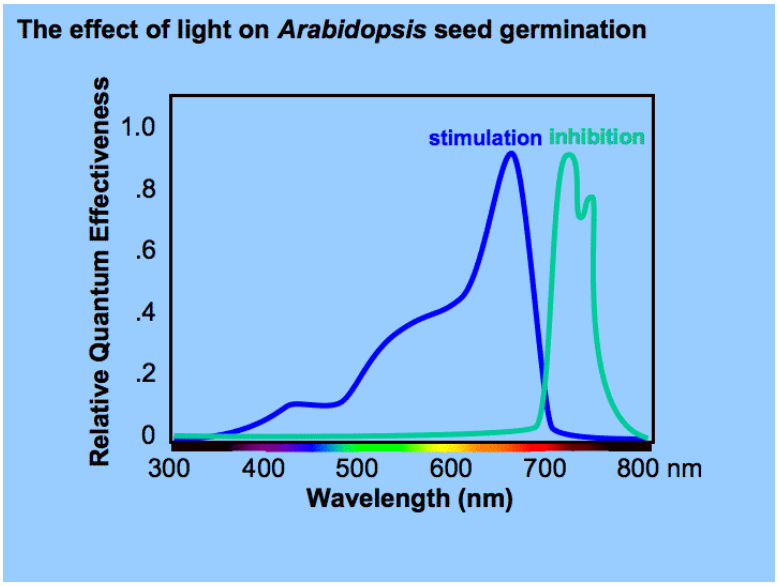
The plant maintains higher levels of phytochrome at its growing points where phytochrome play important roles in growth responses to light. You will also notice the correlation with the zones of greening. Many of the genes for photosynthesis related proteins are regulated by phytochrome. The mechanism for this is shown below for the light harvesting complex b protein.



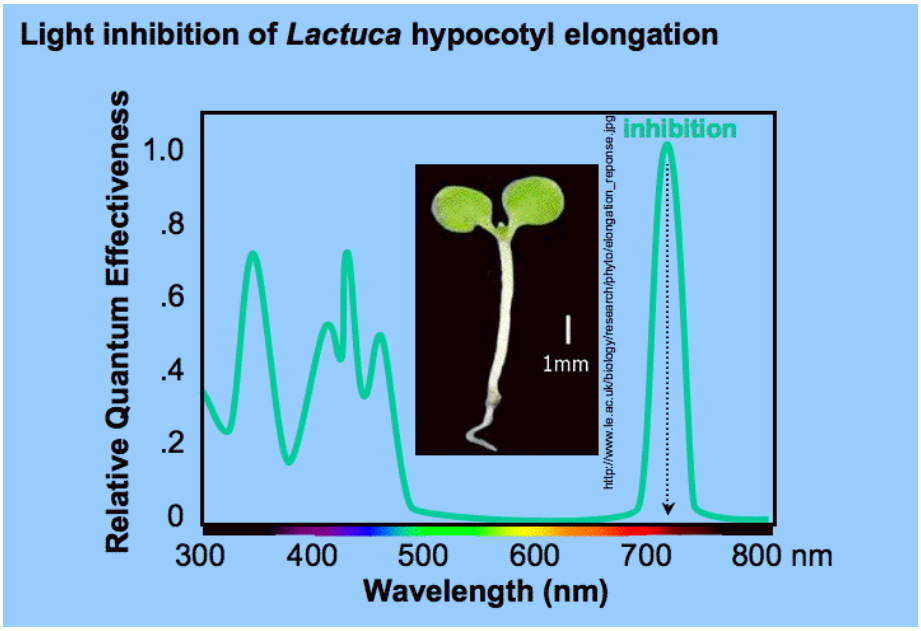
The active phytochrome moves into the nucleus, joins to the dimer of the PIF 3 transcription factors bound to the G-box promoter of the myb genes. The pre-initiation complex (PIC) binds to the TATA box and the myb genes are transcribed and translated. The myb proteins (CCA1 and LHY) are activated and, as transcription factors, bind to the promoter regions of light-stimulated genes such as LHCB.

Some examples of phytochrome responses

There are several famous examples of phytochrome responses including seed germination in *Arabidopsis*.



After a seed germinates, the hypocotyl lifts the cotyledons above the soil in some species (epigeous). This growth is rapid until the plant penetrates the soil and is exposed to light. This rapid water-uptake growth of a seedling is called etiolated growth. The seedling has evolved to include a mechanism to ensure that it rapidly penetrates soil before it runs out of stored nutrients in the seeds. Once in the light, the growth of the hypocotyl is inhibited for strong stocky normal growth of the shoot system.



The growth rate of plants is dependent on their genotype and the environment, as you know from any introductory course

Senescence

The growth of the vascular plant depends upon the activity of meristems, which are, in a sense, always embryonic. Continued indefinitely, this mode of growth could mean immortality; indeed, the longest lived individual organisms ever to have existed on earth have been certain species of trees. Plants and plant parts, however, do die, and death is often not the consequence of accident or environmental stress but of physiological decline aging, or senescence.

Various kinds of physiological senescence and death occur and may affect particular cells, tissues, organs, or the whole plant. In the formation of the vessels of the xylem, cells conclude their differentiation by dying and contribute their empty walls to the conducting tissue. Individual organs such as leaves usually have a limited life span. Entire shoot systems may gradually die back in the aerial parts of perennial plants, which overwinter underground. And, finally, the whole plant may die after a limited period of growth and the

completion of reproduction. This behaviour is found in many annual plants, which complete their life cycle in a single growing season.

Biochemical Changes

The life span may extend to two years, as in biennial plants, or longer, as in banana and certain bamboos, which die after flowering and fruiting. The death of individual cells in tissues such as the xylem appears to be governed by internal factors, but senescence often depends upon interaction of tissues and organs. The presence of young developing leaves often accelerates the aging of older leaves; removal of the younger leaves retards the senescence of the older ones, suggesting control by competition for nutrients. A similar effect is seen in annual plants, in which the development of fruits and seeds is associated with the senescence and, ultimately, the death of the rest of the plant; the removal of reproductive structures slows the rate of aging. In these instances competition obviously has some effect, but it does not sufficiently explain why older, mature organs suffer in competition with those still in active development.

Senescence is the final stage of plant development during which the plant reclaims the valuable cellular building blocks that have been deposited in the leaves and other parts of the plant during growth. Maintaining an efficient senescence process is essential for survival of the plant or its future generations. Senescence is a complex highly regulated process that requires new gene expression and involves the interactions of many signaling pathways.

Reverse senescence: Senescence reversal or suspension indicates that if the decline in cellular constituents during senescence is caused by a sequential decrease in protein synthesis such defects can be overcome. During the reversal process, there must be a reinitiating of transcription / translation of these genes required for synthesis of protein depleted during senescence.

POSSIBLE QUESTION

Unit-IV

PART A (2 Marks)

1. Give an account on ethylene.
2. What are phytochromes?
3. Define senescence.
4. List out the factors influencing endogenous growth of plants.
5. What is the role of phytochrome as light sensor?
6. What is the role of abscisic acid in plant growth?
7. Reversal of senescence.

PART B (8 Marks)

1. Explain in detail the biosynthesis, mode of action and physiological role of Cytokinins.
2. Elaborate the biosynthesis of Auxins and discuss their physiological role and mode of action.
3. Explain the biosynthesis, physiological role and mode of action of gibberillin.
4. What are the factors influencing endogenous growth? Explain.
5. What is called as 'A biological clock'? Explain

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed University established Under Section 3 of UGC Act 1956)

DEPARTMENT OF BIOCHEMISTRY

III B.SC BIOCHEMISTRY – FIFTH SEMESTER

17BCU503A – PLANT BIOCHEMISTRY

MULTIPLE CHOICE QUESTIONS

Unit IV

SL.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4		ANSWER
1	Auxin is found in which region of the plants?	Meristematic region	mature tissue	flowers	fruits		Meristematic region
2	Chemically auxin is	Indole pyruvic acid	Indole 3 acetic acid	Indole butyric acid	2,4 dichloro phenoxy acetic acid		Indole 3 acetic acid
3	Auxin isolated from human urine is	Indole acetic acid	auxonotriolic acid	auxonolonic acid	alpha naphthalene acetic acid		auxonotriolic acid
4	The only true natural auxin of higher plants is	Indole 3 acetic acid	alpha naphthalene acetic acid	2,4 dichloro phenoxy acetic acid	Indole butyric acid		Indole 3 acetic acid
5	The precursor of indole acetic acid is	Tyrosine	methionine	tryptophan	phenyl alanine		tryptophan
6	The enzyme involved in conversion of tryptophan to indole 3 pyruvic acid is	indole pyruvic acid decarboxylase	indole acetaldehyde decarboxylase	amino transferase	tryptophan decarboxylase		amino transferase
7	In tryptamine pathway tryptophan is converted to tryptamine by	tryptophan decarboxylase	indole pyruvic acid decarboxylase	indole acetaldehyde decarboxylase	amino transferase		tryptophan decarboxylase
8	The indole acetaldoxime pathway is characteristic of the family	cruciferae	solanacea	marvacea	malvacea		cruciferae
9	The only non indole auxin is	phenyl pyruvic acid	phenyl acetic acid	alpha naphthalene acetic acid	2,4 dichloro phenoxy acetic acid		phenyl acetic acid

10	Phenyl alanine is converted to phenyl pyruvic acid by the enzyme	aromatic amino transferase	indole pyruvic acid decarboxylase	indole acetaldehyde decarboxylase	amino transferase		aromatic amino transferase
11	Absorption of water is increased by	gibberellins	auxin	cytokinin	ethylene		auxin
12	Shortening of internodes and production of dwarf varieties in apple and pear is due to	gibberellins	auxin	cytokinin	ethylene		auxin
13	The hormone used widely to break seed dormancy is	gibberellins	auxin	cytokinin	ethylene		auxin
14	_____ are weed killers	gibberellins	auxin	cytokinin	ethylene		auxin
15	Early flowering and fruiting is induced by	gibberellins	auxin	cytokinin	ethylene		auxin
16	The precursor for gibberellin biosynthesis is	mevalonic acid	tyrosine	tryptophan	alanine		mevalonic acid
17	Genetic dwarfism is overcome by	gibberellins	auxin	cytokinin	ethylene		gibberellins
18	Bolting and flowering in *Brassica* is induced by	gibberellins	auxin	cytokinin	ethylene		gibberellins
19	Light induced inhibition of stem growth is increased by	gibberellins	auxin	cytokinin	ethylene		gibberellins
20	The production of parthenocarpic fruit in stone fruits is induced by	gibberellins	auxin	cytokinin	ethylene		gibberellins
21	Reduction in number of male flowers and increase in nu	gibberellins	auxin	cytokinin	ethylene		gibberellins
22	Chemically gibberellins are	triterpenoid acids	sesquiterpenoid acids	diterpenoid acids	monoterpenoid acids		diterpenoid acids
23	Seed germination is promoted by	gibberellins	auxin	cytokinin	ethylene		gibberellins
24	Cytokinin is a derivative of	6 furfuryl amino purine	pyrimidine	isopentenyl pyro phosphate	geranyl pyro phosphate		6 furfuryl amino purine
25	Kinetin is formed from	adenosine	guanosine	deoxy adenosine	deoxy guanosine		deoxy adenosine
26	Cell enlargement is induced by all the hormones except	abscisic acid	auxin	cytokinin	ethylene		abscisic acid
27	Enzyme synthesis in plants is regulated by	abscisic acid	auxin	cytokinin	ethylene		cytokinin
28	Sex reversal is induced by	abscisic acid	auxin	cytokinin	ethylene		cytokinin
29	RNA synthesis in elongating zones of onion roots is stimulated by	abscisic acid	auxin	cytokinin	ethylene		cytokinin
30	Abscisic acid is a	triterpene	sesquiterpene	diterpene	monoterpene		sesquiterpenes
31	The precursor for abscisic acid biosynthesis is	mevalonic acid	tyrosine	tryptophan	alanine		mevalonic acid
32	The following hormone is a growth inhibitor	abscisic acid	auxin	cytokinin	gibberellins		abscisic acid

33	Senescence is promoted by	abscisic acid	auxin	cytokinin	ethylene		abscisic acid
34	Loss of chlorophyll and turgor is caused by	abscisic acid	auxin	cytokinin	ethylene		abscisic acid
35	Inhibition of gibberellins induced synthesis of α – amylase and ribonuclease is caused by	abscisic acid	auxin	cytokinin	ethylene		abscisic acid
36	Transcinnamic acid is	gibberellins	auxin	cytokinin	antiauxin		antiauxin
37	RNA synthesis is inhibited by	abscisic acid	auxin	cytokinin	ethylene		abscisic acid
38	Nuclease production is increased by	abscisic acid	auxin	cytokinin	ethylene		abscisic acid
39	_____ is a ripening hormone	abscisic acid	auxin	cytokinin	ethylene		ethylene
40	Precursor for ethylene biosynthesis is	tyrosine	tryptophan	alanine	methionine		methionine
41	Methionine is oxidatively deaminated to	methionol	methionyl phosphate	cystine	cysteine		methionol
42	The cofactor involved in ethylene biosynthesis is	ortho coumaric acid	meta coumaric acid	para coumaric acid	delta coumaric acid		para coumaric acid
43	Resistance to pathogenic infection is induced by	abscisic acid	auxin	cytokinin	ethylene		ethylene
44	The enzyme peroxidase and Polyphenol oxidase in the tissues is stimulated by	abscisic acid	auxin	cytokinin	ethylene		ethylene
45	Epinastic movements is induced by	abscisic acid	auxin	cytokinin	ethylene		ethylene
46	Flowering in pine apple is induced by	abscisic acid	auxin	cytokinin	ethylene		ethylene
47	Radial growth in stems and roots is induced by	abscisic acid	auxin	cytokinin	ethylene		ethylene
48	The level of endogenous auxin is regulated by	abscisic acid	auxin	cytokinin	ethylene		ethylene
49	Leaf abscission is promoted by	abscisic acid	auxin	cytokinin	ethylene		ethylene
50	Genetically dwarf plants can be made taller by	gibberellins	auxin	cytokinin	ABA		Gibberellins
51	Bio assay for gibberellins is	Avena curvature test	soybean callus test	Amylase activity test	Barley leaf disc test for chlorophyll		Amylase activity test
52	During adverse conditions, plants develop a stress hormone	ABA	IAA	ethylene	2,4 – D		ABA
53	Fruit ripening is a	reversible process	irreversible process	light controlled phenomenon	response to light stimulus		irreversible process
54	---------- acts as a wound hormone	Auxin	Gibberellins	Traumatin(traumatic acid)	Cytokinins		Cytokinins
55	Cytokinins ---------- the leaf senescence.	Delay	Induce	Promote	Enhance		Delay

56	One of the most important biological effects of kinetin on plants is	Respiration	Photosynthesis	cell division	Root growth		cell division
57	The following are synthetic analog of auxin molecule except one	Indole acetic acid	2, 4- dichloro phenoxy acetic acid	indolyl butyric acid	indolyl propionic acid		Indole acetic acid
58	Among the following which is antigibberellins	Phenol	Phosphan D	Kaurene	GA 36		Phosphan D
59	Dwarfism can be overcome by the application of	Auxin	Zeatin	Gibberllins	Ethylene		Gibberllins
60	Decarboxylation of tryptophan leads to	Tyrosine	Tryptamine	Indole acetaldehyde	IAA		Tryptamine

UNIT-V

Plant secondary metabolites: Synthesis of secondary metabolites-shikimate pathway. Alkaloids, flavonoids, terpenoids, phenols and glycosteroids-occurrence, distribution and functions. Production of secondary metabolites in plants, stages of secondary metabolite production, PTC-Totipotency, meristematic and nodal cultures-Callus induction. Somatic embryogenesis. Metabolic engineering for increased production of secondary metabolites.

Alkaloids

Alkaloids are a group of naturally occurring chemical compounds (natural products) that contain mostly basic nitrogen atoms. Alkaloid, any of a class of naturally occurring organic nitrogen-containing bases. Alkaloids have diverse and important physiological effects on humans and other animals. Well-known alkaloids include Morphine, strychnine, quinine, ephedrine, and nicotine.

Alkaloids are found primarily in plants and are especially common in certain families of flowering plants. More than 3,000 different types of alkaloids have been identified in a total of more than 4,000 plant species. In general, a given species contains only a few kinds of alkaloids, though both the opium poppy (Papaver somniferum) and the ergot fungus (Claviceps) each contain about 30 different types. Certain plant families are particularly rich in alkaloids; all plants of the poppy family (Papaveraceae) are thought to contain them, for example. The Ranunculaceae (buttercups), Solanaceae (nightshades), and Amaryllidaceae (amaryllis) are other prominent alkaloid-containing families. A few alkaloids have been found in animalspecies, such as the New World beaver (Castor canadensis) and poison-dart frogs (Phyllobates). Ergot and a few other fungi also produce them.

Most alkaloids have one or more of their nitrogen atoms as part of a ring of atoms, frequently called a cyclic system. Alkaloid names generally end in the suffix -ine, a

reference to their chemical classification as amines. In their pure form most alkaloids are colourless, nonvolatile, crystalline solids. They also tend to have a bitter taste.

Pyrrolidine alkaloids

Alkaloids are basically derived from the amino acid called ornithine. This cluster of amino acid comprises the tropane alkaloids, atropine, hyoscine and hyoscyamine from the family of nightshade. Acting as a cluster these alkaloids impede the activities of parasympathetic nerve (originates in the lower part of the spinal cord and brain stem, stimulates digestive secretions, opposes physiological effects of the sympathetic nervous system, constricts the pupils; slows the heart, dilates blood vessels). Incidentally, the pyrrolidine alkaloids also comprise the 'truth medication' scopolamine (also known as hyoscine) and cocaine.

Pyridine and piperidine alkaloids

Pyridine and piperidine alkaloids are a mono carbon hoop including one nitrogen atom and this group of alkaloids comprises numerous species of toxic plants that include venomous hemlock (Conium maculatum), tobacco (Nicotiana tobacum) and lobelia. Incidentally, while tobacco is a member of the nightshade family, scientifically known as Solanaceae, poison hemlock is a constituent of the carrot family or Apiaceae. Coniine, a single ring compound produced in the plant from octanoic acid is a toxic alkaloid present in poison hemlock that is responsible for paralysis, asphyxia (suffocation) and ultimately death. According to history, legendary Greek philosopher, considered by his rulers as an enemy of the people, was provided with an extract of the poisonous hemlock in 399 B.C. and, thus, condemned to death through poisoning. Water hemlock or Cicuta douglasii, which is closely linked with poisonous hemlock, includes cicutoxin - a terpenoid resin. Incidentally, water hemlock is one of the most convulsive or most aggressively lethal indigenous plants found in North America. Ironically, this plant is often mistaken for a parsnip root and in this case the chemicals present in the plant effect the central nervous system directly and most often leads to death.

Pyrrolizidine and quinolizidine alkaloids

Pyrrolizidine and quinolizidine are a complicated group. Incidentally, this group of alkaloids has always proved to be of immense pharmacological interest for researchers and clinical examiners. All these alkaloids are known to have lethal features and may prove to be fatal. While pyrrolizidine is obtained from ornithine and is known to be injurious for the liver, quinolizidine is obtained from amino acid called lysine.

Pyrrolizidine is generally found in ragworts, which is a problem for the grazing animals, comfrey, borage and coltsfoot. In the last instance, the evidence for toxicity is smaller and still unclear.

Indole alkaloids

Indole alkaloids comprise serotonin chemically know as 5-hydroxyltryptamine or 5-HT and others of their kind. These comprise the anesthetizing alkaloids of the passion flower, ophthalmic alkaloids associated with the physostigmine derived from the calabar bean as well as the uterine tonics such as ergotamine. This variety of alkaloids also

comprises the Indian snakeroot or the Rauwolfia serpentaria that consist of reserpine. It may be mentioned here that reserpine contains potent hypotensive and when isolated from the entire plant possess depressive consequences. Among the numerous central nervous stimulants such as strychnine, psilocybin and johimbine, indole alkaloids comprise indole carbon-nitrogen loop. Indole carbon-nitrogen ring is also present in the fungal alkaloids ergine and psilocybin, the neurotransmitter serotonin as well as the mind jerking medication LSD. Researches have shown that these alkaloids may often impede, obstruct or even contend with the action of serotonin in the brain.

Interestingly, one of the strange aspects of alkaloids that occur naturally in the fungi includes ergot, scientifically known as Claviceps purpurea, which is basically a rust fungus that contaminates grains. Incidentally, the alkaloid of ergot is also known as ergine or d-lysergic acid amide. It is popularly known as the natural LSD. Synthetic LSD or d-lysergic acid diethylamide is more powerful than the natural LSD. Two genus of Mexican morning

glory vines also contain natural LSD and these vines are consumed by the native Indians there during significant therapeutic as well as religious ceremonies.

It may be mentioned here that during the Middle Ages, hundreds and thousands of people in Europe were badly affected by a malady known as ergotism. The main symptoms of this disease included festering extremities, spasms as well as insanity. These people suffered from the malady as they consumed bread prepared from rye that was infected with fungus enclosing ergine and various other powerful vasoconstrictor alkaloids that distressed the blood vessels.

Ergotism was also known as 'St Anthony's Fire' and was a deadly malady dreaded by all. Incidentally, the Mecivan peyote cactus or 'Lophopora williamsii as well as San Pedro cactus or 'Trichocereus pachanoi' found in South America contain a different hallucinogenic alkaloid known as mescaline. Incidentally, this variety of alkaloid - mescaline - has a chemical arrangement that is extremely comparable to the brain neurotransmitter dopamine.

Vinblastine and vincristine are two more varieties of indole alkaloids that are found in the Madagascar periwinkle. These varieties also known as 'Catharanthus roseus' are commonly cultivated in the area as a bedding plant belonging to the dogbane family or the 'Apocynaceae'. Commonly known as spindle poisonous, these alkaloids have proved to be very effective in the treatment of chemotherapy for patients suffering from leukemia as well as the Hodgkin's disease that refers to the 'lymph node and spleen cancer'. Researchers have established that this variety of alkaloids helps in terminating or 'depolymerization' of protein microtubules that form the mitotic spindle in cell division. This process efficiently helps in terminating the tumor cells from separating or dividing and, henceforth, resulting to reduction of cancer. It may be noted here that before the periwinkle alkaloids were used by medical practitioners as a treatment there was nearly no hope for relief or survival for patients suffering from Hodgkin's malady. However, researches have established that since the medicos have started utilizing this alkaloid for

treating the disease, there is 90 per cent possibility of survival for patients suffering from Hodgkin's disease.

Plant secondary metabolism produces products that aid in the growth and development of plants but are not required for the plant to survive. Secondary metabolism facilitates the primary metabolism in plants. This primary metabolism consists of chemical reactions that allow the plant to live. In order for the plants to stay healthy, secondary metabolism plays a pinnacle role in keeping all the of plants' systems working properly. A common role of secondary metabolites in plants is defense mechanisms. They are used to fight off herbivores, pests, and pathogens. Although researchers know that this trait is common in many plants it is still difficult to determine the precise role each secondary metabolite. Secondary metabolites are used in anti-feeding activity, toxicity or acting as precursors to physical defense systems.

Flavonoids

Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P or citrin. These metabolites are mostly used in plants to produce yellow and other pigments which play a big role in coloring the plants. In addition, Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities. Flavonoids are also found to be powerful anti-oxidants and researchers are looking into their ability to prevent cancer and cardiovascular diseases. Flavonoids help prevent cancer by inducing certain mechanisms that may help to kill cancer cells, and researches believe that when the body processes extra flavonoid compounds, it triggers specific enzymes that fight carcinogens. Good dietary sources of Flavonoids are all citrus fruits, which contain the specific flavanoids hesperidins,quercitrin,and rutin, berries, tea, dark chocolate and red wine and many of the health benefits attributed to these foods come from the Flavonoids they contain. Flavonoids are synthesized by the phenylpropanoid metabolic pathway where the amino acid phenylalanine is used to produce 4-coumaryol-CoA, and this is then combined with

malonyl-CoA to produce chalcones which are backbones of Flavonoids Chalcones are aromatic ketones with two phenyl rings that are important in many biological compounds. The closure of chalcones causes the formation of the flavonoid structure. Flavonoids are also closely related to flavones which are actually a sub class of flavonoids, and are the yellow pigments in plants. In addition to flavones, 11 other subclasses of Flavonoids including, isoflavones, flavans, flavanones, flavanols, flavanolols, anthocyanidins, catechins (including proanthocyanidins), leukoanthocyanidins, dihydrochalcones, and aurones.

Cyanogenic glycoside

Many plants have adapted to iodine-deficient terrestrial environment by removing iodine from their metabolism, in fact iodine is essential only for animal cells. An important antiparasitic action is caused by the block of the transport of iodide of animal cells inhibiting sodium-iodide symporter (NIS). Many plant pesticides are cyanogenic glycoside which liberate cyanide, which, blocking cytochrome c oxidase and NIS, is poisonous only for a large part of parasites and herbivores and not for the plant cells in which it seems useful in seed dormancy phase. To get a better understanding of how secondary metabolites play a big role in plant defense mechanisms we can focus on the recognizable defense-related secondary metabolites, cyanogenic glycosides. The compounds of these secondary metabolites (As seen in Figure 1) are found in over 2000 plant species. Its structure allows the release of cyanide, a poison produced by certain bacteria, fungi, and algae that is found in numerous plants. Animals and humans possess the ability to detoxify cyanide from their systems naturally. Therefore cyanogenic glycosides can be used for positive benefits in animal systems always. For example, the larvae of the southern armyworm consumes plants that contain this certain metabolite and have shown a better growth rate with this metabolite in their diet, as opposed to other secondary metabolite-containing plants. Although this example shows cyanogenic glycosides being beneficial to the larvae many still argue that this metabolite can do harm.

Terpenoids

The terpenoids, sometimes called isoprenoids, are a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures that differ from one another not only in functional groups but also in their basic carbon skeletons. These lipids can be found in all classes of living things, and are the largest group of natural products.

Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves, and ginger, the yellow color in sunflowers, and the red color in tomatoes. Well-known terpenoids include citral, menthol, camphor, salvinorin A in the plant Salvia divinorum, the cannabinoids found in cannabis, ginkgolide and bilobalide found in Ginkgo biloba, and the curcuminoids found in turmeric and mustard seed.

The steroids and sterols in animals are biologically produced from terpenoid precursors. Sometimes terpenoids are added to proteins, e.g., to enhance their attachment to the cell membrane; this is known as isoprenylation. Terpenoids (or isoprenoids), a subclass of the prenyllipids (terpenes, prenylquinones, and sterols), represent the oldest group of small molecular products synthesized by plants and are probably the most widespread group of natural products. Terpenoids can be described as modified terpenes, where methyl groups are moved or removed, or oxygen atoms added. Inversely, some authors use the term "terpenes" more broadly, to include the terpenoids.

During the 19th century, chemical works on turpentine led to name "terpene" the hydrocarbons with the general formula C10H16 found in that complex plant product. These terpenes are frequently found in plant essential oils which contain the "Quinta essentia", the plant fragrance. They are universally present in small amounts in living organisms, where they play numerous vital roles in plant physiology as well as important functions in

all cellular membranes. They may be defined as a group of molecules whose structure is based on a various but definite number of isoprene units (methylbuta-1,3-diene, named hemiterpene, with 5 carbon atoms). Terpenoids are extraordinarily diverse but they all originate through the condensation of the universal phosphorylated derivative of hemiterpene, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) giving geranyl pyrophosphate (GPP).

Phenols

Polyphenols are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages. Fruits like grapes, apple, pear, cherries and berries contains up to 200–300 mg polyphenols per 100 grams fresh weight. The products manufactured from these fruits, also contain polyphenols in significant amounts. Typically a glass of red wine or a cup of tea or coffee contains about 100 mg polyphenols. Cereals, dry legumes and chocolate also contribute to the polyphenolic intake.

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens.3 In food, polyphenols may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability. Towards the end of 20thcentury, epidemiological studies and associated meta-analyses strongly suggested that long term consumption of diets rich in plant polyphenols offered some protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases. Polyphenols and other food phenolics are the subject of increasing scientific interest because of their possible beneficial effects on human health. This review focuses on the present understanding of the biological effects of dietary polyphenols and their importance in human health and disease.

Occurrence and distribution

Distribution of phenolics in plants at the tissue, cellular and sub cellular levels is not uniform. Insoluble phenolics are found in cell walls, while soluble phenolics are present within the plant cell vacuoles. Certain polyphenols like quercetin are found in all plant

products; fruit, vegetables, cereals, fruit juices, tea, wine, infusions etc., whereas flavanones and isoflavones are specific to particular foods. In most cases, foods contain complex mixtures of polyphenols. The outer layers of plants contain higher levels of phenolics than those located in their inner parts. Numerous factors affect the polyphenol content of plants, these include degree of ripeness at the time of harvest, environmental factors, processing and storage. Polyphenolic content of the foods are greatly affected by environmental factors as well as edaphic factors like soil type, sun exposure, rainfall etc. The degree of ripeness considerably affects the concentrations and proportions of various polyphenols. In general, it has been observed that phenolic acid content decreases during ripening, whereas anthocyanin concentrations increase. Many polyphenols, especially phenolic acids, are directly involved in the response of plants to different types of stress: they contribute to healing by lignifications of damaged areas possess antimicrobial properties, and their concentrations may increase after infection.

Another factor that directly affects the polyphenol content of the foods is storage. Studies have proved that polyphenolic content of the foods change on storage, the reason is easy oxidation of these polyphenols. Oxidation reactions result in the formation of more or less polymerized substances, which lead to changes in the quality of foods, particularly in color and organoleptic characteristics. Such changes may be beneficial, as is the case with black tea or harmful as in browning of fruit. Storage of wheat flour results in marked loss of phenolic acids. After six months of storage, flour contained the same phenolic acids in qualitative terms, but their concentrations were 70% lower compared with fresh. Cold storage, in contrast, has slight effect on the content of polyphenols in apples, pears or onions. Cooking also has a major effect on concentration of polyphenols. Onions and tomatoes lose between 75% and 80% of their initial quercetin content after boiling for 15 min, 65% after cooking in a microwave oven, and 30% after frying.

Functions

Phenols have anti cancer, antidiabetic, antiaging, cardioprotective and neuro protective effects.

Plant tissue culture

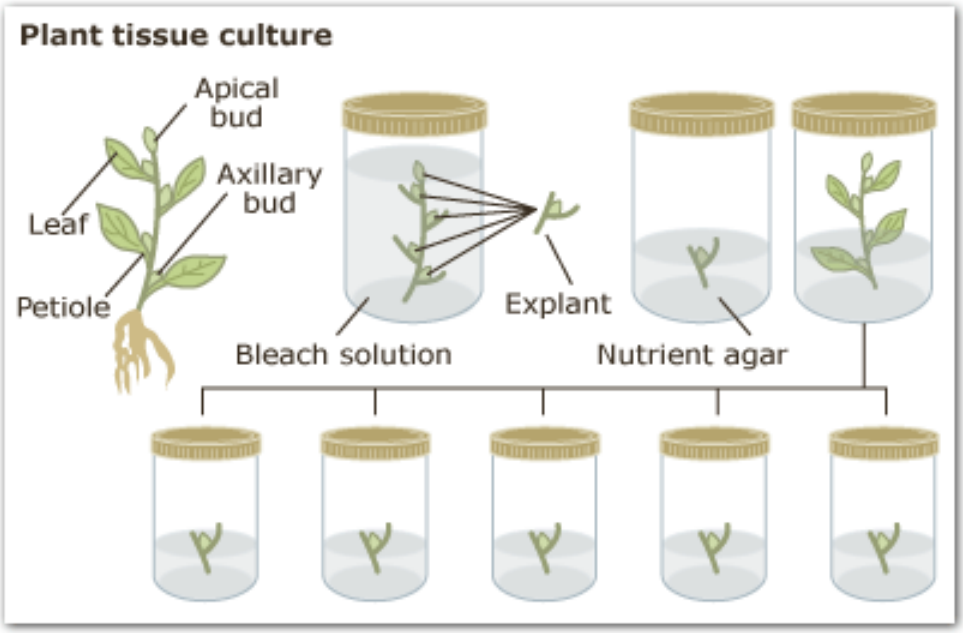
Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation.

Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including:

- The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.
- To quickly produce mature plants.
- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
- The regeneration of whole plants from plant cells that have been genetically modified.
- The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.
- The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e.: orchids and Nepenthes.
- To clean particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

Plant tissue cultures can be initiated from almost any part of a plant. The physiological state of the plant does have an influence on its response to attempts to initiate tissue culture. The parent plant must be healthy and free from obvious signs of disease or decay. The source, termed explant, may be dictated by the reason for carrying out the tissue culture. Younger tissue contains a higher proportion of actively dividing cells

and is more responsive to a callus initiation programme. The plants themselves must be actively growing, and not about to enter a period of dormancy. The exact conditions required to initiate and sustain plant cells in culture, or to regenerate intact plants from cultured cells, are different for each plant species. Each variety of a species will often have a particular set of cultural requirements. Despite all the knowledge that has been obtained about plant tissue culture during the twentieth century, these conditions have to be identified for each variety through experimentation.



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Cell potency is a cell's ability to differentiate into other cell types. The more cell types a cell can differentiate into, the greater its potency. Potency is also described as the gene activation potential within a cell which like a continuum begins with totipotency to designate a cell with the most differentiation potential, pluripotency, multipotency, oligopotency and finally unipotency. Potency is taken from the Latin term "potens" which means "having power."

Totipotency is the ability of a single cell to divide and produce all of the differentiated cells in an organism. Spores and Zygotes are examples of totipotent cells. In the spectrum of cell potency, totipotency represents the cell with the greatest differentiation potential. Toti comes from the Latin totus which means "entirely."

It is possible for a fully differentiated cell to return to a state of totipotency. This conversion to totipotency is complex, not fully understood and the subject of recent research.

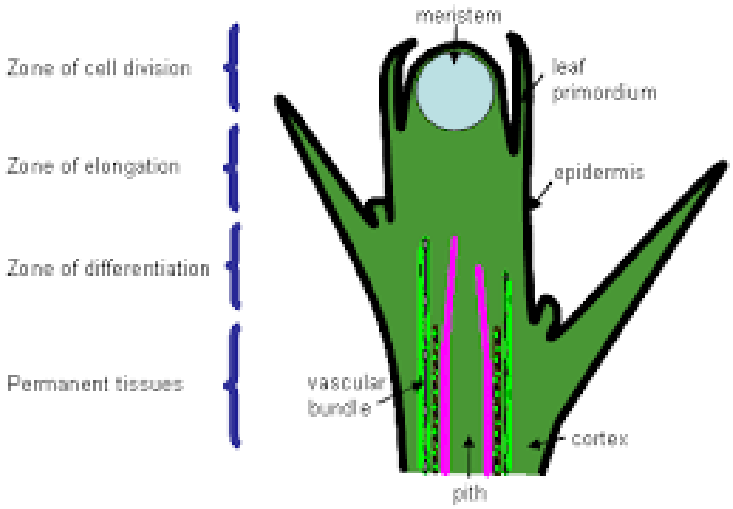
The human development model is one which can be used to describe how totipotent cells arise. Human development begins when a sperm fertilizes an egg and the resulting fertilized egg creates a single totipotent cell, a zygote. In the first hours after fertilization, this zygote divides into identical totipotent cells, which can later develop into any of the three germ layers of a human (endoderm, mesoderm, or ectoderm), into cells of the cytotrophoblast layer or syncytiotrophoblast layer of the placenta. After reaching a 16-cell stage, the totipotent cells of the morula differentiate into cells that will eventually become either the blastocyst's Inner cell mass or the outer trophoblasts. Approximately four days after fertilization and after several cycles of cell division, these totipotent cells begin to specialize. The inner cell mass, the source of embryonic stem cells, becomes pluripotent.

Meristematic cells

Meristematic cells give rise to various organs of the plant and keep the plant growing. The shoot apical meristem (SAM) gives rise to organs like the leaves and flowers, while the root apical meristem (RAM) provides the meristematic cells for the future root growth. SAM and RAM cells divide rapidly and are considered indeterminate, in that they do not possess any defined end status. In that sense, the meristematic cells are frequently compared to the stem cells in animals, which have an analogous behavior and function.

Meristematic cells are incompletely or not at all differentiated, and are capable of continued cellular division (youthful). Furthermore, the cells are small and protoplasm fills the cell completely. The vacuoles are extremely small. The cytoplasm does not contain

differentiated plastids (chloroplasts or chromoplasts), although they are present in rudimentary form (proplastids). Meristematic cells are packed closely together without intercellular cavities. The cell wall is a very thin primary cell wall.



Maintenance of the cells requires a balance between two antagonistic processes: organ initiation and stem cell population renewal. Apical meristems are the completely undifferentiated (indeterminate) meristems in a plant. These differentiate into three kinds of primary meristems. The primary meristems in turn produce the two secondary meristem types. These secondary meristems are also known as lateral meristems because they are involved in lateral growth. At the meristem summit, there is a small group of slowly dividing cells, which is commonly called the central zone. Cells of this zone have a stem cell function and are essential for meristem maintenance. The proliferation and growth rates at the meristem summit usually differ considerably from those at the periphery.

Callus induction: Plant callus (plural calluses or calli) is a mass of unorganized parenchyma cells derived from plant tissue (explants) for use in biological research and biotechnology. In plant biology, callus cells are those cells that cover a plant wound. Callus formation is induced from plant tissues after surface sterilization and plating onto in vitro tissue culture medium. Plant growth regulators, such as auxins, cytokinins, and

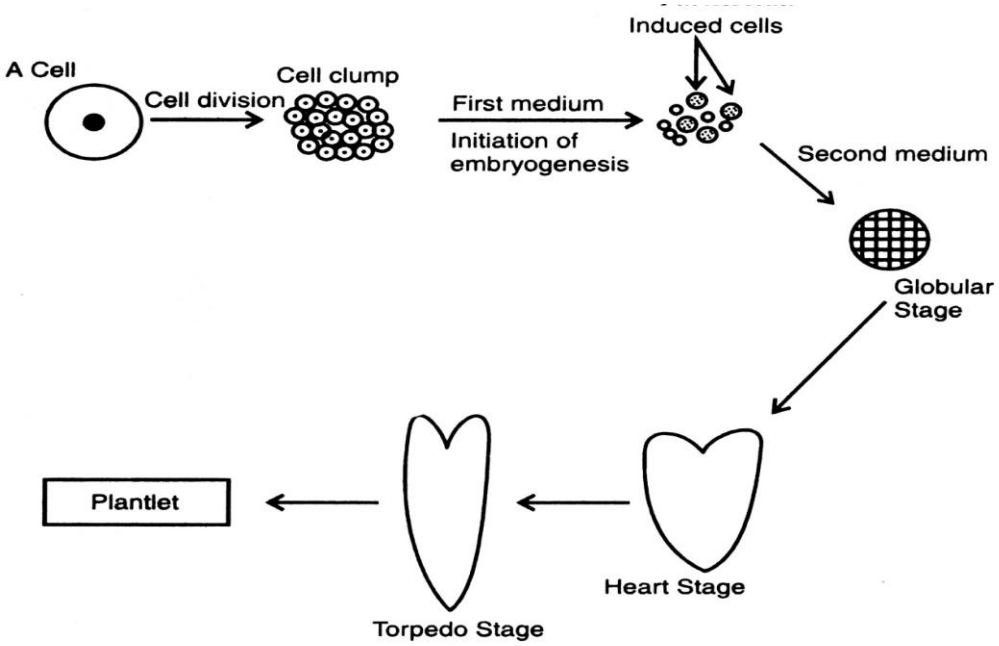
gibberellins, are supplemented into the medium to initiate callus formation or somatic embryogenesis.

A callus cell culture is usually sustained on gel medium. Callus induction medium consists of agar and a mixture of macronutrients and micronutrients for the given cell type. There are several types of basal salt mixtures used in plant tissue culture, but most notably modified Murashige and Skoog medium, White's medium, and woody plant medium. Vitamins are also provided to enhance growth such as Gamborg B5 vitamins. For plant cells, enrichment with nitrogen, phosphorus, and potassium is especially important.

Somatic embryogenesis

Somatic embryogenesis is a process where a plant or embryo is derived from a single somatic cell or group of somatic cells. Somatic embryos are formed from plant cells that are not normally involved in the development of embryos, i.e. ordinary plant tissue. No endosperm or seed coat is formed around a somatic embryo. Applications of this process include: clonal propagation of genetically uniform plant material; elimination of viruses; provision of source tissue for genetic transformation; generation of whole plants from single cells called protoplasts; development of synthetic seed technology. Cells derived from competent source tissue are cultured to form an undifferentiated mass of cells called a callus. Plant growth regulators in the tissue culture medium can be manipulated to induce callus formation and subsequently changed to induce embryos to form from the callus. The ratio of different plant growth regulators required to induce callus or embryo formation varies with the type of plant. Somatic embryos are mainly produced in vitro and for laboratory purposes, using either solid or liquid nutrient media which contain plant growth regulators (PGR's). The main PGRs used are auxins but can contain cytokinin in a smaller amount. Shoots and roots are monopolar while somatic embryos are bipolar, allowing them to form a whole plant without culturing on multiple media types. Somatic embryogenesis has served as a model to understand the physiological and biochemical events that occur plant developmental processes as well as a component to biotechnological advancement.

The first documentation of somatic embryogenesis was by Steward et al. in 1958 and Reinert in 1959 with cell suspension cultures.



Factors and mechanisms controlling cell differentiation in somatic embryos are relatively ambiguous. Certain compounds excreted by plant tissue cultures and found in culture media have been shown necessary to coordinate cell division and morphological changes. These compounds have been identified by Chung et al. as various polysaccharides, amino acids, growth regulators, vitamins, low molecular weight compounds and polypeptides. Several signaling molecules known to influence or control the formation of somatic embryos have been found and include extracellular proteins, arabinogalactan proteins and lipochitooligosaccharides. Temperature and lighting can also affect the maturation of the somatic embryo.

Production of secondary metabolites

Medicinal plants are the most exclusive source of life-saving drugs for majority of the world's population. The utilization of plant cells for the production of natural or recombinant compounds of commercial interest has gained increasing attention over past

decades. The secondary metabolites are known to play a major role in the adaptation of plants to their environment and also represent an important source of pharmaceuticals

Advancements in the production of secondary metabolites

Plant cell and tissue cultures hold great promise for controlled production of myriad of useful secondary metabolites on demand. Discoveries of cell cultures capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants. In order to obtain high yields suitable for commercial exploitation, efforts have been focused on isolating the biosynthetic activities of cultured cells, achieved by optimizing the cultural conditions, selecting high-producing strains and employing precursor feeding, transformation methods, and immobilization techniques. Transgenic hairy root cultures have revolutionized the role of plant tissue culture in secondary metabolite production. They are unique in their genetic and biosynthetic stability, faster in growth, and more easily maintained. Using this methodology, a wide range of chemical compounds has been synthesized. Advances in tissue culture, combined with improvement in genetic engineering of pharmaceuticals, nutraceuticals, and other beneficial substances. Recent advances in the molecular biology, enzymology, and fermentation technology of plant cell cultures suggest that these systems will become a viable source of important secondary metabolites. Genome manipulation is resulting in relatively large amounts of desired compounds produced by plants infected with an engineered virus, whereas transgenic plants can maintain constant levels of production of proteins without additional intervention. Large-scale plant tissue culture is found to be an attractive alternative approach to traditional methods of plantation as it offers controlled supply of biochemical's independent of plant availability.

Production of secondary metabolites from medicinal plants by plant tissue cultures

The major advantages of a cell culture system over the conventional cultivation of whole plants are as follows:

- Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions.
- Cultured cells would be free of microbes and insects.
- The cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites.
- Automated control of cell growth and rational regulation of metabolite processes would reduce labor costs and improve productivity.
- Organic substances are extractable from callus cultures.

Trends in Production of Secondary Plant Metabolites from Higher Plants

Plant cell and tissue cultures can be established routinely under sterile conditions from explants, such as plant leaves, stems, roots, and meristems for multiplication and extraction of secondary metabolites. Strain improvement, methods for the selection of high-producing cell lines, and medium optimizations can lead to an enhancement in secondary metabolite production. The capacity for plant cell, tissue, and organ cultures to produce and accumulate many of the same valuable chemical compounds as the parent plant in nature has been recognized almost since the inception of *in vitro* technology. The strong and growing demand in today's marketplace for natural, renewable products has refocused attention on *in vitro* plant materials as potential factories for secondary phytochemical products and has paved the way for new research exploring secondary product expression *in vitro*. There is a series of distinct advantages to producing a valuable secondary product in plant cell culture, rather than *in vivo* in the whole crop plant.

These include the following:

- Production can be more reliable, simpler, and more predictable.
- Isolation of the phytochemical can be rapid and efficient, when compared with extraction from complex whole plants.

- Compounds produced *in vitro* can directly parallel compounds in the whole plant.
- Interfering compounds that occur in the field-grown plant can be avoided in cell cultures.
- Tissue and cell cultures can yield a source of defined standard phytochemicals in large volumes.
- Tissue and cell cultures are a potential model to test elicitation.
- Cell cultures can be radiolabeled, such that the accumulated secondary products, when provided as feed to laboratory animals, can be traced metabolically.

While research to date has succeeded in producing a wide range of valuable secondary phytochemicals in unorganized callus or suspension cultures, in other cases production requires more differentiated micro plant or organ cultures. This situation often occurs when the metabolite of interest is only produced in specialized plant tissues or glands in the parent plant. A prime example is ginseng (*Panax ginseng*). Because saponin and other valuable metabolites are specifically produced in ginseng roots, root culture is required in vitro. Similarly, herbal plants such as *Hypericum perforatum* (St. John's wort), which accumulates the hypericins and hyperforins in foliar glands, have not demonstrated the ability to accumulate phytochemicals in undifferentiated cells. As another example, biosynthesis of lysine to anabasine occurs in tobacco (*Nicotiana tabacum*) roots, followed by the conversion of anabasine to nicotine in leaves. Callus and shoot cultures of tobacco can produce only trace amounts of nicotine because they lack the organ-specific compound anabasine. In other cases, at least some degree of differentiation in a cell culture must occur before a product can be synthesized (e.g., vincristine or vinblastine from *Catharanthus roseus*). Reliance of a plant on a specialized structure for production of a secondary metabolite, in some cases, is a mechanism for keeping a potentially toxic compound sequestered. Intensive activities have been centered on production of natural drugs or chemoprotective compounds from plant cell culture by one or more of the following strategies:

Organ Cultures for Secondary Metabolite Production

- *Fritillaria unibracteata* can be rapidly propagated, directly from small cuttings of the bulb by the technique of organ culture. The cultured bulb can be harvested after a 50-day culture period in MS media supplemented with 4.44 - M BA and 5.71 - M IAA. The growth rate was about 30–50 times higher than that under natural wild growth conditions. The content of alkaloid and beneficial microelements in the cultured bulbs was higher than found in the wild bulb.
- *In vitro* shoot multiplication of *Frangula alnus* was obtained on woody plant medium with indole-3-acetic acid and 6-benzylaminapurine, the highest metabolite production (1731 mg/100 g of total anthraquinone was in the shoots grown on the MS medium with addition of 1-naphthilaceneacetic (NAA) (0.1 mg/l) and thidiazuron (TDZ) (0.1 mg/l).

Precursor Addition for Improvement of Secondary Metabolite Production

- The treatment of plant cells with biotic and/or abiotic elicitors has been a useful strategy to enhance secondary metabolite production in cell cultures. The most frequently used elicitors in previous studies were fungal carbohydrates, yeast extract, M,J and chitosan. MJ, a proven signal compound, is the most effective elicitor of taxol production in *Taxus chinensis* Roxb. and gonsenoside production in *P. ginseng* C.A. Meyercell/organ culture.
- The involvement of amino acids in the biosynthesis of hyperforin and adhyperforin was reported in *H. perforatum* shoot cultures. Valine and isoleucine, upon administration to the shoot cultures, were incorporated into acyl side chain of hyperforin and adhyperforin, respectively. Feeding the shoot cultures with unlabelled lisoleucine at a concentration of 2 mM induced a 3-7-fold increase in the

production of a hyperforin. Production of triterpenes in leaf-derived callus and cell suspension cultures of *Centella asiatica* was enhanced by the feeding of amino acids. In the callus culture, manifold increase of asiaticoside accumulation was reported with the addition of leucine.

Elicitation of *In vitro* products

- Plants and/or plant cells *in vitro* show physiological and morphological responses to microbial, physical, or chemical factors which are known as "elicitors." Elicitation is a process of inducing or enhancing synthesis of secondary metabolites by the plants to ensure their survival, persistence, and competitiveness. The study was applied in several abiotic elicitors to enhance growth and ginseng saponin biosynthesis in the hairy roots of *P. ginseng*. Generally, elicitor treatments were found to inhibit the growth of the hairy roots, although simultaneously enhancing ginseng saponin biosynthesis. Tannic acid profoundly inhibited the hairy root growth during growth period.
- The production of secondary metabolites in callus, cell suspension, and hairy roots of*Ammi majus* L. is by exposing them to elicitors: benzo (1,2,3)-thiadiazole-7-carbothionic acid S-methyl ester and autoclaved lysate of cell suspension of bacteria-*Enterobacter sakazaki*. GC and GC-MS analysis of chloroform and methanol extracts indicated a higher accumulation of umbelliferone in the elicited tissues than in the control ones. Chitosan was the biotic elicitor polysaccharide and it is eliciting the manifold increase of anthraquinone production in *Rubia akane* cell culture.

Hairy Root Cultures as a Source of Secondary Metabolites

The hairy root system based on inoculation with *Agrobacterium rhizogenes* has become popular in the two last decades as a method of producing secondary metabolites synthesized in plant roots. The hairy root phenotype is characterized by fast hormone-

independent growth, lack of geotropism, lateral branching, and genetic stability. The secondary metabolites produced by hairy roots arising from the infection of plant material by *A. rhizogenes* are the same as those usually synthesized in intact parent roots, with similar or higher yields. This feature, together with genetic stability and generally rapid growth in simple media lacking phytohormones, makes them especially suitable for biochemical studies not easily undertaken with root cultures of an intact plant. During the infection process, *A. rhizogenes* transfers a part of the DNA (transferred DNA, T-DNA) located in the root-inducing plasmid Ri to plant cells, and the genes contained in this segment are expressed in the same way as the endogenous genes of the plant cells. Some A. rhizogenes, such as strain A4, have the T-DNA divided into two sections: the TR-DNA and TL-DNA, each of which can be incorporated separately into the plant genome. Two sets of pRi genes are involved in the root induction process: the *aux* genes located in the TR region of the pRi T-DNA and the *rol* (root loci) genes of the TL region. The hairy roots are normally induced on aseptic, wounded parts of plants by inoculating them with *A. rhizogenes*.

Genetic Manipulation in Hairy Root Culture for Secondary Metabolite Production

Transformed roots provide a promising alternative for the biotechnological exploitation of plant cells. *A. rhizogenes*-mediated transformation of plants may be used in a manner analogous to the well-known procedure employing *Agrobacterium tumifaciens*. *A. rhizogenes*-mediated transformation has also been used to produce transgenic hairy root cultures and plantlets have been regenerated. None of the other T-DNA sequences are required for the transfer with the exception of the border sequences. The rest of the T-DNA can be replaced with the foreign DNA and introduced into cells from which whole plants can be regenerated. These foreign DNA sequences are stably inherited in a Mendelian manner. The *A. rhizogenes*-mediated transformation has the advantage of being able to transfer any foreign gene of interest placed in binary vector to the transformed hairy root clone. An example of a gene of interest with regard to secondary metabolism that was

introduced into hairy roots is the 6-hydroxylase gene of *Hyoscyamus muticus* which was introduced to hyocyamin-rich *Atropa belladonna*by a binary vector system using *A. rhizogenes*. Engineered roots showed an increased amount of enzyme activity and a five-fold higher concentration of scopolamine.

Role of Endophytes in *In vitro* Production of Secondary Metabolites

There are three schools of thought on the origins of secondary metabolism in plants. There is the argument that both plants and endophytic microbes coevolved with pathways to produce these natural products. Another thought is that an ancient horizontal gene transfer took place between plants and microbes. The third suggests that either plants or endophytic fungi produce these secondary metabolites and transfer them to the other symbiont. Biosynthetic pathway studies using radiolabeled precursor amino acids reveal that plants and endophytic fungi have similar but distinct metabolic pathways for production of secondary metabolites. The question is whether bioactive phytochemicals of plants are produced by the plant itself or as a consequence of a mutualistic relationship with beneficial organisms in their tissue. The fact that a combination of inducing factors from both plants and endophytic fungi increased the accumulation of secondary metabolites in plants and fungi, respectively, suggest that the fungal endophyte may play important roles in the biosynthesis of secondary metabolites. Therefore, the symbiotic association and effects of plants and endophytes on each other during the production of other important pharmacological bioactive natural products such as camptothecin, vinblastine, and podophyllotoxin need to be explored. This could provide the framework for future natural product production through genetic and metabolic engineering.

Bioreactors Scaling up of Production of Secondary Metabolites

This is the application of bioreactor system for large-scale cultivation of plant cells for the production of valuable bioactive compounds in an active field. Plant cells in liquid

suspension offer a unique combination of physical and chemical environments that must be accommodated in large-scale bioreactor process.

Immobilization Scaling up of Secondary Metabolite Accumulation

- Advances in scale-up approaches and immobilization techniques contribute to a considerable increase in the number of applications of plant cell cultures for the production of compounds with a high added value. Plant-derived compounds with cancer chemotherapeutic or antioxidant properties use rosmarinic acid and taxol as representative examples.
- Cell cultures of *Plumbago rosea* were immobilized in calcium alginate and cultured in Murashige and Skoog's basal medium containing 10 mM $CaCl_2$ for the production of plumbagin, an important medicinal compound. Studies were carried to find out the impact of immobilization on the increased accumulation of this secondary metabolite. Immobilization in calcium alginate enhanced the production of plumbagin by three-, two-, and one-folds compared with that of control, un-crosslinked alginate and $CaCl_2$ -treated cells, respectively.

Tissue Cultures Producing Pharmaceutical Products of Interest

Research in the area of plant tissue culture technology has resulted in the production of many pharmaceutical substances for new therapeutics. Advances in the area of cell cultures for the production of medicinal compounds have made possible the production of a wide variety of pharmaceuticals such as alkaloids, terpenoids, steroids, saponins, phenolics, flavanoids, and amino acids.

POSSIBLE QUESTION

Unit-V

PART A (2 Marks)

1. What are the functions of phenols?
2. Define callus induction.
3. Define secondary metabolites.
4. Define totipotency.
5. Define somatic embryogenesis.
6. Write the physiological role of cytokinin.
7. What is metabolic engineering?

PART B (8 Marks)

1. What are the stages involved in secondary metabolite production? Explain.
2. Write the occurrence, distribution and functions of alkaloids and flavonoids.
3. Discuss on the metabolic engineering for increased production of secondary metabolites.
4. Explain in detail the callus induction and secondary metabolite production.
5. How will you increase the production of plant alkaloid biosynthesis? Write its Mechanism.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed University established Under Section 3 of UGC Act 1956)

DEPARTMENT OF BIOCHEMISTRY

III B.SC BIOCHEMISTRY – FIFTH SEMESTER

17BCU503A – PLANT BIOCHEMISTRY

MULTIPLE CHOICE QUESTIONS

Unit V

SL.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4		ANSWER
1	The response of a plant to the relative length of light and dark period is called as	phytochrome	photoperiod	cytochrome	photoreceptor		photoperiod
2	The protein pigment that absorb red and far red light most strongly is	cytochrome	phytochrome	photochlorophyllide	bacterial chlorophyllide		phytochrome
3	Coumarin inhibits	seed germination	senescence	flowering	fruiting		seed germination
4	A Study of aging in plants is called as	dormancy	senescence	ripening	fruiting		senescence
5	The photoperiod required to induce flowering for Maryland Mammoth tobacco is	12 hours	15 hours	10 hours	8 hours		12 hours
6	In phytochrome Pr for is converted to Pfr form at the wavelength	730-735 nm	660-665 nm	440-455 nm	540-555 nm		660-665 nm
7	The seeds requiring single exposure of light for germination are called	non-photoblastic seeds	positive photoblastic seeds	negative photoblastic seeds	photoclastic seeds		positive photoblastic seeds
8	Senescence of detached leaves can be delayed by the use of	auxin	gibberellins	cytokinin	abscisic acid		cytokinin
9	*Capsicum annum* is a	short day plant	long day plant	photo-neutral plant	intermediate plant		photo-neutral plant
10	In phytochrome Pfr for is converted to Pr form at the wavelength	730-735 nm	660-665 nm	440-455 nm	540-555 nm		730-735 nm
11	The method employed in softening or weakening the seed coat is called	stratification	scarification	after-ripening	alternating temperature		scarification
12	Senescence is induced by the following hormones except	abscisic acid	ethylene	cytokinin	gibberellins		cytokinin

13	*Hibiscus syriacus* is a	long day plant	short day plant	intermediate plant	photo-neutral plant		long day plant
14	Phytochrome is a	lipoprotein	chromoprotein	glycoprotein	nucleoprotein		chromoprotein
15	Seed germination is induced by the chemical	CMU	DCMU	thiourea	guanidinium		thiourea
16	Rice is an example of	whole plant senescence	sequential senescence	shoot senescence	simultaneous senescence		whole plant senescence
17	Rice is a	long day plant	short day plant	intermediate plant	photo-neutral plant		short day plant
18	The active form of phytochrome is	Pr	Pfr	Ia	Ib		Pfr
19	Respiration rate is increased during	senescence	flowering	fruiting	seed germination		seed germination
20	An example of a plant undergoing shoot senescence is	rice	wheat	banana	mustard		banana
21	The photoperiod required to induce flowering for *Xanthium strumarium* is called	12 hours	15.5 hours	10 hours	18 hours		15.5 hours
22	The photo period of short day plants is	less than 12 hours	less than 10 hours	less than 15 hours	less than 5 hours		less than 12 hours
23	*Xanthium pensylvanicum* is a	short day plant	long day plant	photo neutral plant	intermediate plant		short day plant
24	Chrysanthemum is a	short day plant	long day plant	photo neutral plant	intermediate plant		short day plant
25	Soybean is a	short day plant	long day plant	photo neutral plant	intermediate plant		short day plant
26	The photoperiod required by long day plants is	more than 12 hours	more than 10 hours	more than 15 hours	more than 5 hours		more than 12 hours
27	*Hyoscyamus* is a	short day plant	long day plant	photo neutral plant	intermediate plant		long day plant
28	*Spinacea oleracea* is a	short day plant	long day plant	photo neutral plant	intermediate plant		long day plant
29	*Hibiscus syriacus* is a	short day plant	long day plant	photo neutral plant	intermediate plant		long day plant
30	*Anethum graveolens* is a	short day plant	long day plant	photo neutral plant	intermediate plant		long day plant

31	*Lycopersicum esculentum* is a	short day plant	long day plant	photo neutral plant	intermediate plant		photo neutral plant
32	*Capsicum annum* is a	short day plant	long day plant	photo neutral plant	intermediate plant		photo neutral plant
33	The plants require a photoperiod of 12 to 15 hours for flowering is called	short day plant	long day plant	photo neutral plant	intermediate plant		intermediate plant
34	*Mechania* is a	short day plant	long day plant	photo neutral plant	intermediate plant		intermediate plant
35	*Bryophyllum* is a	short day plant	long day plant	photo neutral plant	long short day plant		long short day plant
36	Pr form is converted to Pfr form at	730-735 nm	660-665 nm	450-550 nm	400-500 nm		660-665 nm
37	Pfr form is converted to Pr form at	730-735 nm	660-665 nm	450-550 nm	400-500 nm		730-735 nm
38	The pigment responsible for the inhibition of flowering	Chlorophyll	carotenoid	xanthophylls	phytochrome		phytochrome
39	Inactive form of phytochrome is	Pr	Pfr	Ia	Ib		Pr
40	Active form of phytochrome is	Pr	Pfr	Ia	Ib		Pfr
41	The conversion of Pfr to Pr is accelerated by	Dithionite	thiourea	CMU	DCMU		Dithionite
42	The conversion of Pr to Pfr is	Oxidation reduction process	decarboxylation process	transamination process	deamination process		Oxidation reduction process
43	Phytochrome is a	lipoprotein	nucleoprotein	chromoprotein	glycoprotein		chromoprotein
44	Number of disulphide linkage in in one phytochrome molecule is	3	6	8	2		6
45	The light absorbing property of phytochrome is due to	protein	amino acids	chromophore	disulphide linkage		chromophore
46	Stimulation of flowering in long day plants during day time is due to the accumulation of	Pr	Pfr	Ia	Ib		Pfr
47	Inhibition of flowering in short day plants during day time is due to the accumulation of	Pr	Pfr	Ia	Ib		Pfr
48	In short day plants flowering is induced by Pr form during	light period	dark period	in presence of sun light	only in evening		dark period
49	In lettuce and tobacco seeds seed germination is induced by	auxin	gibberellins	cytokinin	ethylene		gibberellins
50	The following chemicals induce seed germination except	KNO3	thiourea	ethylene	CMU		CMU
51	Seed germination is induced by	blue light	red light	yellow light	green light		red light

52	During germination the starch in cereal endosperm is converted to	amylase	amylopectin	sucrose	maltose		maltose
53	In cotton seeds lipase synthesis is triggered by	auxin	gibberellins	cytokinin	ethylene		gibberellins
54	The activity of peptidase is stimulated by the endogenous hormone	auxin	GA	cytokinin	ethylene		GA
55	Reserve pool of phosphorus in seeds is	phytin	inorganic phosphate	ortho phosphoric acid	meta phosphoric acid		phytin
56	Lotus seeds can survive for	200 years	400 years	50 years	5 years		400 years
57	The optimum temperature for seed germination is	25-35°C	40-50°C	15-20°C	60-75°C		25-35°C
58	Senescence of detached leaves can be delayed by the use of	auxin	gibberellins	cytokinin	ethylene		cytokinin
59	One of the following statement is false about senescence	cytokinin delay senescence	senescence is accompanied by losses of chlorophyll	gibberellins retard leaf senescence	senescence does not occur due to nutrient competition		senescence does not occur due to nutrient competition
60	The response of plant to the relative length of light and dark period is called	photoperiodism	vernalization	germination	dormancy		photoperiodism